Acquired Demyelinating Syndromes and Pediatric Multiple Sclerosis

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ACQUIRED DEMYELINATING SYNDROMES AND PEDIATRIC MULTIPLE SCLEROSIS

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CHAPTER 1

General introduction

ACQUIRED DEMYELINATION OF THE CENTRAL NERVOUS SYSTEM

Acquired inflammatory demyelinating diseases of the central nervous system (CNS) cause damage to myelin sheaths and typically result in white matter lesions due to inflammation, myelin loss and axonal pathology. Clinically, this may result in transient, relapsing or progressive neurological dysfunction. Multiple sclerosis (MS) is the most common disease within this spectrum. It typically affects adults between 20 and 40 years old. MS is generally assumed to be an autoimmune disease, of which the exact etiology remains unknown. Genetic, immunological and environmental factors each play a role in the pathophysiology of the disease.[1]

Several other immune-mediated demyelinating diseases of the CNS are known. These include Acquired Demyelinating Syndrome (ADS) of childhood, Neuromyelitis Optica (NMO), Acute Disseminated Encephalomyelitis (ADEM), Transverse Myelitis (TM) and Optic Neuritis (ON).[2 3]

All these acquired demyelinating syndromes are rare. In general, clinicians encounter several problems when faced with rare diseases:

- diagnosis is more complex or may be delayed, because a clinician rarely encounters these diseases and thus may be inexperienced with or unaware of them
- the disease course and future may be hard to predict because of lack of insight into pathogenesis
- treatment is often based on expert-opinion instead of large randomized controlled trials, making it difficult to establish the optimal treatment and timing of treatment
- research is challenged by the low number of patients.

For patients, it is more challenging to find the right information or to find fellow sufferers of the same disease.[4]

This thesis focuses on pediatric ADS and MS, ADEM and NMO and describes the clinical features of these diseases. The goal of these studies is to find disease-specific characteristics that will improve early and accurate diagnosis.

MULTIPLE SCLEROSIS (MS) IN CHILDREN: HISTORY AND RESEARCH BACKGROUND

MS is a well-known disease in young adults. However, most people are unaware that 3-5% of all MS patients experience their first attack in childhood.[5-8] Even physicians rarely recognize or diagnose MS in children. However, it is not 'new', as most people think. Perhaps the first patient in history that could be diagnosed with MS was Lidwina of Schiedam (the Netherlands), living in the 14th century, who developed the first symptoms at the age of 14 years. She fell while skating and developed a recurrent and progressive disease.[9] The first case series of 13 children with MS dates from 1883.[10]

Although MS in children is increasingly recognized in recent decades, it was mostly considered and treated equally as adult MS. The Multiple Sclerosis International Federation

(MSIF), and several of its member societies, finally became aware of the need for special care, education and research in pediatric MS. They supported and facilitated the founding of the International Pediatric MS Study Group (IPMSSG) in 2007. This global network unites more than 150 physicians and researchers treating and/or studying MS in children. The IPMSSG aims to structure and improve clinical care and research for example by determining clinical definitions and finding tools for accurate and early diagnosis. They also aim to improve treatment for children with MS, by defining design, outcome measures and feasibility of clinical trials (www.ipmssg.org).[11-14] Since the establishment of the IPMSSG, research in pediatric MS and related variants flourishes. Worldwide, pediatric MS care centers are founded and national research collaboration programs are organized.

The term ADS was introduced in 2009 and refers to a first episode of inflammatory CNS demyelination occurring in childhood.[3] Because of the unawareness of ADS and its variants in children it is often considered too late to be a possible diagnosis. In general, ADS is associated with several uncertainties, for example about recovery, future development and a subsequent diagnosis of MS. The risk of MS following such an initial attack is unknown. It is important to determine the environmental, immunological, genetic and neuroimaging features predictive of MS for two reasons. First, this will aid in the adequate characterization of patients at first demyelinating event. This may have important prognostic and treatment implications. Secondly, insights into predictive factors are essential to understand the pathophysiology of MS in general and ideally to find new therapeutic or even preventive strategies.

In the Netherlands a nationwide prospective study was started investigating ADS variants and pediatric MS in 2007.

The aims of this study are:

- defining the incidence of pediatric ADS in the Netherlands
- defining the proportion of these children with a subsequent diagnosis of MS
- gathering epidemiological data to identify possible risk factors for final MS diagnosis (e.g. family history, ethnicity)
- characterizing clinical and neuroimaging features of ADS, and define which of these features are prognostic for MS in children
- gathering blood, cerebrospinal fluid (CSF) and DNA samples for research on prognostic biomarkers for MS
- gathering long-term follow-up data in this cohort to provide information about relapse rate, disease course and neuropsychological consequences of ADS
- comparing the features of ADS and MS in children with clinically isolated syndrome (CIS) and MS in adults.

This ongoing study is termed 'PROUD*kids*' (PRedicting the OUtcome of a Demyelinating event in children), and is similar to the PROUD study performed in adults with CIS. Children with ADS are referred to us either directly by members of the PROUD*kids* study group, which is a network of pediatric neurologists of the eight Dutch academic hospitals

and of five nonacademic neuropediatric hospitals, or by the NSCK (Netherlands Pediatric Surveillance Unit). The latter reaches all Dutch pediatricians monthly by e-mail and aims to provide insight in the epidemiology of rare pediatric diseases in the Netherlands. This research is performed in collaboration with European physicians and researchers[15] and with the IPMSSG.

ACQUIRED DEMYELINATING SYNDROMES (ADS) OF CHILDHOOD

ADS is a group of disorders characterized by acute inflammatory demyelination of the CNS in children.[3] These disorders differ in clinical presentation, as well as in outcome. The dysfunction caused by the demyelination may either be at a single site (monofocal) or may be due to simultaneous demyelination of multiple sites in the brain, optic nerves or spinal cord (polyfocal). They may present either as one single event (monophasic illness) or as part of chronic disease with recurrent episodes of demyelination which results most often in a diagnosis of MS. In a retrospective French cohort of 296 children with ADS, 57% had a final diagnosis of MS after a median follow-up period of 1.9 years.[16] In the Netherlands, data from our retrospective cohort showed that only 37 of the 117 children (32%) fulfilled the clinical McDonald criteria for MS after a median follow-up of 3.6 years.[17] In the Canadian prospective cohort, by now 63 of the 302 children with ADS (21%) have a diagnosis of MS, after a median follow-up of 3.14 years.[18]

The variants of ADS are summarized in Table 1.1. The definitions of these variants in children were revised in 2013.[12] Distinguishing different subtypes at clinical presentation can be useful because of the difference in course and outcome of the subtypes. Strict definitions are also needed to be able to compare different studies. However, substantial similarities between the different variants of ADS exist and they may be difficult to distinguish with certainty at a first event. Therefore these definitions are still under debate.

The most well-known ADS in children is *acute disseminated encephalomyelitis* (*ADEM*). But ADEM is also the most challenging disorder to define. ADEM is very rare in adults and affects mostly children younger than 10 years old. It is generally thought that a child with ADEM is more likely to have a monophasic disease with good outcome. The symptoms of the event may fluctuate within 3 months. However, relapses of ADEM may occur, making it even more difficult to distinguish ADEM from a first event of MS. The numbers of patients who were diagnosed with MS after an initial diagnosis of ADEM differ in retrospective studies between 0% and 29%.[16 21 22] Because ADEM and MS can appear as clinically similar demyelinating disorders, especially in young children, it can be problematic to distinguish them at first presentation. The IPMSSG stated that the clinical presentation of ADEM is characterized by polyfocal neurological deficits and must include encephalopathy, defined as alteration of behavior or consciousness.[11] Although this may be overly restrictive, they argued that encephalopathy is typically uncommon in MS[16 23], and therefore can differentiate between

Table 1.1. Proposed 2013 IPMSSG criteria (derived from Krupp et al. Mult Scler. 2013)[12]

Pediatric CIS (all are required)	- A monofocal or polyfocal, clinical CNS event with presumed inflammatory demyelinating cause - Absence of a prior clinical history of CNS demyelinating disease - No encephalopathy* - The diagnosis of MS based on baseline MRI features[19] is not met
Pediatric ADEM (all are required)	- A first polyfocal, clinical CNS event with presumed inflammatory demyelinating cause - Encephalopathy* - No new clinical and MRI findings emerge three months or more after onset - Brain MRI is abnormal during the acute (three-month) phase - Typically on brain MRI: • diffuse, poorly demarcated, large (>1-2 cm) lesions involving predominantly the cerebral white matter • T1 hypointense lesions in the white matter are rare • deep grey matter lesions (e.g. thalamus or basal ganglia) can be present
Pediatric MS (any of the following)	- Two or more nonencephalopathic (e.g. not ADEM-like), clinical CNS events with presumed inflammatory cause, separated by more than 30 days and involving more than one area of the CNS - One nonencephalopathic episode typical of MS which is associated with MRI findings consistent with 2010 Revised McDonald criteria for DIS and in which a follow up MRI shows at least one new enhancing or nonenhancing lesion consistent with DIT MS criteria[19] - One ADEM attack followed by a nonencephalopathic clinical event, three or more months after symptom onset, that is associated with new MRI lesions that fulfill 2010 Revised McDonald DIS criteria[19] - A first, single, acute event that does not meet ADEM criteria and whose MRI findings are consistent with the 2010 Revised McDonald criteria for DIS and DIT (applies only to children ≥ 12 years old)
Pediatric NMO (all are required)	- Optic neuritis - Acute myelitis - At least two of three supportive criteria: • contiguous spinal cord MRI lesion extending over three vertebral segments • brain MRI not meeting diagnostic criteria for MS • anti-aquaporin-4 IgG seropositive status

* Encephalopathy is defined as alteration in consciousness or behavior, which cannot be explained by fever.
MRI= magnetic resonance imaging, DIS= dissemination in space, DIT= dissemination in time.

ADEM and a first episode of MS.[24] Of course this does not apply for all children. The absence of encephalopathy does not rule out ADEM.[17 25] Conversely, children with a final diagnosis of MS can present with encephalopathy at onset. For example in the retrospective study of Dutch children with ADS, children with a polyfocal onset without encephalopathy were indeed more likely to have a final diagnosis of MS (in 26% of children) compared to children with a polyfocal onset with encephalopathy (17%), but this difference was not significant.[17] In a study in children and adults with ADEM and MS confirmed by brain biopsy or autopsy, the IPMSSG criteria had a sensitivity of 80% and they were 91% specific for a pathological diagnosis of ADEM. This means that in 9% of these patients with pathologically confirmed MS and a clinical MS diagnosis at last follow-up, an incorrect diagnosis of ADEM was made.[26] Although encephalopathy is the most helpful disease characteristic, one must keep

Table 1.2. Differences between ADEM and MS[11 27 28]

	ADEM	MS
Age	<10 years	> 10 years
Sex	possible male preponderance	female preponderance
Presentation	polyfocal	more often monofocal
Symptoms	encephalopathy, fever, headache, meningismus, seizures, ataxia, brainstem symptoms	pyramidal signs, brainstem symptoms, partial myelitis, (unilateral) optic neuritis
Preceding infection	frequent	possible
Brain MRI lesions	- large (>2 cm), multiple - confluent, ill-defined lesions - bilateral deep grey matter lesions (thalamus, basal ganglia) - perifocal edema - mass effect - absence of previous demyelinating lesions	- well-defined lesions - perpendicular to corpus callosum, periventricular or juxtacortical - asymptomatic lesions - black holes (hypointense lesions on T1-weighted images)
Follow-up MRI	(in)complete resolution of lesions	new lesions and black holes
CSF	- oligoclonal banding: possible - mild pleocytosis: frequent	- oligoclonal banding: frequent - mild pleocytosis: possible
Disease course	mostly monophasic	multiphasic

These differences are relative and cannot be used as strict criteria
MRI= magnetic resonance imaging, CSF= cerebrospinal fluid.

in mind some other characteristics that can aid in distinguishing ADEM and MS at onset. These are summarized in Table 1.2.

Clinically isolated syndrome (CIS) is assumed to have a higher risk of subsequent MS diagnosis, ranging from 38-46%.[17 24] The presentation can be monofocal (optic neuritis, transverse myelitis or a brainstem syndrome) or polyfocal (affecting multiple sites of the CNS), but the main difference with ADEM is that these children do not present with encephalopathy.[11]

A first episode of *optic neuritis (ON)* is generally more straightforward to identify. ON is caused by inflammation of the optic nerve and can present with sudden reduced visual acuity, decreased color perception and painful eye movements. ON can be unilateral or bilateral, manifest as a monofocal symptom, or in the context of polyfocal neurological deficits. In general, recovery is good in children[29], with a visual acuity of at least 20/40 at follow-up in 83-96% of the affected eyes.[30 31] The risk to develop MS is about 19-36%.[29-32] Unilateral ON is more often observed in older children (> 10 years). The risk to develop MS is higher in older children and when white matter lesions on brain MRI are present at onset.[30-33] Mild unilateral optic neuritis in a young child may remain undiagnosed, because a young child does not complain about moderate visual loss and parents may not recognize it.

In *transverse myelitis (TM)* a demyelinating lesion in the spinal cord can cause motor, sensory and autonomic deficits, as well as pain. An isolated acute TM is usually a monophasic

event. Outcome can range from complete recovery to very poor outcome or even death.[34 35] Studies showed that 13-43% of patients remain non-ambulatory, 22-80% had persisting bladder dysfunction[34-36] and 46-75% still experienced sensory symptoms.[34 36] A younger age at onset was associated with poor outcome, especially for bladder function.[34 35] Approximately 13% of children with TM have a final diagnosis of MS[35] and 8% of children with MS had an isolated TM as first attack.[16] TM can also occur as part of a polyfocal demyelinating syndrome or in ADEM.

When TM manifests together with ON (simultaneously or consecutively) a diagnosis of *neuromyelitis optica (NMO)* should be considered. This disease should especially be suspected when the spinal cord lesion extends at least 3 vertebrae on MRI, which is then defined as longitudinally extensive transverse myelitis (LETM).[37] NMO will be discussed in more detail separately.

EPIDEMIOLOGY OF MS IN CHILDREN

Recent studies in respectively Canada and the British Isles report an annual incidence of ADS of 0.9 and 1.0/100,000 children.[3 38] A reliable prevalence or incidence of pediatric MS is still unknown. The estimated incidence is between 0.3 and 0.5/100,000 children. However, these figures are derived from studies that are not nationwide and prospective.[39 40] Incidence numbers may vary by geographic region and by follow-up duration. They may be underestimated, because subjective and transient symptoms in children may be missed. At first, other diseases in childhood may be more plausible, delaying a final diagnosis of MS.[41]

An increased female preponderance is observed only with higher age, as is an increased incidence of MS. In children younger than 10 years, the female-to-male ratio is about equal.[8 42] About 8% of children with MS have a family history of MS. This number may be higher, because the family members are still in the age range at risk of developing MS.[42] There appears to be an increased susceptibility to pediatric-onset MS in non-Caucasian populations living in northern countries like the US and Canada.[8 40 43]

PROGNOSTIC FACTORS FOR MS IN CHILDREN

The following clinical characteristics have already been identified as associated with a higher MS risk:[16-18 22 24 31 44]

- a first presentation above the age of 10 years
- clinical presentation:
    - monofocal
    - without encephalopathy
- CSF:
    - elevated IgG index

- presence of oligoclonal bands (OCB)

- brain MRI abnormalities at baseline, including:
  - non-symptomatic lesions
  - optic nerve lesion or MS suggestive lesions
  - pediatric MS MRI criteria.

In clinical practice, performing an MRI scan will be one of the first steps in the diagnostic process. Together with the clinical symptoms and laboratory results, the appearance of the white (and/or grey) matter lesions on MRI, can aid in the differential diagnosis of ADS and predict MS outcome. Until 2004, only the McDonald criteria were available for MS diagnosis, which incorporated the Barkhof MRI criteria.[45 46] These MRI criteria were developed for adults. In adults the differential diagnosis differs from that in children and includes aspecific white matter lesions or vascular lesions. In 2004 the French group developed the KIDMUS criteria, in a pediatric ADS population. These were very specific, but lacked sensitivity.[47] Searching for more sensitive criteria, the Canadian group proposed two sets of criteria. The first was a modification of the McDonald criteria (Callen diagnostic pediatric MS criteria) and was developed to distinguish children with clinically definite MS from children with other non-demyelinating relapsing neurologic disorders (SLE and migraine), with 85% sensitivity and 98% specificity.[48] Because in this study MRI scans performed at the second attack (which can be clinically the MS defining attack) were used, these criteria are of no value to define prognosis at first event. Preferably, MRI can aid in distinguishing ADEM from MS at first attack, because this is the most challenging distinction in clinical practice. The Canadian group showed that Callen diagnostic pediatric MS criteria were not specific for this particular purpose, because 70-75% of the children with ADEM fulfilled these criteria. The KIDMUS criteria are very specific (100%) also for this purpose, but not sensitive (29%). Therefore the Canadian group developed a second set of criteria, called the Callen MS-ADEM criteria. These were 81% sensitive and 95% specific for distinguishing ADEM from MS at onset in their original study.[49] All the proposed MRI criteria and their ability to predict MS diagnosis at onset are summarized in Table 1.3.

ENVIRONMENTAL RISK FACTORS IN MS

Epidemiological studies revealed that genetic as well as environmental factors are involved in the etiology of MS. Epidemiological, especially migration studies, showed that the environmental risk factors are specifically important during childhood.[54] It is known that MS is particularly prevalent in countries remote from the equator, thus in countries with a colder climate and less exposure to sunlight. In addition, studies showed that place of residence seems to be more important as a risk factor than ethnicity. When a child is born in a country of low MS prevalence, and moves during childhood years to a country of high MS prevalence, it adopts the higher MS risk of the new country.[55 56] This suggests that in a child

Table 1.3. MRI characteristics of pediatric MS

MRI criteria	Original purpose	Sensitivity (%)	Specificity (%)	References
McDonald MRI criteria [46] (at least 3 out of 4) - ≥ 9 lesions on T2-weighted imaging - ≥ 3 periventricular lesions - ≥ 1 juxtacortical lesion - ≥ 1 infratentorial lesion	Prognostic for MS in adult population	52-69	63-92	17 47 50 51 52
KIDMUS criteria [47] (both) - corpus callosum long axis perpendicular lesions - the sole presence of well-defined lesions	Prognostic for MS in ADS population	8-49	96-100	17 47 48 51 52
Callen diagnostic pediatric MS criteria [48] (at least 2 out of 3) - ≥ 5 lesions on T2-weighted imaging - ≥ 2 periventricular lesions - ≥ 1 brainstem lesion	Distinguish MS from non-demyelinating recurrent diseases (migraine, SLE)	74-89	68-90	51-53
Callen MS-ADEM criteria [49] (at least 2 out of 3) - absence of a diffuse bilateral lesion pattern - black holes - ≥ 2 periventricular lesions	Distinguish MS from ADEM	95	90	51

Sensitivity and specificity are given for studies in ADS populations, not for the original studies (except for the KIDMUS study, which is an ADS cohort study).

with a certain genetic background, exposure to certain environmental factors can initiate the disease. Several environmental factors involved in the etiology of MS in adults have been studied more specifically.

It is generally thought that viral exposure during childhood can induce MS in adulthood. This means that children provide a unique opportunity to study these effects, because of a shorter time window between exposure to common environmental viruses and disease-onset. Epstein-Barr virus (EBV) has been of particular interest because it infects B lymphocytes and persists latently in memory B cells. A consistent relation between a remote EBV infection and increased MS susceptibility in adults has been described.[57] Also in children, numerous studies have confirmed the significant increased frequencies of EBV seropositivity in children with MS compared to healthy controls, with higher mean EBV nuclear antigen (EBNA titers) in MS patients.[18 58-62]

Another important environmental risk factor is vitamin D deficiency. Vitamin D may have immunomodulatory effects and a protective effect in MS.[63] Lower vitamin D levels in patients with a first demyelinating event are associated with a higher risk for a subsequent diagnosis of MS.[18 64] In adults as well as in children with MS, higher serum vitamin D levels are associated with lower MS risk and with lower relapse rate.[65 66]

Studies on the association between smoking and MS risk in adults show contrasting results but most showed that smoking increases risk of adult MS.[67] One French study found that the risk to develop MS in a child is increased by 2-fold when a child is exposed to passive smoking. This risk increased with a longer duration of exposure in older children.[68]

Also obesity in childhood or early adulthood is related to an increased risk of MS later in life.[69 70 71] This is confirmed by one study in children showing that higher body mass index is associated with an increased risk of MS and CIS in adolescent girls.[72]

Vaccination has been linked to acquired demyelination, especially ADEM, but this association is largely based on case-reports and case series and not confirmed in case control or cohort studies.[27] A French cohort study showed that hepatitis B vaccination did not increase risk of ADS.[73 74] Hepatitis B or tetanus vaccination was not associated with an increased risk of conversion to MS in an ADS cohort.[75]

GENETIC RISK FACTORS IN MS

The general lifetime risk to develop MS as an adult is 1 in 1,000. However, having a first-degree relative with MS increases this risk to 2-5%. Having a monozygotic twin sibling with MS, increases the risk further to approximately 27%.[55 76] This indicates that only for a small part a genetic component is involved. For years, the only known risk allele was HLA-DRB 1*15 in MS patients of European descent. This increased susceptibility was similar in pediatric-onset MS.[18 77 78] HLA-DRB1 is a locus in the major histocompatibility complex class II. To date, numerous genome-wide association studies (GWAS) have already been performed, but only in adult MS patients. As a result of large research collaborations, more than 100 risk SNPs (single nucleotide polymorphisms) outside the HLA region have already been identified.[79] However, they account for a less significant effect with lower odds ratios.

DISEASE COURSE AND PROGNOSIS OF MS IN CHILDREN

It is important to accurately diagnose MS as early after disease-onset as possible, because of the impact of the prognosis and because of the available therapies that can alter disease course. Almost all children have a relapsing-remitting type of disease course.[42] It is generally thought that children have a more benign disease than adults, because a young brain has better repair capacities and possesses additional compensatory mechanisms. Children have a higher annualized relapse rate and initially a more favorable recovery. In general the

disease progression is slower and it takes a longer time before they enter the secondary progressive state of the disease. However, because of their young age at the time of disease-onset, they tend to reach this secondary progression at a younger age than patients with adult onset MS.[5 80-83]

MS can have severe psychosocial consequences, especially in children undergoing personal and cognitive development. The diagnosis of a rare chronic illness, with unpredictable attacks of neurological impairment, may be difficult to cope with.[84] On top of this, cognitive decline, fatigue and depression, which are common features of MS in adults, also occur in children with MS. More than 31% of the children with MS have a decrease in cognitive functioning in the first years of their disease, irrespective of disability status and number of relapses. The occurrence of cognitive impairment early in the disease course may be explained by the interference of the disease in the processes of CNS myelination and maturation of neuronal networks in children.[85-87] Fatigue and depression are also common features of MS in children and is described in up to 75% of patients.[85 87 88]

MANAGEMENT AND TREATMENT

Severe acute exacerbations of ADS are treated according to the adult guidelines with intravenous methylprednisolone. In case of treatment failure one can opt for plasma exchange or intravenous immunoglobulins (IVIg).[15]

As children with MS are likely to have a more inflammatory disease and exacerbations tend to recover better in children than in adults, they are also likely to benefit from the currently available disease-modifying therapies. In adults interferon-beta 1 and b and glatiramer acetate are currently used as first line treatment. These drugs have proven to reduce the number (by approximately 30%) and severity of relapses, and potentially reduce cognitive and physical disability.[89] Randomized controlled trials of these MS medications are only available in the adult MS population. When prescribing 'adult' drugs in adult dosing to a child, one has to consider a different tolerance to medication and the unknown effects on child development. Multiple safety reports (mostly small case series) on MS medication in children have now been published, providing information about effect, tolerability and safety.[15] The results are summarized in Table 1.4. They show that these therapies are beneficial in children as well, with reduction in relapse rate and comparable side effects. However, the effect on long term disease course is still uncertain. The drugs can be initiated in a lower dose, increasing the dose in several weeks to reach the full dose that has been proven effective in adults.

When first line treatment does not suffice, an option is to switch first line drugs before passing on to second line treatment. Treatment failure is difficult to define, but must be considered when:

- severe side effects occur
- the number of relapses during at least one year of treatment is stable or progressive

- more than two attacks occur during 1 year of treatment.[14 15]

Second line treatment seems to be more effective in adults than first line treatment, but also raises more concerns because of severe or even fatal side effects observed. These drugs include natalizumab and fingolimod.[90-92] The number of children treated with natalizumab is increasing. Limited numbers of reports describe it to be very effective with no serious side effects reported.[93] However, the duration of exposure is too short to reliably address the safety issues. In general, progressive multifocal leukoencephalopathy (PML) occurs very rarely in children younger than 12 years old and children have lower JC polyomavirus infection rates than adults.[94] Accordingly, the risk to develop PML may be lower in children. On the other hand, we know that in adults the chance to develop PML increases with longer treatment duration.[95] So when a young child is treated with natalizumab, the chance to develop PML may be considerable.

No data on the use of fingolimod in children are as yet available. One of the greatest benefits of this drug is the oral formula, which is expected to increase treatment compliance. Two fatal infections (disseminated primary varicella zoster and herpes simplex encephalitis) occurred in adults using fingolimod.[92]

Table 1.4. Current available 1st and 2nd line disease-modifying therapies in children

Drug	Effect	Side effects	Tolerability
Interferon beta 1a and 1b [99-108] (Avonex 30 µg IM weekly, Rebif 22 or 44 µg SC 3 times per week, Betaferon 0.25 mg SC every other day)	Decrease in ARR (from 2.5-1.7 pre-treatment to 0.4-0.04 post-treatment)	- Flu-like symptoms - Leukopenia -Thrombocytopenia - Anemia - Transient elevation in transaminases - Injection site reactions (subcutaneous formulation) - Myalgia - Headache - Fatigue	Discontinuation up to 60%
Glatiramer acetate [104 106 107 109] (Copaxone 20 mg daily SC)	Decrease in ARR (from 2.1-2.8 pre-treatment to 0.25-0.2 post-treatment)	- Injection site reactions - Transient systemic reaction (flushing, dizziness) - Chest pain - Fatigue	Discontinuation up to 35%
Natalizumab [93 110] (Tysabri 300 mg IV monthly)	- Decrease in ARR (from 3.7-2.4 pre-treatment 0.4-0.1 post-treatment) - Mild reduction in disability score (EDSS from 2.7-2.0 to 1.9-1.0)	- Hypersensitivity reaction - Severe (opportunistic) infections, such as PML	Discontinuation up to 10%

IM= intramuscular, SC= subcutaneous, IV= intravenous, ARR= annualized relapse rate, EDSS= Expanded Disability Status Scale, PML= progressive multifocal leukoencephalopathy.

There are only case reports or case series on other immune modulating drugs like cyclophosphamide[96], mitoxantrone[97] and rituximab[98]. Because of the reported side effects and limited experience in adult MS, these therapies are currently not recommended for use in children.

Symptomatic therapies, including treatment of fatigue, depression, spasticity and bladder dysfunction, have not been evaluated in children with MS yet. Non-pharmacological intervention includes physical and exercise therapy, assistive devices, speech therapy and neuropsychological assessment with appropriate intervention when necessary.[84 111]

LESSONS TO BE LEARNED FROM CHILDREN WITH MS

Although MS in children may just be one end of the broad MS spectrum, there are several problems to address in this specific age group, including developmental, biological, social en treatment issues. From a clinical point of view MS in children is rather similar to MS in adults, especially in children older than 10 years. Nevertheless some distinct features have already been reported (Table 1.5).

Whether the immunological background is different in children compared to adults remains to be determined. It has already been demonstrated that the presence of OCB in the CSF is comparable with adults and is reported to be as frequent as 92% in children with MS.[116]

Assessment of risk factors of MS in children provides a unique opportunity to explore relevant preceding factors that are important for the onset and course of the disease in general. In adults the time between probable disease-onset and disease presentation is long. Adults have been exposed to many potential, but also many irrelevant risk factors. Children have

Table 1.5. Characteristics of pediatric onset MS that differ from adult onset MS[17 42 43 50 80-82 85 112-115]

Sex	Females = males in children < 10 years old Females > males in children > 10 years old
Ethnicity	More often non-Caucasian ancestry
Presenting symptoms	More often polyfocal More often cerebellar symptoms; Ataxia is especially common in younger patients
MRI	McDonald MS criteria cannot be applied < 10 years old Larger lesions and fewer well-defined T2 hyperintense lesions in younger children Probably a higher lesion burden in older children More often infratentorial lesions
Disease course	Relapsing remitting in > 95% of cases
Relapse rate	Higher
Recovery after relapse	Usually good and more rapid
Disability	Lower disability scores (controlled for disease duration)
Progression	Slower, although overall secondary progression is reached at a younger age
Cognition	Impairment already revealed early in the disease course

not been exposed to as many environmental factors as adults, but must have encountered some relevant agents because they developed MS at such an early age. Furthermore they can be studied during the period of first exposure to environmental factors, and the time between exposure and disease initiation is shorter than in adults (displayed in Figure 1.1).

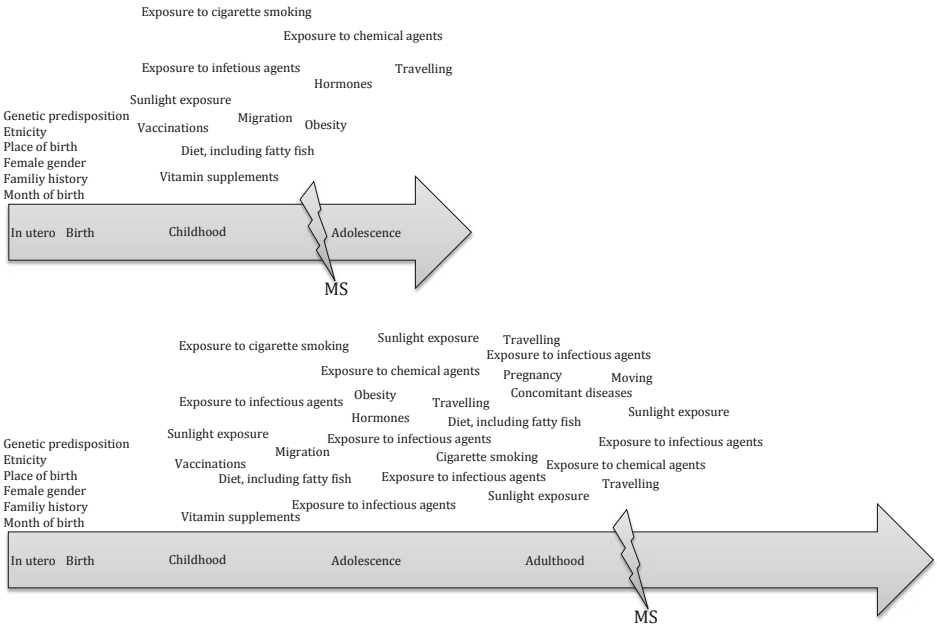

Figure 1.1. Potential factors involved in pediatric and adult MS onset or disease course

NEUROMYELITIS OPTICA AND NMO SPECTRUM DISORDERS

NMO, previously called Devic's disease, was long considered a severe, disabling but rare variant of MS. It is characterized by ON and TM, which is more extensive on MRI compared to TM in MS. These symptoms may occur simultaneously or consecutively (many years apart). The identification of an autoantibody that was very specific for this disease led to a great breakthrough and NMO is now considered a disease entity distinct from MS. The clinical definition of (pediatric and adult) NMO is mentioned in Table 1.1.[20]

The disease-associated antibody is a serum autoantibody directed against aquaporin-4 (AQP4), a major CNS water channel found predominantly on astrocytes. AQP4-antibodies can be detected in more than 60% of patients, and can reliably distinguish NMO from MS with almost 100% specificity. [117 118 119] Other variants of NMO are now recognized, such as a subgroup of patients with

- relapsing ON, severe and bilateral ON
- (relapsing) LETM

- Asian optico-spinal MS
- ON or LETM associated with systemic autoimmune disease.[19 37]

Brain lesions in adult NMO patients may also occur and especially at sites of high AQP4 expression.[120]

NMO is considered to have a poor prognosis.[121 122] A cohort of NMO patients, described before the detection of the AQP4-antibody, showed that most patients experienced incomplete recovery and early incremental disability due to frequent and severe relapses. Within 5 years of onset, more than half of the patients with relapsing NMO were blind (in one or both eyes) or had permanent monoplegia or paraplegia. One third died because of respiratory failure.[122] The discovery of the AQP4-antibody also confirmed that NMO is a humoral B-cell mediated disease, which has implications for the choice of therapy. More than 80% of adults with NMO have a recurrent disease, but disease-modifying therapies that are beneficial in MS, are ineffective in NMO. Azathioprine is now the first-choice treatment.[37 123]

NMO in children occurs in about 3.2-8.5% of all children with ADS.[124] The mean age of disease-onset lies between 10-14 years. Also in children, it occurs more often in females, and it seems to be more frequent in non-white children, like Afro-Americans.[37 124-126] In AQP4-antibody positive children, cerebral lesions may occur, and about half of these are symptomatic, making it difficult to distinguish NMO from ADEM at first presentation.[124-126] The outcome of NMO is variable. A small study including nine children with monophasic NMO showed good clinical recovery and prognosis.[127] In a group of eight monophasic and nine recurrent patients, 24% had persistent severe visual loss and 6% of children were wheelchair dependent.[124] In a large cohort of 58 AQP4-antibody positive children, 93% had a recurrent disease. Only 6% of these children had a normal neurological clinical examination after a median follow-up of 12 months. About half of the children had persistent disability due to visual loss or poor motor recovery.[125] In children with NMO, the AQP4-antibody test is also very specific and moderately sensitive, in the same range as in adult NMO.[124]

IN SEARCH OF DISEASE-SPECIFIC BIOMARKERS: AUTOANTIBODIES TO IDENTIFY ACQUIRED DEMYELINATING SYNDROMES

Ideally, disease-specific autoantibodies would reliably distinguish the different acquired demyelinating syndromes. An encouraging example is the AQP4-autoantibody that has been identified in NMO.[117 118] In the same way as this AQP4-antibody was discovered in adult NMO patients, researchers hope to identify an autoantigen specific for children with a monophasic, and thus a more ADEM-like presentation, to distinguish them from a chronic recurrent MS-like disease. One of the promising autoantibodies is directed against myelin oligodendrocyte glycoprotein (MOG).[128] Other potential candidates in MS include autoantibodies targeting myelin peptides[129-131] or the potassium channel KIR4.1.[132]

SCOPE OF THIS THESIS

This thesis focuses on disease characteristics of acquired demyelinating syndromes, especially the pediatric variants and pediatric MS, as well as ADEM and NMO in adults. These syndromes are often difficult to recognize and diagnose, because of the substantial clinical overlap with MS. We aimed to improve the diagnostic process and to enhance the insight into these disorders by describing their clinical features. Furthermore the objective of the current study is to find disease-specific diagnostic and prognostic markers. An early and reliable diagnosis is important, because of the implications for prognosis and treatment.

In **chapter 2** the incidence and clinical features of ADS in children in the Netherlands are described. **Chapter 3** further elaborates on the clinical features of ADEM. ADEM is considered a disease of young children, and much less is known about ADEM in adults. Disease characteristics and long-term follow-up data of children and adults with ADEM are described and compared between both groups. Because of the difficulty to distinguish ADEM and MS in children at first presentation, we compared the different sets of available MRI criteria to make this distinction (**chapter 4**). Most studies on the sequelae of MS in children focus on cognitive functioning, but little is known about the impact of the disease on daily life. We performed a study (**chapter 5**) to assess fatigue and depression and the impact on quality of life in children with MS as well as in children with monophasic disease. In **chapter 6** we demonstrate that the AQP4-antibody assay is a reliable marker for the diagnosis of NMO. We studied whether the MOG-antibody test is able to distinguish different ADS subtypes in **chapter 7**. These investigations showed that this test is useful in particular cases with a polyfocal disease-onset with encephalopathy, at a young age and who are unlikely to develop MS. The main findings of this thesis and interpretation of our results are discussed in **chapter 8**, as well as suggestions for future research.

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CHAPTER 2

Incidence of acquired demyelinating syndromes of the CNS in Dutch children: a nationwide study

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ABSTRACT

Background Acquired demyelinating syndromes (ADS) can be a first presentation of multiple sclerosis (MS) in children. The incidence of these disorders in Europe is currently unknown.

Methods Children (<18 years old) living in the Netherlands who presented with ADS were included from January 1, 2007 to December 31, 2010 by the Dutch pediatric MS study group and the Dutch surveillance of rare pediatric disorders. Demographic and clinical data were collected.

Results Eighty-six patients were identified over four years, resulting in an incidence of 0.66/100,000 per year. Most patients presented with polyfocal ADS without encephalopathy (30%), followed by polyfocal ADS with encephalopathy (24%), optic neuritis (ON, 22%), monofocal ADS (16%), transverse myelitis (3%) and neuromyelitis optica (3%). Patients with polyfocal ADS with encephalopathy were younger (median 3.9 years) than patients with ON (median 14.6 years, $p<0.001$) or monofocal ADS (median 16.0 years, $p<0.001$). Patients with polyfocal ADS without encephalopathy (median 9.2 years) were also younger than monofocal ADS patients (median 16.0 years, $p<0.001$). There was a slight female preponderance in all groups except the ON group, and a relatively large number of ADS patients (29%) reported a non-European ancestry. Familial autoimmune diseases were reported in 23%, more often in patients with relapsing disease than monophasic disease (46% vs. 15%; $p=0.002$) and occurring most often in the maternal family (84%, $p<0.001$). During the study period, 23% of patients were subsequently diagnosed with MS.

Conclusion The annual incidence of ADS in the Netherlands is 0.66/100,000 children/year. A polyfocal disease-onset of ADS was most common.

INTRODUCTION

In the last decade, knowledge about pediatric multiple sclerosis (MS) and other demyelinating diseases of the CNS has increased considerably. As a group, these first immune-mediated demyelinating events of the CNS are referred to as acquired demyelinating syndromes (ADS).[1] They share common clinical characteristics and they can all represent a first episode of MS. Due to increased awareness among clinical professionals, these diagnoses are likely to be made more often. At present, only one prospective study reported about the incidence of ADS.[1] Other available incidence studies had a retrospective design[2] or focused on subgroups of ADS, like acute disseminated encephalomyelitis (ADEM) and MS[3].

In 2007, we started a nationwide prospective surveillance study to define the incidence of ADS in the Netherlands. We used the network of collaborators of the Dutch pediatric MS study group and participated in a nationwide surveillance program to detect rare pediatric diseases in which all Dutch pediatricians are involved. We here describe the incidence as well as clinical and demographic characteristics of children with ADS in the Netherlands.

METHODS

Patient inclusion

Children younger than 18 years and living in the Netherlands and suspected of a first inflammatory demyelinating event of the CNS who were detected by our surveillance from 2007 to 2010 were included in this study.

Diagnoses were made in accordance to the criteria proposed by the International Pediatric MS Study Group (IPMSSG).[4] Based on clinical and MRI data, patients were divided in six ADS groups; 1. optic neuritis (ON), 2. transverse myelitis (TM), 3. monofocal ADS (mono ADS), 4. polyfocal ADS (poly ADS) without encephalopathy, 5. polyfocal ADS with encephalopathy and 6. neuromyelitis optica (NMO). We avoided the term ADEM[5], because of inconsistent use of this term in previous studies and chose to define this group more transparently as 'poly ADS with encephalopathy' according to the definition proposed by the IPMSSG.

A diagnosis of MS was made in case of a second demyelinating attack of the CNS with clinical and/or MRI evidence of a new lesion localization at least one month after onset. A patient who presented with poly ADS with encephalopathy, required at least two new episodes without encephalopathy, at least three months after onset, for a diagnosis of MS.[4]

Patients were excluded if another cause of the neurological symptoms was demonstrated, including infectious, metabolic, toxic or systemic immunological causes.

The patients were identified using two methods, in order to reach nationwide inclusion of patients:

- In the PROUD*kids* study (PRedicting the OUtcome of a Demyelinating event in children) pediatric neurologists of the eight Dutch academic hospitals and of five non-academic neuropediatric hospitals are involved. The aim of this study is to investigate prognostic factors that predict MS diagnosis in children after ADS.
- The NSCK (Netherlands Pediatric Surveillance Unit) reaches all Dutch pediatricians monthly by e-mail and aims to provide insight in the epidemiology of rare pediatric diseases in the Netherlands. Pediatricians were asked to report whether they did or did not see a patient suspected of a CNS inflammatory demyelinating disease.

Patients were included in the study after written informed consent was obtained from parents and patients older than 12 years. A standardized scoring template was used to gather demographic and clinical information of all reported patients. Demographic data consisted of sex, date and place of birth, ethnic background and family history in first- and second-degree relatives. Country of birth and ancestry was asked to both the child and his parents: patients with at least one parent of non-European origin were classified as of non-European origin. Whenever possible, we asked both parents about their family history with emphasis on familial autoimmune diseases like autoimmune thyroid disease, rheumatoid diseases and type I diabetes. Clinical data consisted of date of disease-onset, clinical symptoms, concomitant diseases, infection or vaccination in preceding four weeks, hospitalization and treatment. MRI, blood and CSF results were also collected for diagnostic evaluation. Follow-up data were provided by the treating physician and by telephone interview of the parents at least once two years after disease-onset. Clinical records were then evaluated. We assessed whether diagnoses changed during follow-up and the patient had to be excluded. We also assessed whether a final diagnosis of MS could be made.

The clinical, laboratory and imaging data were reviewed (by IAK) in order to ensure that patients met the inclusion criteria and to diagnose them appropriately.

This study was approved by the Medical Ethical Committees of the Erasmus University Medical Center in Rotterdam and of the other participating centers.

Analysis

Demographical data of the Dutch population were derived from Statistics Netherlands.[6]

Statistical analysis was performed using SPSS 17.0. The Kruskal-Wallis and Chi-square tests were used to test differences in clinical and demographic characteristics between the six groups. Mann-Whitney *U* tests were used to follow-up differences in numerical data between groups, whereas categorical data were compared using Chi-square or Fisher's exact tests. A Bonferroni correction was applied, so all effects are reported at a 0.0083 significance level.

The Chi-square test was also used to compare the ethnic background of our patients with the Dutch population, to compare the autoimmune family history between monophasic and relapsing patients and to compare seasonal distribution in the entire group. Results were

considered significant if $p<0.05$. Unknown or not reported data in all groups were removed from the analyses.

RESULTS

From January 1, 2007 to December 31, 2010 111 children were reported. One child and her parents refused inclusion. Eighty-six of the reported patients met the inclusion criteria and were analyzed. Twenty-four reported patients (22%) had an alternative diagnosis and were subsequently excluded. The final diagnoses of the excluded patients are listed in Table 2.1. The correct diagnoses were made by laboratory tests, blood and CSF studies, MRI/ MR angiography, as well as clinical course and response to treatment.

Table 2.1. Diagnosis of the excluded patients

Diagnosis	Number (*n*=24)
Infectious disease	11
- Viral encephalitis	*6*
- Postinfectious TM	*3*
- Meningitis	*2*
Systemic inflammatory or autoimmune disease	6
- (Cerebral) vasculitis	*3*
- Celiac disease	*1*
- Susac's syndrome	*1*
- Hashimoto encephalopathy	*1*
Mitochondrial disease	4
- Leber Hereditary Optic Neuropathy (LHON)	*1*
- Other	*3*
Neoplastic disease	2
Posterior Reversible Encephalopathy Syndrome (PRES)	1

Given the number of children in the Netherlands younger than 18 years in 2007 (3,360,433), 2008 (3,344,945), 2009 (3,329,173) and 2010 (3,314,663)[6], the average incidence of ADS is 0.66 per 100,000 Dutch children per year (0.60/100,000 in 2007, 0.60/100,000 in 2008, 0.72/100,000 in 2009, 0.72/100,000 in 2010).

Thirty percent of patients presented with poly ADS without encephalopathy, 24% with poly ADS with encephalopathy, 22% with ON, 16% with mono ADS, 3% with TM and 3% with NMO.

Figure 2.1 shows the distribution of the patients' age at clinical presentation. Children with poly ADS with encephalopathy were younger (median 3.9 years) than children with ON (median 14.6 years) (U=29, $p<0.001$) and children with monofocal ADS (median 16

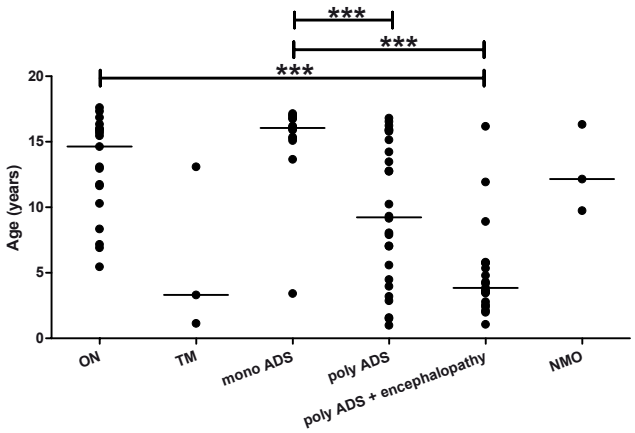

Figure 2.1. Age distribution of the patients, categorized by clinical presentation
The median age per group is shown. The horizontal lines above the groups indicate statistical differences between the groups (Mann-Whitney *U* test; *** $p< 0.001$).
ON = optic neuritis; TM = transverse myelitis; ADS = acquired demyelinating syndrome; mono= monofocal, poly= polyfocal; NMO = neuromyelitis optica.

years) (U=22, p<0.001). Also children with poly ADS without encephalopathy (median 9.2 years) were younger than children with mono ADS (U=63, p<0.001). No significant differences were observed between the other groups.

The demographic characteristics of the included patients are summarized in Table 2.2. The female-male distribution was similar between the groups. After stratification of all children with ADS in a group younger (n=41) and a group older than 10 years (n=45), we also found a similar female:male ratio of 1.3:1 in the younger and of 1.1:1 in the older group.

Twenty-five patients (29%) were of non-European origin. This proportion was higher than the proportion of children of non-European origin (< 18 years old) in the general pediatric population in the Netherlands (16%)[6] (χ^2=13.992, p<0.001). The incidence of ADS in children of European origin is 0.52/100,000 per year, in contrast to 1.16/100.000 per year in children of non-European origin in the Netherlands. Most of these non-European patients (84%) were born in the Netherlands themselves.

A familial history of autoimmune diseases was present in 23% of all patients. No difference was observed in the presence of autoimmune diseases (including MS) in the first- and second-degree relatives between the six ADS subgroups. Autoimmune thyroid diseases and rheumatoid arthritis were most frequently reported (both in five cases). Only three ADS patients reported MS, all in the maternal family. Familial autoimmune diseases occurred more often in patients with relapsing disease than in patients with monophasic disease (46% vs 15%, χ^2=9.51, p=0.002). A maternal family history of autoimmune diseases was

Table 2.2. Demographic characteristics of all patients, categorized by clinical presentation

	ON	TM	Mono ADS	Poly ADS without encephalopathy	Poly ADS with encephalopathy	NMO	*p*-value[a]	All
	n=19	*n*=3	*n*=14	*n*=26	*n*=21	*n*=3		*n*=86
Female:Male	0.9:1	2:1	1.3:1	1.4:1	1.1:1	2:1	0.97	1.2:1
European ethnicity, *n* (%)	12 (63)	2 (67)	6 (43)	16 (62)	15 (71)	3 (100)	0.70	54 (63)
Non-European ethnicity, *n* (%)	5 (26)	1 (33)	5 (36)	9 (35)	5 (24)	0	0.70	25 (29)
- Middle-Eastern	2 (11)	0	0	2 (8)	1 (5)	0		5 (6)
- African	1 (5)	0	4 (29)	2 (8)	1 (5)	0		8 (10)
- Middle-American	1 (5)	0	1 (7)	1 (4)	0	0		3 (4)
- Asian	0	0	0	1 (4)	1 (5)	0		2 (2)
- Mixed[b]	1 (5)	1 (33)	0	3 (12)	2 (10)	0		7 (8)
Unknown ethnicity	2 (11)	0	3 (21)	1 (4)	1 (5)	0	0.42	7 (8)
Place of birth: outside Europe, *n* (%)	1 (5)	0	1 (7)	2 (8)	0	0	0.81	4 (5)
Autoimmune family history, *n* (%)	1 (5)	0	7 (50)	8 (31)	3 (14)	1 (33)	0.05	20 (23)
- MS	1 (5)	0	1 (7)	0	0	1 (33)	0.18	3 (3)
- Other auto-immune disease	0	0	6 (43)	8 (31)	3 (14)	0	0.02[c]	17 (20)
Unknown family history	5 (26)	2 (67)	3 (21)	7 (27)	7 (33)	0	0.54	24 (28)

[a] Patients between the six ADS groups are compared (χ^2 test for categorical data). [b] Mixed ethnicity: one parent of European origin and one parent of non-European origin. [c] Not significant after Bonferroni correction.

ON= optic neuritis; TM= transverse myelitis; ADS= acquired demyelinating syndromes, mono= monofocal, poly= polyfocal; NMO= neuromyelitis optica; MS= multiple sclerosis.

Table 2.3. Clinical characteristics of all patients, categorized by clinical presentation

	ON	TM	Mono ADS	Poly ADS without encephalopathy	Poly ADS with encephalopathy	NMO	*p*-value[a]	All
	n=19	*n*=3	*n*=14	*n*=26	*n*=21	*n*=3		*n*=86
Previous infection, *n* (%)	1 (5)	2 (67)	1 (7)	9 (35)	10 (48)	0	0.01[b]	23 (27)
Previous vaccination, *n* (%)	0	0	0	0	1 (5)	0	0.70	1 (1)
Cerebral MRI pathology, *n* (%)	8 (42)	0	14 (100)	23 (88)	21 (100)	1 (33)	<0.001[c]	67 (78)
Follow-up time, months, median (range)	14.3 (1.1-42.3)	9.9 (2.3-15.6)	21.4 (2.8-45.9)	12.5 (1.5-51.6)	13.2 (0.3-44.8)	7.3 (1.8-19.1)	0.66	12.9 (0.3-51.6)
Relapsing disease, *n* (%)	6 (32)	0	7 (50)	9 (35)	2 (10)	0	0.07	24 (28)
MS, *n* (%)	4 (21)	0	7 (50)	9 (35)	0	0	0.01[b]	20 (23)
Second clinical attack ≤ 2 years, *n* (% of relapsing patients)	5 (83)	0	6 (86)	5 (56)	2 (100)	0	0.37	18 (75)

[a] Patients between the six ADS groups are compared (χ^2 test for categorical data and Kruskal-Wallis test for numerical data). [b] Not significant after Bonferroni correction. [c] Mono ADS compared to ON (Fisher's exact test, p=0.001); poly ADS with encephalopathy compared to ON (Fisher's exact test, p<0.001).
ON= optic neuritis; TM= transverse myelitis; ADS= acquired demyelinating syndromes, mono= monofocal, poly= polyfocal; NMO= neuromyelitis optica; MRI= magnetic resonance imaging; MS= multiple sclerosis.

much more frequent than a paternal family history of autoimmune diseases (84% vs 16%, $p<0.001$).

In the ADS patients, we found no difference between a disease-onset in Winter (36%), Spring (21%), Summer (24%) or Autumn (19%).

Table 2.3 shows the other clinical characteristics at first demyelinating attack of the included patients. The differences in preceding infection between the groups did not reach significance. Only one child received a vaccination (measles mumps and rubella vaccine) before onset of a polyfocal disease with encephalopathy.

All patients, except one child with TM, underwent brain MR imaging. Sixty-seven patients with ADS presented with demyelinating lesions on brain MRI. Cerebral MRI lesions were more frequent in children with mono ADS (Fisher's exact test, $p=0.001$) and poly ADS with encephalopathy (Fisher's exact test, $p<0.001$) as compared to children with ON.

Twenty-eight percent of the patients experienced at least one relapse. One patient experienced two episodes of ON after a poly ADS with encephalopathy (with resolution of previous clinical symptoms and MRI abnormalities and without new MRI lesions), one patient suffered from another episode of poly ADS with encephalopathy and two patients had recurrent ON without cerebral MRI lesions. By now, 20 patients have been diagnosed as MS. More patients with a monofocal onset (Fisher's exact test, $p=0.001$) or a polyfocal onset without encephalopathy (Fisher's exact test, $p=0.003$) were subsequently diagnosed with MS compared to patients with a polyfocal onset with encephalopathy. Five patients were diagnosed as MS based on asymptomatic new MRI lesions. The proportion of patients with a relapsing disease who experienced their second clinical attack within two years after onset (excluding patients with only MRI evidence of new disease) was comparable between the different diagnostic groups. Only three children younger than 10 years experienced a second clinical attack. The patients who were subsequently diagnosed with MS were all older than 10 years at the time of their first demyelinating attack.

DISCUSSION

The incidence of ADS in children in the Netherlands is 0.66/100,000 per year. It is difficult to compare this number with the incidence in other countries because only two studies defined and analyzed the incidence of ADS[1 2] whereas another study focused on solely ADEM and MS[3].

A nationwide prospective study in Canada reported an annual incidence of ADS of 0.9/100,000.[1] Both our study and the Canadian study succeeded to reach nationwide coverage by using recently initiated centralized national databases. The lower incidence observed in the Netherlands may be explained in part by geographical difference and differences in demographic characteristics of the pediatric patients, especially regarding ethnicities. In the

Canadian group 37% of children were first-generation Canadians (i.e. both parents born outside Canada)[1] whereas in the Netherlands this count was 18%.

A recent paper showed that the incidence of ADS in the United States is 1.63/100,000 per year. [2] This is much higher than our or the Canadian incidence. The main difference between this US and both our and the Canadian study is the methodology: the US investigators retrospectively searched a large health maintenance organization database in one area. The incidence in this multiethnic cohort of Southern Californian children was then used to extrapolate the incidence in the US. The question can be raised whether this study population is representative for the entire US. Furthermore, the higher incidence may be due to the large ethnic diversity in this cohort.[2]

A nationwide German survey focused on children (< 16 years old) with ADEM and MS. They reported an annual incidence of MS of 0.3/100,000.[3] So far, we found an annual incidence of MS of 0.15/100,000 in Dutch children. This difference may be caused by the still short follow-up time of the patients in our study. The real incidence of pediatric MS in the Netherlands can only be calculated when the youngest child of our cohort reaches the cut-off age for pediatric ADS of 18 years. The higher incidence of MS in Germany can also be due to their inclusion of both suspected as definite MS patients.[3]

A remarkable finding in the German study was the much lower incidence of ADEM (0.07/100,000).[3] The authors speculated that this low number of ADEM patients could be a consequence of geographical differences with other cohorts.[3] Although ADEM was not defined in their study, we found an annual incidence of patients with a polyfocal onset with encephalopathy (ADEM according to the IPMSSG[4]) of 0.16/100,000. So we could not confirm this low incidence of ADEM in a geographical comparable area.

In our study poly ADS without encephalopathy was the most common presentation (30%), followed by poly ADS with encephalopathy (24%) and ON (22%). TM was very rare in our cohort (3%) in contrast to the Canadian cohort (22%).[1] The Canadian children with TM differed from the Dutch children with TM: they were much older (mean age at onset of 11 years) and MRI abnormalities (either cerebral or spinal) were present in 91%. So there could be a discrepancy in defining TM. We classified children with TM and clinical and/or MRI evidence of a localization outside the spinal cord as either polyfocal ADS or NMO. Children who were reported but turned out to have a proven acute and active infectious etiology were excluded.

Most patients had a European ancestry. However, the incidence of ADS was twice as high in children of non-European origin as in children of European origin in the Netherlands. Most of these children were born in the Netherlands themselves. Previous studies in Canada and the US also showed that pediatric MS patients more frequently had a non-European ancestry or were non-Caucasian in contrast to adult MS patients, indicating that the pediatric MS population reflects the changing immigration patterns in countries with high MS prevalence. The difference may be explained by the possibility that children with ancestors from a

country with low MS prevalence may lack protective genetic factors or are more vulnerable to environmental factors when they grow up in a country with high MS prevalence.[2 7 8]

Familial MS was reported in only 3% of ADS patients, which is in agreement with previous reports of numbers varying between 3 and 8%.[1 3 9] This proportion is higher in retrospective studies on pediatric MS and in studies with longer follow-up durations.[10 11] Next to MS, we found that other autoimmune diseases in first- and/or second-degree relatives were present in 20% of our patients. Although this number is higher than reported in the Canadian study[1], it is likely to be an underestimation, because the family history was not reported in detail in 28% of patients. Data on the presence of autoimmune diseases in the Dutch pediatric population are not available. We observed that familial autoimmune diseases were especially present in patients with relapsing disease. Studies on the presence of autoimmune diseases in first-degree relatives of adult MS patients show contrasting results[12 13], but the most recent rigorously performed study suggests that autoimmune diseases are not more frequent in families of MS patients.[14]

Of particular interest is the observation that autoimmune diseases were especially frequent in the maternal family. It is not likely that this difference in autoimmune diseases between the mother's and father's family is caused by the possibility of only the mothers being the parent interviewed. In all, except for one child, we were able to obtain information on both parents. A maternal parent-of-origin effect has been suggested for MS in adult patients.[15-17] To our knowledge, a possible maternal transmission of autoimmune diseases has neither been described before in pediatric ADS or MS patients nor in adult MS patients.

It is plausible to expect that the incidence of ADS we describe in this study is an underestimation because despite all efforts, there are multiple reasons why it is impossible to identify every patient in one country. Theoretically, some ADS patients older than 16 years could have been missed, because they may have been directly referred to adult neurologists. We do not expect this to be a large number of patients in our study, as our MS center is a national referral site for both pediatric and adult ADEM, MS, and ADS variants. Secondly, patients with mild or self-limiting symptoms may not be referred to a pediatrician or neurologist at all. Thirdly, ophthalmologists did not participate in this study, so we may have missed a number of ON cases. Still, it is of note that the number of patients with ON we observed is in accordance with a previously reported incidence.[1] Finally, some physicians simply may fail to report patients. However, the response rate in the Dutch NSCK surveillance system is quite high. The number of pediatricians that participated was 86.6% in 2007, 85.5% in 2008 and 84% in 2009.[18] Strength of our study is that we used two complementary methods to enroll patients. By the NSCK, 44% of the patients were identified and the remaining patients were identified by the PROUD*kids* study group.

Our study illustrates that other disorders may mimic ADS at first presentation (Table 2.1). It is important to consider these disorders in the differential diagnosis of ADS, because of potential treatment and prognosis. ADS can be a challenging diagnosis and knowing

the incidence can increase the awareness of these disorders in children. This is relevant as there are indications of increasing incidence, especially among certain ethnic groups in the Western world.

The research on ADS in the Netherlands is still in progress and the aim is to provide long-term follow-up data in the future.

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CHAPTER 3

Disease course and outcome of acute disseminated encephalomyelitis is more severe in adults than in children

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ABSTRACT

Background Acute disseminated encephalomyelitis (ADEM) affects children more frequently than adults. Current studies investigating ADEM in different age groups are difficult to compare.

Objective To investigate whether the clinical presentation, outcome and disease course of ADEM differ between adults and children.

Methods Disease characteristics of 25 adults and 92 children suffering from ADEM between 1988 and 2008 were compared.

Results The most common presenting symptoms of ADEM in both groups were pyramidal signs and encephalopathy. Ataxia occurred more frequently in children (p=0.002). In general, MRI showed ill-defined and large white matter lesions, whereas periventricular lesions were more prevalent in adults (p=0.001). In adults duration of hospitalization was longer (p=0.002) and ICU admission was more frequently required (p=0.043). Three adults (12%) and one child (1%) died (p=0.030). Fewer adults had complete motor recovery after their first clinical event (p<0.001). In 73 patients follow-up time was ≥ 2 years and most of these patients remained monophasic. Although relapses after ADEM can occur, only one adult (5%) and five children (6%) converted to MS.

Conclusions The clinical presentations in children and adults share similarities, but the disease course and outcome of ADEM is more severe in adults with respect to hospitalization, ICU admission, recovery and mortality.

INTRODUCTION

Acute disseminated encephalomyelitis (ADEM) is a rare monophasic inflammatory demyelinating disorder of the CNS. ADEM is characterized by multifocal neurological symptoms, which usually include encephalopathy. The disease is often associated with an infectious illness or vaccination. MRI typically shows multifocal large demyelinating lesions in the CNS white matter. Gray matter involvement (e.g. lesions in thalamus and basal ganglia) also occurs.[1-3]

Specific biological markers to establish the diagnosis are as yet not available. Diagnosis is based upon a combination of clinical and radiological features and exclusion of diseases that mimic ADEM.[1 2] In most patients, ADEM is associated with complete symptom resolution and a good long-term prognosis, but the outcome varies. Although ADEM is considered a monophasic disease, in children the risk of relapses is reported to be as high as 30%.[2-4] The number of children eventually developing MS is probably lower, because this diagnosis may only be made if multiple subsequent attacks occur at least three months after the initial event.[3] Early and accurate distinction between ADEM and other demyelinating disorders is important for therapy and prognosis.[5]

ADEM in adults is even rarer than in children and less well studied.[6-9] It is unclear whether disease characteristics of ADEM in adults differ from those in children. Dissimilar inclusion criteria make it hard to compare these studies.[5]

The aim of this study was to examine the clinical, laboratory and radiological characteristics, the outcome and the disease course of ADEM in adults and in children and to compare these features between these two groups.

MATERIALS AND METHODS

Study population

We included patients diagnosed with ADEM between 1988 and 2008. These patients were referred to the Erasmus University Medical Center or were identified by the Dutch study group for pediatric MS. Patients were identified by hospital database searches with the terms 'ADEM', 'acute disseminated encephalomyelitis' and 'encephalomyelitis'. This study was approved by the Medical Ethical Committees of the Erasmus University Medical Center in Rotterdam and of the other participating centers.

Clinical records were reviewed by the authors (I.A.K. and I.E.R.V.) in order to ensure accuracy of diagnosis. ADEM was defined in accordance to the criteria proposed by the International Pediatric MS Study Group (IPMSSG) [10], as a first clinical event with a presumed inflammatory or demyelinating cause and with an acute or subacute onset of polyfocal neurological symptoms and brain MRI abnormalities. These abnormalities were defined as the presence of hyperintense white matter lesions and/or lesions in the gray matter of basal ganglia and thalamus on fluid-attenuated inversion recovery (FLAIR) or T2-weighted brain MRI images. We

also included patients without encephalopathy at clinical onset, but with an otherwise typical presentation of ADEM. Encephalopathy was defined as altered consciousness or evident change of behavior at the time of attack onset not related to seizures or antiepileptic treatment.

Patients were excluded if another cause of neurological symptoms was suspected, including primary infectious, metabolic, toxic or systemic immunological causes. Patients with neuromyelitis optica (NMO) were also excluded.

Data collection

Individuals who were 18 years of age or older were considered adults. All available data were collected and registered in a central database. These data consisted of demographic features (age, gender), clinical symptoms, laboratory blood and CSF results, MRI and follow-up data. Information on the presence of infection or vaccination in the four weeks preceding disease-onset were collected.

Available brain MRI scans were reviewed by two experienced investigators (I.A.K. and I.E.R.V.) using a predefined scoring template.[11] Lesions were scored on T2-weighted, proton density, and FLAIR images. Gadolinium was not administered routinely. We studied spinal cord MRI when available. The Barkhof MRI criteria [12] were scored and we assessed whether these criteria could aid in predicting conversion to MS. All available follow-up scans were reviewed for either the presence of new lesions or lesion resolution.

Follow-up and outcome

Length of follow-up time was determined by the last contact with a neurologist or pediatrician. Complete recovery was defined as a total resolution of initial clinical symptoms. Residual cognitive impairment or behavioral change without other clinical symptoms was not designated as completely recovered. Cognitive impairment or behavioral change was assessed either by neuropsychological evaluation or by parents and patients reports or by the treating primary care physician. All residual symptoms that persisted after the initial clinical attack were not present prior to this event.

Monophasic disease was defined as a relapse-free interval of at least two years after the initial event. A relapsing disease was defined as a new clinical demyelinating event of the CNS at least three months after the initial event. Treatment related fluctuations were not considered a relapse. A diagnosis of MS was made when two non-ADEM demyelinating episodes subsequently occurred at least three months after the initial attack, fulfilling the McDonald criteria for dissemination in time and space.[10]

Statistical analysis

Statistical analysis was performed using SPSS 15.0. Chi-square or Fisher's exact test were used to compare categorical data between adults and children and Student's *t*-test to com-

pare continuous data. Results were considered significant if *p*-values were below 0.05. Not significant results are denoted as *NS*.

RESULTS

A total of 117 patients (25 adults and 92 children) were included. Basic characteristics are summarized in Table 3.1. The mean age at presentation was 40.8 years in adults and 6.3 years in children. More males than females were reported in the pediatric group (male:female ratio=1:0.8) compared to the adult group (male:female ratio=1:1.3), which was not a significant difference.

Table 3.1. Patient characteristics

	Adults	Children
Total number of patients, *n*	25	92
Age		
Range, years	18.0 – 82.0	0.5 – 16.2
Mean ± SD, years	40.8 ± 17.6	6.3 ± 4.2
Male, n (%)	11 (43)	51 (55)
Follow-up		
Range	7 days – 14.1 years	9 days – 19.2 years
Dead, *n* (%)	3 (12)	1 (1)
Lost to follow-up, *n* (%)	2 (8)	5 (5)
Number of patients included in follow-up analyses, *n* (%)	20 (80)	86 (94)
Mean ± SD, years	5.8 ± 5.2	5.2 ± 4.9

Clinical presentation

The presenting clinical features are shown in Table 3.2. Overall, a disease-onset in winter was most common. A previous infection occurred in 18 adult and 64 pediatric patients, with flu-like symptoms (6 adults and 25 children, 25% and 28% respectively) and upper respiratory tract infections (4 adults and 14 children, 22% and 16% respectively) being the most prevalent.

Five children developed ADEM within one month after receiving a vaccination, including influenza, quadruple, MMR (measles, mumps and rubella) and meningococcus C vaccination.

All patients had a polyfocal clinical presentation. Most patients presented with pyramidal signs and more than half of the patients presented with encephalopathy. Fever, headache and brainstem symptoms were also frequently observed. Ataxia is more often present in children than in adults (p= 0.002).

Laboratory investigation

Blood test results were available in 25 adults and 81 children and were abnormal in respectively 14 (56%) and 55 (68%) patients. Results are summarized in Table 3.2. Leukocytosis occurred more often in children than in adults (p=0.045).

CSF examination results were available in 25 adults and 84 children and were abnormal in 18 adults (72%) and 69 children (82%). Pleocytosis was mild (defined as a leukocyte count between 4-100/µl) in most cases (73% of adults and 91% of children). There were no differences between adults and children with respect to CSF pleocytosis, OCB or elevated IgG index.

Table 3.2. Clinical features at disease-onset

	Adults, *n* (%)	Children, *n* (%)	*p*-value
Seasonal distribution			
Winter	9 (36)	34 (37)	*NS*
Spring	10 (40)	18 (20)	0.034
Summer	2 (8)	25 (27)	0.044
Autumn	4 (16)	15 (16)	*NS*
Preceding infection[a]	18/24 (75)	64/90 (71)	*NS*
Preceding vaccination[a]	0/19 (0)	5/84 (6)	*NS*
Symptoms			
Encephalopathy	13 (52)	63 (69)	*NS*
Pyramidal	20 (80)	66 (72)	*NS*
Bilateral ON	3 (12)	13 (14)	*NS*
Unilateral ON	2 (8)	6 (7)	*NS*
Sensory	9 (36)	17 (19)	*NS*
Brainstem	9 (36)	40 (44)	*NS*
Ataxia	5 (20)	51 (55)	0.002
Extrapyramidal	1 (4)	5 (5)	*NS*
Meningism	2 (8)	24 (26)	*NS*
Headache	9 (36)	41 (45)	*NS*
Fever	9 (36)	43 (47)	*NS*
Seizures	3 (12)	24 (26)	*NS*
Blood investigation[a]			
Leukocytosis[b]	7/21 (33)	43/74 (58)	0.045
Elevated CRP and/or ESR[c]	11/22 (50)	37/73 (51)	*NS*
CSF analysis[a]			
Pleocytosis[d]	15/22 (68)	66/77 (86)	*NS*
Oligoclonal bands[e]	1/18 (6)	3/37 (8)	*NS*
Elevated IgG index[f]	8/20 (40)	8/39 (21)	*NS*

[a] Data are presented as number of available data. [b] Leukocyte count > $10*10^9$/liter. [c] C-reactive protein (CRP) > 10 mg/l Erythrocyte sedimentation rate (ESR) > 15 mm/h (men), > 20 mm/h (women), >13 mm/h (children). [d] Leukocyte count > 4/µl. [e] Positive when > 2 CSF oligoclonal bands are present (detected with isoelectric focusing). [f] IgG index > 0.60 (adults), > 0.68 (children).

Neuroimaging

MRI scans of 24 adult and 78 pediatric patients that had been performed in the acute stage of disease were available for evaluation in this study (Table 3.3). Almost all showed lesions with poorly defined margins. More than 75% showed large cerebral white matter lesions. Infratentorial lesions and lesions in the thalamus and basal ganglia were also common. The latter tended to occur more often in children (p=0.05), whereas adults had a higher prevalence of periventricular lesions (p=0.001).

Table 3.3. Brain and spinal cord MRI

	Adults, *n* (%)	Children, *n* (%)	*p*-value
Brain MRI	24	78	
Large lesions (> 2cm)	18 (75)	67 (86)	*NS*
Ill-defined lesions	22 (92)	76 (97)	*NS*
Supratentorial	22 (92)	72 (92)	*NS*
Infratentorial	16 (67)	55 (71)	*NS*
Brainstem	16 (67)	44 (56)	*NS*
Cerebellar	10 (42)	32 (41)	*NS*
Subcortical	8 (33)	37 (47)	*NS*
≥ 9 T2-weighted hyperintense lesions	5 (21)	15 (19)	*NS*
≥ 3 periventricular lesions	8 (33)	4 (5)	0.001
Perpendicular to corpus callosum	4 (17)	3 (4)	*NS*
Thalamus / basal ganglia	11 (46)	53 (68)	0.05
Barkhof MRI criteria: at least 3 of 4	7 (29)	10 (13)	*NS*
Spinal cord MRI[a]	6	30	
Spinal cord lesions	4 (66)	20 (66)	*NS*
> 2 segments	2 (33)	13 (43)	*NS*

[a] Data are presented as number of available data.

Spinal cord scans were only performed in the acute stage in 6 adult and 30 pediatric patients, to investigate clinical suspicion of myelitis, and showed intramedullary lesions in approximately two-thirds of patients in both groups.

At first MRI, 17 patients (7 adults and 10 children) had a polyfocal clinical presentation and multiple MRI lesions, which fulfilled at least three out of four of the Barkhof criteria. However, these lesions had other aspects that were not typical for MS, such as large and diffuse lesions and/or significant lesions in the deep gray matter. None of these patients converted to MS within a mean follow-up time of 3.3 years in adults (range 0.1-13.9 years) and 4.4 years in children (range 1-8.5 years).

Hospitalization and management

Duration of hospitalization in the adult group ranged from 5 to 167 days with the exception of one patient who was hospitalized for 397 days. Excluding this patient, the mean duration of hospitalization in adults was 48 days. In children the duration of hospitalization ranged from 2 to 142 days with a mean of 23 days, which was significantly shorter than in adults (p=0.002). Two children did not need hospital admission.

Nine adults (36%) and 14 children (15%) required admission to the intensive care unit (ICU) (p=0.043). Twenty-one adult patients (84%) and 67 children (73%) received high-dose corticosteroid treatment at first attack in a dose of 500 or 1000 mg IV methylprednisolone per day in adults and 20-30 mg/kg per day in children for 3-5 days (*NS*). Additional treatments included intravenous immunoglobulin, plasma exchange and oral prednisolone. Two adults (8%) and 15 children (16%) did not require any immunosuppressive treatment.

Outcome

Follow-up time ranged from 7 days to 14.1 years in the adult patients and from 9 days to 19.2 years in the pediatric patients (Table 3.1). Two adult patients and five children were

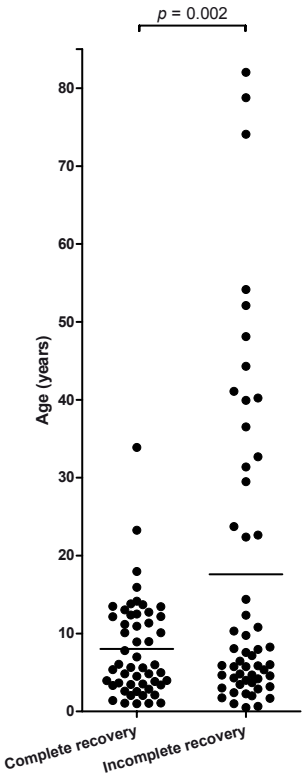

Figure 3.1. Age distribution of completely and incompletely recovered patients

lost to follow-up. Three adult patients (12%) and one child (1%) died as a consequence of the disease (p=0.030).

Excluding these patients, the mean follow-up period was 5.8 years (range: 2 months - 14.1 years) in the adult group (n=20) and 5.2 years (range: 2 months -19.2 years) in the pediatric group (n=86) (*NS*). Only three adult patients (15%) had complete motor recovery after their first clinical event in contrast to 50 pediatric patients (58%) (p<0.001). Figure 3.1

Table 3.4. Monophasic patients: clinical characteristics

	Monophasic patients, *n* (%)	
	Adults	Children
Total number of patients, *n*	8	46
Male	3 (38)	25 (54)
Age at disease-onset, median, years	35.2	5.4
Range, years	18.0-78.8	0.5-13.5
Symptoms at 1st event		
Encephalopathy	3 (38)	32 (70)
Pyramidal	5 (63)	31 (67)
Bilateral ON	1 (13)	8 (17)
Unilateral ON	0 (0)	3 (7)
Sensory	3 (38)	5 (11)
Brainstem	2 (25)	18 (39)
Ataxia	3 (38)	23 (50)
Extrapyramidal	0 (0)	4 (9)
Meningism	0 (0)	11 (24)
Headache	3 (38)	21 (46)
Fever	4 (50)	24 (52)
Seizures	0 (0)	13 (28)
CSF at 1st event[a]: oligoclonal bands present[b] and/or elevated IgG index[c]	1/6 (17)	6/32 (19)
MRI at 1st event[a]		
Barkhof MRI criteria: at least 3 of 4	1/8 (13)	6/43 (14)
Spinal cord lesions	1/2 (50)	7/11 (64)
> 2 segments	1/2 (50)	3/11 (27)
MRI at follow-up[a]		
New brain lesions	0/3 (0)	0/31 (0)
Barkhof MRI criteria: at least 3 of 4	1/3 (33)	2/31 (7)
Resolution of MRI lesions	1/3 (33)	26/31 (84)
Follow-up, median, years	8.7	4.1
Range, years	3.0-14.1	2.0-19.2

[a] Data are presented as number of available data. [b] Positive when > 2 CSF oligoclonal bands are present (detected with isoelectric focusing). [c] IgG index > 0.60 (adults), > 0.68 (children).

shows that the complete recovered patients were younger than the patients who did not completely recover (p=0.002). Four adult patients (20%) reported having cognitive impairment or behavioral change at last follow-up, in contrast to 28 children (33%) (*NS*).

Of the twelve adults who reached a follow-up time of two years or longer, eight patients remained monophasic (67%). Of the 61 children who reached a follow-up time of at least two years, 46 remained monophasic (75%) (*NS*). The clinical characteristics of the monophasic patients are summarized in Table 3.4. No significant differences between groups were found.

In 11 adults and 60 children follow-up MRI was performed after a mean of 1.5 years in both groups. Resolution of lesions was observed in six adults (55%) and 43 (72%) children (*NS*). Two adults (18%) and 11 (19%) children showed new lesions on MRI (*NS*). They also showed new clinical signs.

Relapsing cases

Of all patients included in the follow-up analyses, a total of four adults (20%) and 16 children (19%) had a relapsing disease (*NS*). The mean time to the second clinical episode was 2.1 years (range: 4 months – 6.4 years) in adults and 2.0 years (range: 3 months – 6.6 years) in children.

Two adults (10%) and five children (6%) experienced another ADEM event. They did not develop any other subsequent events (within a mean follow-up time of 5.5 years) and follow-up MRI showed resolution of lesions. One adult (5%) and six children (7%) developed optic neuritis, without other new clinical symptoms (within a mean follow-up time of 10.1 years) and with resolution of lesions on follow-up MRI. Four of these patients were tested for antibodies against aquaporin-4, a marker for NMO [13], and were negative.

One adult (5%) and five children (6%) developed MS (*NS*). They experienced at least two demyelinating events and new brain MRI lesions without normalization of MRI. The diagnosis MS was made after 10.9 years in the adult patient and after a mean of 3.3 years in the pediatric patients (range 1.4-6.4 years).

No significant differences in clinical characteristics were found between the monophasic ADEM patients, the patients with relapsing demyelination and the patients with MS in the adult and pediatric groups (Chi-square and Mann-Whitney *U* test, Bonferroni corrected; data not shown).

DISCUSSION

In this study we found that the clinical presentation and outcome of ADEM in adults differ from those in children. Children presented more often with ataxia and pleocytosis in blood and less often with periventricular lesions on MRI. There is a tendency that thalamus and basal ganglia involvement on MRI occurs more frequently in children. Disease course

was worse in adults: more than one third of adult patients required admission to an ICU and duration of hospitalization was two times longer than in children. Outcome was also worse: complete motor recovery was less frequent (only 15% of adults in contrast to 58% of children) and more adult patients died. There was no difference in the occurrence of relapses or conversion to MS. To our knowledge only in one previous study, in an Asian population, clinical and radiological findings and outcome of ADEM in different age groups were compared.[14] Sample sizes in our study were larger and follow-up time was longer. The mean follow-up time of more than five years in both groups was longer than [6-8 15 16] or comparable to most previous studies [17-21] and the number of patients with a follow-up time of at least 2 years (73 patients) is high.

In contrast to some other studies we did not strictly adhere to the criteria proposed by the IPMSSG.[10] The presence of encephalopathy at onset can facilitate the distinction between ADEM and MS, because encephalopathy is very unusual in MS.[6 8 11 17 21] On the other hand (pathologically confirmed) ADEM without encephalopathy as well as more severe presentations of MS with encephalopathy may exist.[11 20-23] In our study, the patients without encephalopathy had other signs and symptoms that are considered typical of ADEM, including large and ill-defined demyelinating lesions on MRI. The majority of these patients remained monophasic. In contrast, three of the six patients who converted to MS presented with encephalopathy at their first demyelinating episode. This again emphasizes the overlap between ADEM and MS and the difficulty making an accurate distinction based solely on clinical diagnostic criteria. When comparing the clinical symptoms and MRI patterns of only the adult and pediatric patients with encephalopathy at onset (13 and 60 patients respectively) we found there were still no large differences in clinical presentation between the groups. The difference in ataxia disappeared (p=0.068), but the higher frequency of periventricular lesions in adults remained (p=0.004).

The prevalence of most of the studied features of ADEM was in accordance with previous studies in adults [6-9] and in children [15-21 24 25].

OCB and an elevated IgG index in CSF were reported in only a few cases, in accordance with previous studies.[6-8 14-21 24] An elevated IgG index was found more frequently than OCB. An elevated IgG index in pediatric ADEM patients is reported in only one other study.[21] Although in general the presence of OCB predisposes for a relapsing disease [8 17 19 21], none of the patients that converted to MS in our study presented with OCB.

MRI abnormalities consisted of ill-defined lesions and large lesions that were often located in the cerebral white matter, thalamus, basal ganglia and brainstem. We confirmed previous findings that lesions in thalamus and basal ganglia tend to occur more often in children than in adults.[14] This frequent involvement of basal ganglia is often asymptomatic. Only two patients with lesions in this area presented with extrapyramidal signs. Periventricular involvement was more frequent in adults than in children, as is described previously.[14] This difference cannot be explained by the older patients in the adult group that may already

have some degree of leukoencephalopathy, because the difference still existed after excluding the patients older than 50 years of age (p=0.001). The percentage of periventricular lesions we found was lower than that reported in other studies in children [15-17 19 20 24 25] and adults [6 8]. We counted the presence of at least three periventricular lesions, whereas in most other studies they probably counted the presence of at least one periventricular lesion.

In the adult and pediatric groups, none of the 17 patients that fulfilled the Barkhof criteria developed MS.

In general ADEM is considered to have a good prognosis, but we found that the clinical course of ADEM is more severe and recovery is worse in adults than in children. This finding is supported by the conclusion of the Asian study that children have a more favorable functional outcome than adults.[14] Recovery rates were lower in both age groups in our study than in most previous studies [6 7 9 15 16 18 20 24 25], because we considered patients with very mild non-disabling symptoms as not fully recovered.

Cognitive impairment and/or behavioral change were reported in a substantial number of patients (20% of adults and 33% of children). Twelve children (14%) needed special education, which is higher than the national average of 4% in the Netherlands.[26] The children with cognitive or behavioral sequelae were below 12 years old at time of disease-onset. It has been shown in children with ADEM and with acquired brain injury (e.g. tumor treatment) that a younger age at the time of disease-onset is a risk factor for these long-term complications.[27 28] Patients that seemed fully recovered can show subtle cognitive or behavioral impairments when tested years after the diagnosis.[15 17 27 29] To identify cognitive impairment, neuropsychological investigation should be a routine investigation in all patients.

The less favorable outcome in adults compared with children cannot be explained by differences in clinical presentation (preceding factors, symptoms, blood and CSF parameters or radiological features). So, rather than by a difference in pathophysiology at onset, this may be explained by reduced plasticity of the aging brain. Consistent with this age-related worse disease course is the observation that disability and disease progression in MS are an effect of age and not an effect of clinical course or relapses.[30] It is important to be aware that the recovery and prognosis of ADEM in adults is worse and that rapid and more aggressive treatment is indicated in these patients.

ADEM is generally considered a monophasic disease and 67% of adults and 75% of children remained monophasic after at least two years of follow-up. A cut-off of two years was chosen since relapses are likely to occur within 2 years.[7 15-21 24] The number of relapsing patients (20% of adults and 19% of children) in our study is within the range of previous studies,[6-8 15-20 24] but our detected risk of subsequent conversion to definite MS is low: one adult (5%) and five children (6%). This could be because we did not diagnose all patients who experienced a relapsing disease as MS, according to the strict definitions by the IPMSSG.[10] Most patients have only one relapse and remain relapse-free afterwards. Seven patients developed recurrent optic neuritis, without other new clinical symptoms. It is

debatable whether they have MS or a distinct disease entity, because follow-up MRI showed no new lesions. In a few patients, aquaporin-4 antibodies were tested and turned out to be negative, making an NMO spectrum disorder less likely. It is important to keep in mind that a relapse can occur even years after the first event. In this small group of relapsing patients we could not detect any parameters that could predict conversion to MS.

We conclude that there are no large differences in clinical presentation between adult and pediatric patients with ADEM. The main differences between adult and pediatric ADEM patients are the more severe disease course, less favorable recovery and higher mortality in adults. Even though a relapsing disease course can occur, the development of MS after ADEM is rare, both in children as in adults.

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CHAPTER 4

A comparison of MRI criteria for diagnosing pediatric ADEM and MS

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ABSTRACT

Background Brain MRI is a useful tool for diagnosing inflammatory demyelinating disorders in children. However, it remains unclear which are the most reliable criteria for distinguishing multiple sclerosis (MS) from monophasic disorders such as acute disseminated encephalomyelitis (ADEM). We therefore compared the four current sets of MRI criteria in our Dutch pediatric cohort and determined which are the most useful in clinical practice for distinguishing ADEM from MS.

Methods We included 49 children who had had a demyelinating event and an MRI scan within 2 months of their first clinical attack. Twenty-one patients had ADEM and remained relapse-free after at least 2 years of follow-up. Twenty-eight patients had a definitive diagnosis of MS. We assessed the sensitivity and specificity of the following MRI criteria: Barkhof criteria, KIDMUS criteria, Callen MS-ADEM criteria and Callen diagnostic MS criteria.

Results The Callen MS-ADEM criteria had the best combination of sensitivity (75%) and specificity (95%). The KIDMUS criteria had higher specificity (100%), but much lower sensitivity (11%). The Barkhof criteria had a sensitivity of 61% and a specificity of 91%. The Callen diagnostic MS criteria were the most sensitive (82%), but were only 52% specific for distinguishing a first attack of MS from ADEM.

Conclusions The results in our cohort demonstrate that the new Callen MS-ADEM criteria are the most useful for differentiating a first attack of MS from monophasic ADEM. Although the Callen diagnostic MS criteria are more sensitive, they lack the specificity necessary to differentiate MS from ADEM.

Introduction

In children who have a first attack of immune-mediated demyelination it is usually difficult to distinguish multiple sclerosis (MS) from acute disseminated encephalomyelitis (ADEM). This distinction is important, because ADEM is considered a monophasic disease with a more benign course, while MS is a lifelong chronic illness. Multiple subsequent attacks leading to a diagnosis of MS occur in approximately 20% of children initially diagnosed with ADEM.[1-5]

MRI is a useful tool in confirming the diagnosis of acute immune-mediated demyelination and may become more useful for recognizing patients who are at risk of future attacks of demyelination, or MS. Until recently, two sets of criteria were available for predicting conversion to MS after a first demyelinating event: the Barkhof MRI criteria were developed for adult patients[6] and the MRI KIDMUS criteria were the first criteria developed for children.[7] In our Dutch cohort of pediatric MS patients, we have already confirmed that both sets of criteria have a high specificity and positive predictive value.[4] However, their sensitivity is poor, especially in children younger than 10 years old.[4 5 7 8]

Two recent studies by one group emphasize the need to develop MRI criteria in children that can reliably distinguish the first attack of MS from ADEM and other neurological diseases.[8 9] The first study developed criteria that can help distinguish patients with MS at first attack from monophasic ADEM patients.[8] The second study proposed modifications to the Barkhof criteria that would enhance the diagnostic accuracy of these criteria for MS in children. These criteria were initially designed to distinguish between MS (at second attack) and other, non-demyelinating, diseases (SLE and migraine). As the authors state, it is necessary to evaluate the usefulness of these criteria in predicting conversion to MS at the first demyelinating attack.[9]

Although all these criteria were developed for a slightly different purpose, it is of interest to assess to what extent these criteria are useful to distinguish MS at first attack from monophasic ADEM, and in that way can predict conversion to MS. Therefore we compared the test properties of the existing MRI criteria for MS in distinguishing MS at first attack from ADEM and validated the recently proposed criteria in our Dutch pediatric cohort.

METHODS

Patients and definitions

We included consecutive patients under 17 years old whose first demyelinating event of the CNS had occurred between 1995 and 2008. All children were identified by the Dutch Study Group for Pediatric MS, which consists of 15 major pediatric neurology centers in the Netherlands. Uniform definitions were used across sites. Clinical data were retrieved from a central database.[4]

Children were only eligible for this study when a cerebral MRI scan had been obtained within 2 months of the first clinical event and when a follow-up time of at least 2 years was reached. We classified children as either monophasic ADEM or MS (based on clinical features).[10] The only difference with the International Pediatric MS Study Group consensus definition for monophasic ADEM outcome[10] was that we did not strictly require the presence of encephalopathy at onset. In all MS cases the diagnosis was based on the clinical course (exacerbations), and not solely on new MRI activity. All MS cases in this study had more than 2 additional exacerbations after the initial clinical attack. We excluded patients with multiphasic or recurrent variants of ADEM as well as patients with neuromyelitis optica.[10]

Standard protocol approvals, registrations, and patient consents

This study was approved by the Medical Ethical Committees of the Erasmus University Medical Center in Rotterdam and of the other participating centers. Written informed consent was obtained from all patients or their parents.

MRI analysis

All MRI scans were performed on a 1.5 Tesla MRI scanner with slice thicknesses of 3-5 mm. The presence of lesions was determined on T2-weighted and fluid-attenuated inversion recovery (FLAIR) sequences. T1-weighted images were used to determine the presence of black holes. All scans were scored blinded to diagnosis by two experienced raters (I.A.K. and R.F.N.). All lesions were located and measured in accordance with the technique described previously.[9] We defined small lesions as being less than 2 centimeters in diameter in the axial dimension, and large lesions as having a maximum diameter of more than 2 centimeters.

MRI scans were classified as meeting the published Barkhof [6], KIDMUS [7], or Callen criteria (Table 4.2).[8 9]

Statistical analysis

We counted the total number of patients in the MS group and in the ADEM group who fulfilled the four sets of MRI criteria. Subsequently we calculated the sensitivity, specificity, positive predictive value and negative predictive value of the different sets of criteria. We also assessed the interrater reliability (Cohen K).

Analyses were performed using SPSS version 14.0.

RESULTS

Twenty-eight patients with MS and 21 patients with ADEM met our inclusion criteria (Figure 4.1). Their clinical characteristics are shown in Table 4.1. All patients in the ADEM group were polysymptomatic at presentation. Encephalopathy occurred more frequently in the

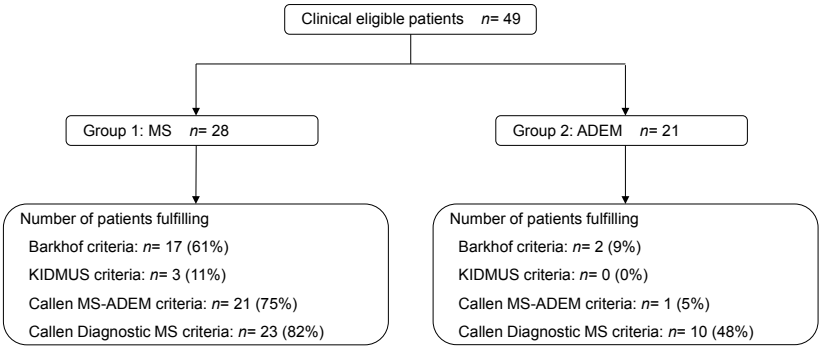

Figure 4.1. STARD flow diagram
ADEM=acute disseminated encephalomyelitis; MS=multiple sclerosis.

Table 4.1. Patient characteristics

	Multiple Sclerosis *n*=28	Acute disseminated encephalomyelitis *n*=21
Female / Male	14 / 14	5 / 16
Age at onset disease, y, mean ± SD	12.41 ± 3.8	6.76 ± 4.3
Follow-up time, y, mean ± SD	3.6 ± 2.9	3.8 ± 2.6
Clinical presentation at first attack, *n* (%):		
monofocal	13 (46)	0 (0)
polyfocal without encephalopathy	13 (46)	10 (48)
polyfocal with encephalopathy	2 (7)	11 (52)

ADEM group (52% versus 7% in the MS group). All patients with MS experienced subsequent clinical attacks. Two of the MS patients had an initial demyelinating event consistent with the diagnosis of ADEM (with encephalopathy), but later went on to have multiple relapses typical for MS and demonstrated new lesion accrual on MRI.

Table 4.2 shows the test properties of the four sets of MRI criteria. The application of the newly proposed Callen MS-ADEM criteria had the best sensitivity (75%) and specificity (95%) for distinguishing MS at first attack from ADEM. Their proposed diagnostic MS criteria were 82% sensitive and 52% specific (Table 4.2).

The Cohen K value for interrater reliability was 0.84 for the MS-ADEM criteria and 1.0 for the other criteria.

DISCUSSION

In our cohort, we confirmed the high sensitivity and specificity of the newly proposed Callen MS-ADEM criteria.[8] The sensitivity of these criteria in distinguishing between ADEM

Table 4.2. Test properties of the available sets of MRI criteria when applied to distinguish between ADEM and MS at first attack (fulfilling the criteria at first MRI correlates with conversion to MS)

	Barkhof (at least 3 out of 4) - ≥ 9 lesions on T2-weighted images or 1 gadolinium enhancing lesion - ≥ 3 periventricular lesions - ≥ 1 juxtacortical lesion - ≥ 1 infratentorial lesion	KIDMUS (1 out of 2) - lesions perpendicular to long axis of corpus callosum - the sole presence of well-defined lesions	KIDMUS (both) - lesions perpendicular to long axis of corpus callosum - the sole presence of well-defined lesions	Callen MS vs ADEM criteria (at least 2 out of 3) - absence of a diffuse bilateral lesion pattern - presence of black holes - ≥ 2 periventricular lesions	Callen Diagnostic MS criteria (at least 2 out of 3) - ≥ 5 lesions on T2-weighted images - ≥ 2 periventricular lesions - ≥ 1 brainstem lesion
Sensitivity (%)	61	57	11	75	82
Specificity (%)	91	95	100	95	52
Positive predictive value (%)	90	94	100	96	70
Negative predictive value (%)	63	63	46	74	69

ADEM=acute disseminated encephalomyelitis; MS=multiple sclerosis.

and MS after a first demyelinating event in children is higher than of the previous available Barkhof and KIDMUS MRI criteria.

Unlike the original study, we did not require the presence of encephalopathy for the clinical diagnosis of ADEM in the present study. It has previously been shown that ADEM-like disease also exists without encephalopathy.[1 4] Children with a polysymptomatic disease with encephalopathy are likely to have different MRI lesion characteristics, including larger and more diffuse bilateral cerebral lesions, than children without encephalopathy. Since 'the absence of a diffuse bilateral lesion pattern' is included in the criteria to distinguish MS at first attack from ADEM[8], we wanted to investigate whether these criteria were still applicable in children with a monophasic polyfocal disease without encephalopathy at onset. It is interesting that irrespective of a monophasic polyfocal disease with or without encephalopathy, the specificity of these criteria remained the same when applied to our pediatric population. In addition to this, we reclassified our groups according to the international consensus definition for ADEM, thus excluding patients without encephalopathy at onset. This did not significantly change the sensitivity and specificity of the criteria (data not shown).

We found a perfect interrater reliability. This is notable because 'diffuse bilateral lesions' remains a subjective criterion, lacking a precise definition in the literature.[8]

Large lesions are more frequently seen in children with a monophasic disease than in children with MS, but this difference is only observed after the age of 10 years.[4] We would therefore recommend studying these criteria in two larger groups of children: one group below 10 years old and one group above this age.

We confirmed that the newly proposed Callen diagnostic MS criteria have a high sensitivity, but the lowest specificity when used to distinguish between ADEM and MS at first attack.[8] In their original study, these criteria were designed to distinguish between MS at the time of second attack and other, non-demyelinating, diseases.[9] However, at the initial presentation, the MRI distinction between a first attack of MS and other demyelinating disease such as ADEM is usually more difficult, which may explain the lower sensitivity and specificity when applied at the time of first attack.

Of all criteria currently available, the Callen MS-ADEM criteria appear to be the most useful in clinical practice for children with a first demyelinating event, specifically for distinguishing between children with a monophasic polysymptomatic demyelinating event (ADEM) and those who are at risk of developing a second demyelinating attack and thus of converting to MS. Still, these findings need to be interpreted with caution because of the limited sample size in our study and in previous studies. Furthermore, although the length of follow-up time is comparable in both groups, the time in the ADEM group is still relatively short to state with complete certainty that they will remain monophasic in the future. It will be important to examine whether these criteria reliably predict future conversion to MS in a prospective cohort of children with a first attack of demyelination. Multinational collaborations would significantly help this endeavour.

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CHAPTER 5

Fatigue and depression in children with multiple sclerosis and monophasic variants

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ABSTRACT

Background Fatigue is an important symptom in adult multiple sclerosis (MS) and it is likely to occur in children with MS. It is currently unknown whether children who experienced a monophasic inflammatory demyelinating event of the central nervous system in the past also suffer from fatigue.

Methods We studied the presence and severity of fatigue in 32 children (18 boys, 14 girls) between 11-17 years old (mean: 14 years, 10 months) with a monophasic inflammatory demyelinating disease (*n*=22) or definite MS (*n*=10). This was measured with the Checklist Individual Strength. A score of ≥ 40 on the severity of fatigue subscale indicated the presence of severe fatigue. We also examined the relation between fatigue and depression (assessed by the Child Depression Inventory). Additionally we measured the health-related quality of life (HRQoL), using the TNO-AZL Child Quality of Life child form. We compared the scores of the MS and monophasic patients with the scores of healthy Dutch children.

Results The highest scores on the fatigue scales subjective fatigue and physical activity were found in the children with MS. Only 1 of the monophasic patients suffered from severe fatigue in contrast to 4 of the MS patients. In the MS group fatigue and depression were correlated. MS patients experienced a lower HRQoL on the scales locomotor functioning, cognitive functioning and interaction with peers.

Conclusion The occurrence of fatigue is very rare after a monophasic inflammatory demyelinating event in the past. As expected, fatigue occurs more frequent in pediatric MS patients.

INTRODUCTION

Fatigue is the most commonly reported symptom of multiple sclerosis (MS) in adults and affects almost all patients in various degrees.[1] MS can present before the age of 18 years in up to 10% of patients.[2] Previous studies on childhood MS estimate that fatigue is present in 20-73% of the patients. However, these studies focus particularly on cognitive dysfunction and not on severity and impact of fatigue.[3-5]

It is also known that a single neuroinflammatory attack like Guillain-Barré syndrome (GBS) can result in chronic fatigue.[6] So it is likely that children with a monophasic inflammatory demyelinating disease of the central nervous system (CNS), which also has an autoimmune etiology like GBS, will suffer from fatigue as well. Examples of these monophasic diseases occurring in childhood are acute disseminated encephalomyelitis (ADEM), optic neuritis (ON) and transverse myelitis (TM). Yet no data on fatigue as a long-term complication in these patients have been published.

When studying fatigue in pediatric patients, it is important to consider that fatigue is common in healthy adolescents as well and that there is a relation with depression.[7] The prevalence of affective disorders in children with MS is not systematically assessed and estimates vary from 6 to 46 %.[3-5 8] Children who have experienced ADEM in the past, may also be vulnerable to behavioral and emotional problems.[9 10]

In the present study we determined whether children with either a monophasic or a chronic inflammatory demyelinating disease of the CNS develop fatigue. We also studied whether this is related with the simultaneous occurrence of depression and if the health-related quality of life (HRQoL) is affected. We used self-report questionnaires that are validated for adolescents in the Netherlands and compared the results of the monophasic and chronic patients with the results of healthy peers.

METHODS

Participants

We recruited children between 11 and 17 years old referred to four MS centers in the Netherlands who experienced an idiopathic inflammatory demyelinating disease of the CNS. We included patients with a diagnosis of ADEM, ON, TM and MS.[11] All ADEM patients had a polyfocal onset, with multiple CNS lesions on MRI and were relapse-free at the moment of testing.[11 12] All patients with a diagnosis of MS had experienced at least one clinical relapse. None of the children had a relapse in the three months preceding the evaluation.

Patient characteristics and detailed clinical information were obtained from medical records or by physician telephone interview (in case of a last hospital visit more than 2 months earlier). We ascertained that all patients and parents were able to read and under-

stand Dutch. All participants gave informed consent. This study was approved by the Medical Ethical Committee of the Erasmus University Medical Center in Rotterdam.

Measures

Self-report paper and pencil questionnaires in Dutch, as well as a letter with instructions, were sent to the patients by mail. Parents were instructed not to assist their child. Every questionnaire concerned the preceding 2 weeks.

All questionnaires had satisfactory psychometric properties, including reliability and validity.[7 13-16] For all patients adequate clinical information was collected and Expanded Disability Severity Scale (EDSS) scores were assigned by a trained physician (I.A.K.).[17]

Fatigue

Fatigue was measured using the Checklist Individual Strength (CIS) on 4 subscales representing several aspects of fatigue: subjective experience of fatigue (8 questions), concentration (5 questions), motivation (4 questions) and physical activity (3 questions). The sum of all the sub scores composes a multidimensional total fatigue score. The response to each question was rated on a 7-point Likert scale, with a higher score indicating a higher level of subjective fatigue and concentration problems and a lower level of motivation and physical activity.[7 15] Severe fatigue was defined as a score of 40 or more on the subjective experience of fatigue subscale, according to a study on chronic fatigue syndrome in adolescents in the Netherlands.[18]

The CIS questionnaire was originally developed in the Netherlands for adults, but has been validated for teens older than 10 years.[18] We compared our data with data of 128 Dutch children between 12 and 17 years old without any current illness. These children participated as a reference group in an earlier study on chronic fatigue syndrome in children in the Netherlands.[19]

Depression

A validated Dutch translation of the Children's Depression Inventory (CDI) was used to assess depression. The questionnaire consists of 27 items representing a range of depressive symptoms. For each item, the child was asked to choose the one statement (out of three) that best reflected his feelings. The item scores (ranging from 0-2) are added into a total score, with higher scores being predictive of a depressive disorder.[13 14] A cut-off score of 19 or higher (based on the raw CDI total score) can be used to detect depressive disorders in children and adolescents.[20]

Data of a group of 36 healthy Dutch adolescents aged 12-18 years, derived from a control group used in a previous study on chronic fatigue syndrome in children, were available as a reference.[21]

Health-related quality of life (HRQoL)

The TNO-AZL Child Quality of Life Child Form 12-15 (TACQOL CF 12-15) measures the child's feelings about his HRQoL. HRQoL is defined as the child's health status weighted by the subjective emotional response to reported health status problems. It offers the child the possibility to distinguish between his functional problem and the way he feels about it. The questionnaire is a multidimensional construct (consisting of 44 items), covering six domains: pain and physical complaints (Body), locomotor functioning (Motor), cognitive functioning (Cognition), interaction with peers (Peers), the experience of positive emotions (Emopos) and negative emotions (Emoneg). Higher scale scores indicate a better HRQoL. No total score is calculated.

Reference data were derived from an available sample of healthy children from the general population in the Netherlands.[16]

Data analysis

We classified the patients into a group of patients with monophasic inflammatory demyelinating diseases of the CNS (i.e. ADEM, ON, TM: 'monophasic') and a group of patients with a chronic multiphasic inflammatory demyelinating disease (i.e. clinically definite MS patients: 'MS'). All (scale) scores were calculated for the three questionnaires.

One-way analysis of variance (ANOVA) with Bonferroni correction was performed to test the statistically significant differences in scores between the monophasic patients, the MS patients and the control groups. Spearman's rank correlation coefficient was used to study the association between fatigue and depression, as well as fatigue and EDSS in both patient groups. A significance level of 0.05 was chosen for all analyses.

All data analyses were performed with SPSS version 14.0 for Windows.

RESULTS

A total of 38 children with one or more inflammatory demyelinating events of the CNS, were suitable to participate in this study. Thirty-two children responded. The characteristics of these patients are presented in Table 5.1. The patient group consisted of 10 children with clinically definite MS, all relapsing-remitting, and 22 children with a monophasic inflammatory demyelinating event ('monophasic'), of whom 16 had ADEM, 3 had ON and 3 had TM. All the ADEM patients had clinical symptoms suggestive of ADEM[12], however 10 patients did not fulfill the newer ADEM criteria of the International Pediatric MS Study Group because they had no encephalopathy at onset.[11] Twelve of the 22 monophasic patients had mild residual disability, but did not experience any further relapses. The length of follow-up time was longer in the monophasic group than in the MS group. None of the patients with ON and TM had brain MRI lesions and abnormalities in cerebrospinal fluid suspected of MS (i.e. normal IgG index and no oligoclonal bands).

Table 5.1. Characteristics of patients and control groups

	Patients (*n*=32)		Healthy controls		
	MS (*n*=10)	Monophasic (*n*=22)	CIS (*n*=128)	CDI (*n*=36)	TACQOL (*n*=388)
Boys / Girls	18 / 14		49 / 79	12 / 24	199 / 189
	4 / 6	14 / 8			
Age: Mean (SD) Median (range)	14y 10mo (1.75) 15y 2mo (11.35-17.34)		14y 9mo (1.16)	16y 9mo (1.38)**	13y 5mo (1.05)**
	15y 7mo (1.47) 16y 4mo (12.83-17.34)	14y 5mo (1.78) 14y 6mo (11.35-16.77)			
Age at 1st attack: Mean (SD) Median (range)	10y 7mo (4.34) 11y 7mo (0.5-15.96)		-	-	-
	12y 2mo (3.55) 12y 10mo (5.98-15.96)	9y 11mo (4.56) 11y 1mo (0.5-15.9)			
Time since first event: Mean (SD) Median (range)	4y (3.56) 2y 10mo (0.4-13)		-	-	-
	3y 5mo (3.10) 3y 1mo (0.4-9.38)	4y 3mo (3.79) 2y 10mo (0.47-13)			
Time since last clinical episode: Mean (SD) Median (range)	3y 4mo (3.48) 1y 9mo (0.25-13)		-	-	-
	1y 3mo (1.17) 1y (0.25-4.16)	4y 3mo (3.79)* 2y 10mo (0.47-13)			
Total follow-up time: Mean (SD) Median (range)	5y (3.79) 4y (0.5-13.17)		-	-	-
	4y 5mo (3.10) 4y 2mo (0.67-10.67)	5y 3mo (4.11) 3y 11mo (0.5-13.17)			
EDSS score: Mean (SD) Median (range)	1.5 (1.8) 1 (0-7.5)		-	-	-
	2.5 (2.5) 1.5 (0-7.5)	1.1 (1.2) 1.0 (0-4.0)			
Current treatment	8		0	0	0
	6[a]	2*			

[a] 5 MS patients used interferon beta-1a and 1MS patient used glatiramer acetate.
* $p<0.01$ MS patients vs. monophasic patients (Mann-Whitney U test and Fisher's exact test). ** $p<0.001$ patients vs. controls (One-way ANOVA with Bonferroni corrected Student's *t*-test).

Fatigue

We found the highest scores in the MS group on the subscales subjective fatigue and physical activity, indicating more fatigue related symptoms in this group (Figure 5.1). ANOVA showed that the total fatigue scores were not significantly different between MS patients versus controls and versus monophasic patients. But the score on the subscale subjective fatigue was significantly higher in the MS patients than in the control group (F=4.030, df=159, 95% C.I. 0.50-19.03, p=0.035) and in the patients with a monophasic disease (F=4.030, df=159, 95% C.I. 1.64-23.16, p=0.018). There was also a significant difference in the subscale physical activity between MS patients and controls (F=3.032, df=159, 95% C.I. 0.02-6.20, p=0.048). The subscales concentration and motivation showed no significant differences between the three groups.

Using a cut-off score of 40 on the subscale subjective fatigue, five patients suffered from severe fatigue: four MS patients and only one patient with ADEM. Two of the fatigued MS patients had their last relapse within one year before testing. The others were relapse-free for more than one year. Three MS patients were treated with interferon, the other patients did not receive any treatment.

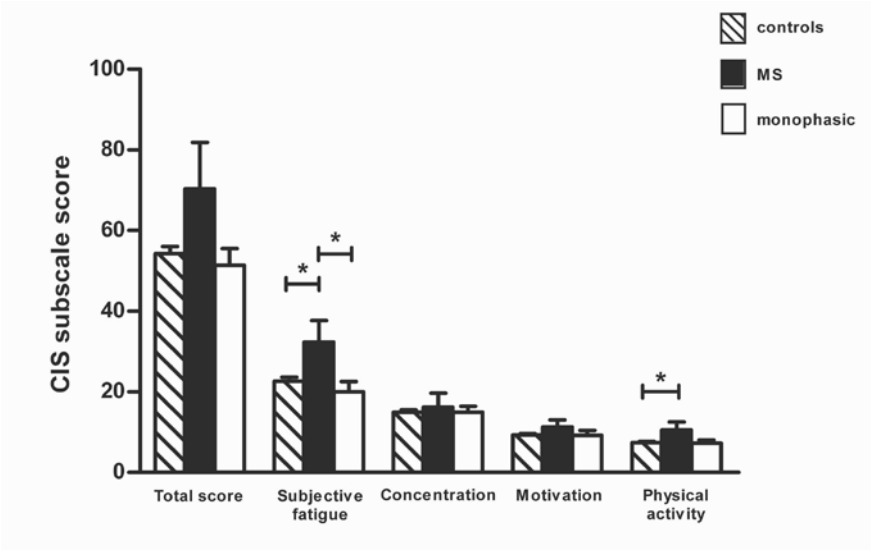

Figure 5.1. Assessment of fatigue in control subjects, MS patients and patients with a monophasic disease course

Data are presented as mean with SEM. *p<0.05. Higher scores indicate a higher level of subjective fatigue and concentration problems, and a lower level of motivation and physical activity.

Depression

The differences between the scores of the MS patients, monophasic patients and controls on the CDI questionnaire were not statistically significant (Figure 5.2).

Two patients with MS had a score higher than the cut-off value of 19, indicating a depressive disorder. All patients in the monophasic group had scores below this value.

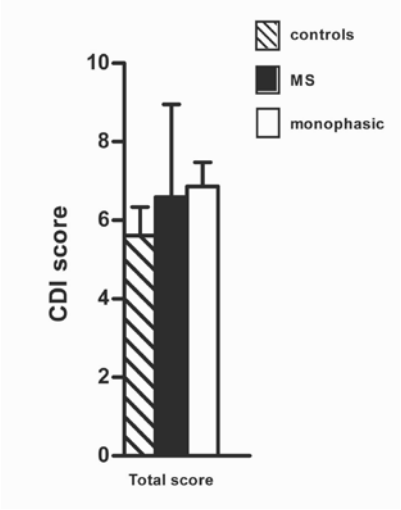

Figure 5.2. Assessment of depression in control subjects, MS patients and patients with a monophasic disease course
Data are presented as mean with SEM. Higher scores are predictive of a depressive disorder.

Correlations

A more severe outcome on the fatigue questionnaire correlated with a higher depression score, only in the MS group (r_s=0.841, 95% C.I. 0.449-0.961, p=0.002) and not in the monophasic group (r_s=0.284, 95% C.I. -0.156-0.630, p=0.201). A correlation was found between fatigue and disability (as measured by EDSS) in all patients, with the strongest correlation in the MS patients (r_s=0.703, 95% C.I. 0.132-0.924, p=0.023).

Both MS patients with the highest score on the subjective fatigue subscale (i.e. 53 and 56), had the highest scores on the CDI questionnaire (i.e. 20 in both) and the highest EDSS scores (i.e. 6.5 and 7.5) as well.

Health-related quality of life (HRQoL)

The MS patients scored significantly lower on the scales Motor (F=30.526, df=419, 95% C.I. -9.36- -4.66, p<0.001), Cognition (F=3.544, df=419, 95% C.I. -6.42- -0.20, p=0.033) and Peers (F=8.097, df=419, 95% C.I. -6.04- -1.48, p<0.001) than the healthy controls. Their score was also significantly lower on the scales Motor (F=30.526, df=419, 95% C.I. -7.57- -1.98, p<0.001) and Peers (F=8.097, df=419, 95% C.I. -5.94- -0.50, p=0.014) than

the monophasic patients. The patients with a monophasic disease course had a significantly lower score on the Motor scale than control subjects (F=30.526, df=419, 95% C.I. -3.84--6.31, p=0.003) (Figure 5.3).

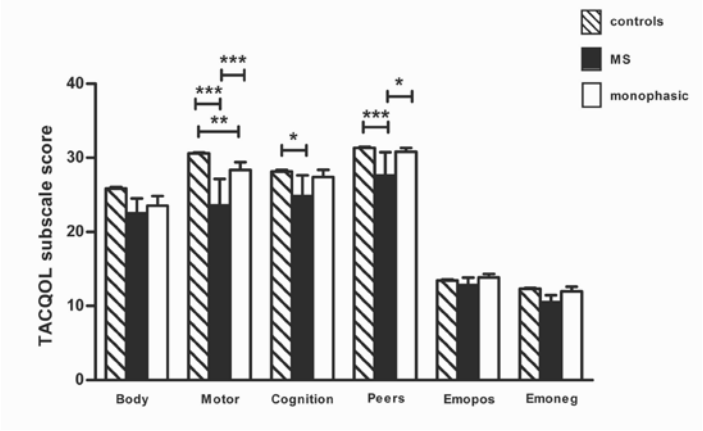

Figure 5.3. Assessment of health-related quality of life (HRQoL) in control subjects, MS patients and patients with a monophasic disease course
Data are presented as mean with SEM. *p<0.05 **p<0.01 ***p<0.001. Higher scores indicate a better HRQoL.
Body = pain and physical complaints, Motor = locomotor functioning, Cognition = cognitive functioning, Peers = interaction with peers, Emopos = the experience of positive moods, Emoneg = the experience of negative moods.

DISCUSSION

This study shows that the scores on the severity of fatigue and physical activity scales of the CIS questionnaire were higher in the group of MS patients than in healthy teenagers and in patients with a monophasic disease course. However the total scores were not significantly different between the three groups. We found that only one patient with a monophasic disease (ADEM) suffered from severe fatigue, in contrast to 4 of the 10 MS patients. The latter is in accordance with two previous studies showing that fatigue was problematic in 20-49% of children with MS (assessed by subjective patient reports).[3 4] In an Italian cohort, 73% of 63 patients with MS reported severe fatigue, when compared to healthy children, rated with the Fatigue Severity Scale (FSS). When using the established cut-offs of the FSS for adults, this percentage was much lower (14%).[5] For this reason it is important that applied questionnaires are validated for children, like the CIS questionnaire that was used in our study.

As has been shown in healthy adolescents and in adults with MS, there is a possible relation between fatigue and depression.[7 22] We observed that two MS patients reporting severe fatigue, suffered from depression as well. These patients also had severe disability. None of the monophasic patients suffered from depression. Fatigue and depression were

correlated only in the MS group, although these findings must be interpreted carefully given the small number of patients and hence the large confidence intervals. There was also a relation between fatigue and disability. The latter is in contrast to findings in adult MS, in which fatigue is present at all stages of the disease independent of disease duration and disability.[1]

The relation between fatigue and depression in adult MS patients is complex, due to the multidimensional nature of fatigue.[22] It is possible that the experience of fatigue could be caused by depressive feelings. On the other hand, severe fatigue can induce depression. The exact etiology and pathophysiology of fatigue in MS are not well understood. These symptoms may be centrally mediated and can be resultant to the underlying pathologic alterations (demyelination, inflammation and axonal injury) in the CNS or can be a psychological reaction to the illness.[1 22 23]

In general, it has been shown that changes in white matter structure are associated with depression.[24] Relations between certain brain lesions and fatigue as well as depression have been reported in adult patients with MS.[1 22] Our study indicates that an attack to the CNS white matter does not necessarily lead to increased fatigue or depression, as only 1 of the 16 ADEM patients suffered from severe fatigue and none of these monophasic patients suffered from depression.

Symptoms of fatigue, as well as depression, may also be secondary to hospitalization at a young age or the impact of the diagnosis of a neurological disease. During adolescence rapid physical and psychological changes occur. It is a critical period for socialization, development of self-esteem and identity and planning for the future. For this reason the diagnosis of a possibly chronic, progressive disease with a very uncertain course can have a significant impact on the perspective of future life of teenagers.[25] To further assess the influence of these psychological factors, future studies are needed to compare children with MS to children with chronic diseases not involving the CNS.

In addition, we showed that children with MS experienced a worse HRQoL in locomotor functioning, cognitive functioning and interaction with peers. In adults with MS depression and fatigue are both independently associated with impaired quality of life next to advancing neurological disability.[22] Moreover, depression and fatigue seem to be the most important contributors of HRQoL.[26]

The present study was undertaken to assess the presence and severity of fatigue, possibly induced by depression, in patients with monophasic demyelination of the CNS next to patients with a multiphasic disease course like MS. One of the strengths of our study is that we applied questionnaires that were validated for children and adolescents in the Netherlands. Because the prevalence of fatigue among a healthy population of adolescents seems to be high as well, we used comparative data of healthy controls of the same age in the Netherlands. In that way we corrected for age-related occurrence of fatigue and affective disorders during puberty. In contrast to most other studies, we also assessed the occurrence of depression and whether the HRQoL was affected. Apart from the variation in

time between disease-onset and testing, the small sample size is an important limitation of our study. The latter is a problem in most research on pediatric MS, due to the rarity of the disease. Because of the small sample size the results of this study need to be interpreted with caution and future studies are needed including more patients that are tested on the same time point in their disease course. In that way it is easier to correct for possible confounders.

Contrary to our expectations, fatigue does not seem to be a problem in children that experienced a monophasic inflammatory demyelinating disease of the CNS in the past. We found that more children with MS suffered from severe fatigue compared with healthy peers, which is in accordance with previous data. Furthermore it is important for clinicians to be aware of the possible association of fatigue with depression and the impact on the health-related quality of life in childhood. Therefore we advise to include evaluation of complaints of fatigue and depression in the regular long-term follow-up of pediatric MS patients in order to offer an adequate and timely intervention program focused on these socially impairing consequences of disease.

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CHAPTER 6

Antibodies against aquaporin-4 in neuromyelitis optica: distinction between recurrent and monophasic patients

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Multiple Sclerosis, 2011

ABSTRACT

The detection of antibodies against aquaporin-4 (AQP4) has improved the diagnosis of neuromyelitis optica (NMO). We evaluated a recently established cell-based anti-AQP4 assay in 273 patients with inflammatory CNS demyelination. The assay had a specificity of 99% and a sensitivity of 56% to detect all NMO patients and of 74% to detect the recurrent NMO patients, similar to the initial studies reported. AQP4-antibodies were absent in monophasic NMO patients, while samples in recurrent cases remained positive during follow-up. We conclude that the pathogenesis of monophasic NMO may be different from that of relapsing NMO.

INTRODUCTION

The discovery of an autoantibody specific for neuromyelitis optica (NMO), directed against the aquaporin-4 (AQP4) water channel, contributed significantly to the differentiation of NMO from typical MS[1 2] and led to the identification of NMO spectrum disorders (NMOsd).[3]

The distinction from MS is important, because the prognosis of NMO is worse and more aggressive therapy is needed.[4] In particular, the early and accurate prediction of a recurrent disease course can lead to initiation of early treatment to prevent further relapses, before severe permanent disability is reached.

We have recently set up an assay to detect AQP4-antibodies for use in a national reference laboratory and studied antibody presence in patients with recurrent and monophasic NMO.

PATIENTS AND METHODS

Patients

Patients with NMO or NMOsd referred to the Erasmus Medical Center and VU Medical Center between 2000 and 2008 were included (group 1). In September 2008 we started to run the assay nationwide. All samples of NMO and NMOsd that were applied for AQP4-antibody testing from September 2008 to May 2009 were included in this study (group 2).

The Medical Ethical Committees of the Erasmus Medical Center and VU Medical Center approved this study and all patients (group 1) provided informed consent. Clinical information of the patients in group 2 was obtained from treating physicians.

The diagnosis of NMO was made based on the revised diagnostic criteria[5], irrespective of NMO-IgG status. We defined NMO as having both optic neuritis (ON) and longitudinally extensive transverse myelitis (LETM) without radiological evidence for MS. These patients were classified as either recurrent (multiple episodes of ON, myelitis or both) or monophasic (simultaneous occurrence of both ON and myelitis without subsequent clinical episodes). Follow-up time of at least six months was required for all patients. Patients with either LETM or recurrent ON, as well as patients with both ON and myelitis <3 segments, but without brain MRI lesions that are typical for MS[6] were diagnosed as having NMOsd.

As controls we randomly selected definite MS patients[6] and neurological controls with non-demyelinating diseases (ONDs).

Anti-AQP4 assay

AQP4-antibodies were routinely assayed in a cell-based assay using a fluorescence activated cell sorter (FACS, LSRII and DIVA software, Becton Dickinson, San Jose, USA). Plasmids containing EGFP-tagged AQP4-M1 or AQP4-M23 complementary DNA were kindly provided by Prof. A. Vincent (University of Oxford, Oxford, UK).[7] HEK293T cells were transiently

transfected with both EGFP-tagged AQP4 isoforms using standard transfection techniques. AQP4- or sham-transfected cells were incubated with patient or control samples (1:30) and bound antibodies were detected with goat anti-human IgG Allophycocyanin (APC) conjugated secondary antibody (Jackson Immunoresearch Laboratories, Brunschwig Chemie B.V., Amsterdam, The Netherlands). To correct for aspecific staining of HEK 293T cells, the APC-channel mean fluorescence intensity (MFI) from sham-transfected cells was subtracted from that of AQP4-transfected cells for each individual sample (ΔMFI).

Anti-AQP4 IgG was considered detectable if the MFI of a sample was higher than the assay cut-off value (assay cut-off = average MFI + 10x standard deviation of eight individual negative control sera from apparently healthy lab workers tested in every assay).

Statistical analysis

Statistical analysis was performed using SPSS 15.0. Chi-square or Fisher's exact test were used to compare categorical data between monophasic and recurrent NMO patients and the Mann-Whitney *U* test to compare continuous data. Results were considered significant if *p*-values were <0.05.

RESULTS

We included 188 patients in group 1 and 183 in group 2. We were unable to retrieve the clinical data of 10 patients in group 2 (these patients were all seronegative). The test results are shown in Table 6.1.

Overall, AQP4-antibodies were present in 56% of the NMO patients included in both groups. Among NMOsds, the antibodies could only be detected in four patients who were highly susceptible of having recurrent NMO but with a spinal cord lesion that did not extend

Table 6.1. AQP4-antibody test results in local (Group 1) and nationwide (Group 2) cohorts of NMO and NMOsd

	Group 1 (*n*=188) Positive/*n* (%)	Group 2 (*n*=173) Positive/*n* (%)	Total (*n*=361) Positive/*n* (%)
NMO	12/22 (55)	8/14 (57)	20/36 (56)
NMOsd	1/31 (3)	3/48 (6)	4/79 (5)
Myelitis (<3 segments) and ON	1/18 (6)	3/5 (60)	4/23 (17)
LETM or recurrent ON	0/13 (0)	0/43 (0)	0/56 (0)
MS	2/74 (3)	0/84 (0)	2/158 (1)
OND	0/61 (0)	0/27 (0)	0/88 (0)

AQP4 = aquaporin-4. NMO= neuromyelitis optica. NMOsd= neuromyelitis optica spectrum disorders. ON= optic neuritis. LETM= longitudinally extensive transverse myelitis. MS= multiple sclerosis. OND= other neurologic diseases (neurological controls with non-demyelinating diseases).

over three vertebral segments. None of the patients with solely LETM or recurrent ON tested positive.

AQP4-antibodies were absent in monophasic NMO patients and present in 74% of the patients with a recurrent disease. More female patients had a recurrent disease.

The monophasic and recurrent NMO patients did not differ in receiving treatment, age at disease-onset, follow-up time and time-interval between disease activity and serum sampling (Table 6.2).

Table 6.2. Clinical characteristics of NMO patients (*n*=36)

	Monophasic (*n*=9)	Recurrent (*n*=27)	*p*-value
AQP4-antibody positive, *n* (%)	0 (0)	20 (74)	<0.001
Female, *n* (%)	3 (33)	24 (89)	0.003
Immunomodulatory therapy, *n* (%)	3 (33)	12 (44)	0.705
Age at disease-onset, mean ± SD	40.2 ± 10.8 y	32.7 ± 11.1 y	0.065
Follow-up time, mean ± SD	4.7 ± 5.1 y	8.2 ± 5.4 y	0.120
Time-interval between clinical disease activity and obtaining serum sample, mean ± SD	2.6 ± 4.4 y	1.4 ± 1.9 y	0.812
Time-interval ≤ 4 months, *n* (%)	5 (56)	11 (41)	0.470

NMO= neuromyelitis optica. AQP4 = aquaporin-4.

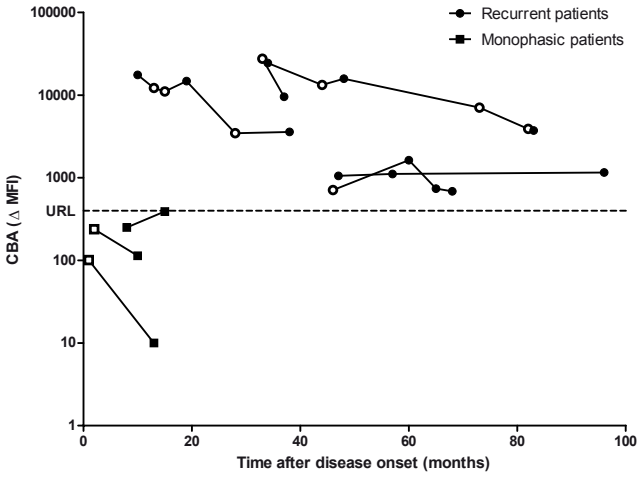

Figure 6.1. Antibody titre change in NMO patients during disease course
All longitudinal samples from one patient were measured in the same assay. Results are expressed as ΔMFI (MFI of AQP4-transfected cells – MFI of sham-transfected cells). Open symbols denote clinical disease activity.
URL= upper reference limit. CBA= cell-based assay. MFI= median fluorescence intensity

In eight patients multiple samples were obtained at different time points during their disease course (Figure 6.1). The recurrent patients (n=5) remained positive irrespective of disease activity, whereas the monophasic patients (n=3) remained negative.

The assay has a specificity of 100% to differentiate NMO from neurological controls and of 99% to differentiate NMO from MS patients. AQP4-antibodies were present in two patients initially diagnosed as having MS. Although both patients presented with bilateral ON and symptoms related to the spinal cord, cerebral MRI showed lesions typical of MS. During follow-up these patients developed a recurrent disease that is more suspected of an NMOsd.

DISCUSSION

We have established a cell-based assay that is useful to detect AQP4-antibodies in NMO patients with a sensitivity between 56% and 74% and to discern patients with NMO from MS with a high specificity (99-100%). Our findings are within the range of previously reported assays to detect NMO-IgG and AQP4-antibodies.[1 7 8]

Antibodies against AQP4 were not present in monophasic NMO patients. Interestingly, a previous study also found a difference in seropositivity rates between monophasic and recurrent NMO patients (12.5% *vs.* 78% seropositivity)[8 9], emphasizing the validity of our observation.

One possible explanation for absence of AQP4-antibodies in monophasic NMO patients would be a long time interval between disease activity and serum sampling[8], which we could not confirm. Another hypothesis is that the monophasic patients rather have a postinfectious disease, although there were no strong clinical indications in this direction. Also the use of immunotherapy could not explain the seronegativity in monophasic patients. This was underlined by the fact that the number of seropositive patients did not differ between patients with and without immunosuppressive treatment (respectively, 8/15=53% *vs.* 12/21=57%, p=0.821). Additionally we showed that under treatment with immunosuppressive therapy and during remission titres can be lower in recurrent NMO patients, but are still detectable.

This study had both a retrospective and a prospective design. An advantage of retrospective inclusion of patients is the long follow-up time and of prospective inclusion is the short time-interval between disease activity and serum sampling. Prospective studies with a much longer follow-up time are needed to assess whether an anti-AQP4 positive status is prognostic for a recurrent disease course. Future research is needed to assess whether the pathogenesis of monophasic NMO is distinct from that of recurrent NMO.

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CHAPTER 7

Myelin oligodendrocyte glycoprotein antibodies plead against MS diagnosis in an ADS cohort

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Submitted

ABSTRACT

Background Acquired demyelinating syndromes (ADS) in children are a group of distinct first immune-mediated demyelinating events of the central nervous system (CNS). They can all represent a first episode of multiple sclerosis (MS). Predictive biomarkers for future diagnosis are lacking. A putative target antigen in ADS is myelin oligodendrocyte glycoprotein (MOG). Previous studies, which mainly focused on specific diagnostic subgroups of adults and/or children with demyelinating diseases, showed that these antibodies could be detected in a subset of (young) patients with acute disseminated encephalomyelitis (ADEM), pediatric onset MS, anti-aquaporin-4 (AQP4) negative NMO or NMO spectrum disorders. We analyzed the presence of MOG-antibodies in a cohort of ADS patients, stratified by clinical presentation, in order to identify disease characteristics of anti-MOG seropositive patients.

Methods 117 children with ADS were analyzed with a cell-based anti-MOG assay, utilizing a stably transfected LN18 cell line. The patients were divided in 5 groups: optic neuritis (ON; *n*=20), transverse myelitis (TM; *n*=7), other monofocal ADS (*n*=22), polyfocal ADS without encephalopathy (*n*=44) and polyfocal ADS with encephalopathy at onset (*n*=24). Additionally, 13 children with other neurological diseases (OND), 31 healthy children and 29 adult ADEM patients were tested.

Results Nineteen of the 117 children with ADS tested anti-MOG seropositive (16%). Of these, the disease-onset was polyfocal in 17 patients, whereas 2 had isolated ON. The group of patients with polyfocal ADS plus encephalopathy (ADEM) had the highest prevalence of anti-MOG seropositivity (42% versus 16% in non-encephalopathic polyfocal ADS patients). None of the OND or healthy controls had MOG-antibodies. After a mean follow-up time of 4.7 years, 47 ADS children had a final diagnosis of MS. In none of them MOG-antibodies were detected. Of the 70 ADS children without MS diagnosis after similar follow-up time, 27% were anti-MOG seropositive. Four children had a specific course of ADEM onset followed by multiple episodes of ON and all were anti-MOG seropositive. Of the adult ADEM patients, only 1 out of 29 tested anti-MOG seropositive.

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

INTRODUCTION

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

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

In the search for disease-specific autoantibodies in ADS, myelin oligodendrocyte glycoprotein (MOG) is a putative target antigen. This protein is expressed on the surface of myelin sheaths and oligodendrocytes, and thus specific to the CNS. Previous studies already showed that MOG-antibodies can cause demyelination in vitro and can induce experimental autoimmune encephalomyelitis (EAE).[6 7] Based on the current knowledge, antibodies to MOG appear specific for CNS demyelinating diseases[8 9] and are especially present in patients with ADEM[10 11], in children with very early-onset MS[12], and with higher titers in the youngest children and children with ADEM.[11 13 14] But the sensitivity of MOG-antibody assays in demyelinating diseases varies and is reported to be only as high as 47%.[15] This is in part due to the patients included, as the antibodies lack sensitivity for the overarching group of ADS patients. Previous studies focused mainly on specific subgroups of ADS patients based on diagnosis, like pediatric onset MS[10-12 14 16], ADEM[10 11 13 14 16] and CIS.[11 13 14] Recently MOG-antibodies were also detected in patients with anti-AQP4 negative NMO or NMO spectrum disorders such as recurrent ON and longitudinally transverse myelitis.[17-20]

To date, it is unsure which subgroup the children with MOG-antibodies represent within the spectrum of ADS.[21] In the current study we investigated the presence of MOG-antibodies in a cohort comprised of all ADS subtypes and compared their presence between the different ADS subtypes based on clinical presentation. We hypothesized that the MOG-antibodies are prevalent in the younger ADS children who are more likely to have a polyfocal onset with encephalopathy, which is the strict definition for ADEM.[22]

METHODS

Patients and controls

Children with a first demyelinating event of the CNS (ADS), younger than 18 years, enrolled in the Dutch study on pediatric MS[2 23], were consecutively included in this study. At first event, patients were divided into five groups, based on clinical presentation: ON, TM, other monofocal disease-onset (mono ADS), polyfocal disease-onset without encephalopathy (poly ADS –) and polyfocal disease-onset with encephalopathy (poly ADS +).[2] A diagnosis of MS could be made when a second demyelinating attack occurred, with clinical and/or MRI evidence of dissemination in time and space at least one month after onset.[24] After a first attack with encephalopathy, two subsequent attacks without encephalopathy were needed, at least three months after onset, for a diagnosis of MS.[22] Follow-up information was provided by the clinical physician and by telephone interview of the parents.

As control groups we included healthy children and children with other neurological diseases (OND). Furthermore we tested a group of adult patients with a clinical diagnosis of ADEM.

This study was approved by the Medical Ethical Committees of the Erasmus University Medical Center in Rotterdam and of the other participating centers.

Anti-MOG assay

In order to detect antibodies to native intact MOG we used a LN18 cell line (a kind gift of Prof. B. Hemmer, Technical University of Munich, Germany) that stably expressed full length MOG protein on the surface. For the detection and quantification of antibodies binding to MOG expressed on the cell surface we used FACS analysis. In each assay we tested 8 individual negative control sera (apparently healthy lab workers), one strongly positive and one low positive control serum. Antibodies against MOG were considered detectable if the difference in median fluorescence intensity (ΔMFI) between MOG transfected and untransfected LN18 cells of a sample was higher than the assay cut-off value (assay cut-off = average ΔMFI + 10x standard deviation of 8 individual negative control sera). For the present paper a total of seven individual experiments were performed and the cut-off was determined as average ΔMFI + 10x standard deviation of all individual negative control sera tested in these experiments.

Statistical analysis

Statistical analysis was performed using SPSS 20.0. Chi-square and Fisher's exact tests were used to compare categorical data and Kruskal-Wallis and Mann-Whitney *U* tests to compare continuous data. Differences in continuous data between two groups were compared using Student's *t*-test.

Results were considered significant if *p*-values were < 0.05. Bonferroni corrections were made when appropriate.

RESULTS

We included 117 patients with ADS, 13 children with other neurological disorders (OND) and 31 healthy children. The OND group consisted of patients with epilepsy (*n*=4), viral encephalitis (n=4), other autoimmune diseases (*n*=2), migraine (*n*=1), trauma (*n*=1) and severe hypertension (*n*=1). In addition, 29 adult ADEM patients were tested. Demographic characteristics are shown in Table 7.1.

Table 7.1. Demographics of patients and controls

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

MOG-antibodies were present in 19 of the 117 children with ADS (16%) and in none of the healthy or OND control children (Fisher's exact test, *p*=0.002). Seventeen anti-MOG seropositive patients had a polyfocal disease-onset. Two children had isolated ON as the initial event, whereas all other 47 patients with a monofocal onset were seronegative.

Figure 7.1 shows the presence of MOG-antibodies in the 5 separate clinical subgroups. The group of children with a polyfocal disease-onset plus encephalopathy had the highest frequency of anti-MOG seropositivity (42%). Also 16% of children presenting with polyfocal ADS without encephalopathy had MOG-antibodies. Most of them fulfilled the International Pediatric MS Study Group (IPMSSG) criteria for ADEM, although they presented without encephalopathy, and did not qualify as suffering from MS.[22] The clinical presentation and disease course of individual MOG-antibody positive children are outlined in Table 7.2.

The characteristics of the MOG-antibody positive and negative pediatric patient groups are shown in Table 7.3. The MOG-antibody positive patients were 4.3 years younger on average than the MOG-antibody negative patients (*p*<0.001).

In the ADS cohort, 47 children had a final diagnosis of MS (mean follow-up time of 4.7 years) and they were all seronegative. In contrast, of the 70 patients without MS diagnosis (mean follow-up time of 5 years) 27% was seropositive (Pearson Chi-Square, *p*<0.001). Twelve of these 70 children developed a relapsing disease without fulfilling diagnosis of MS. In eight of these 12 children MOG-antibodies could be detected (Table 7.2). Figure

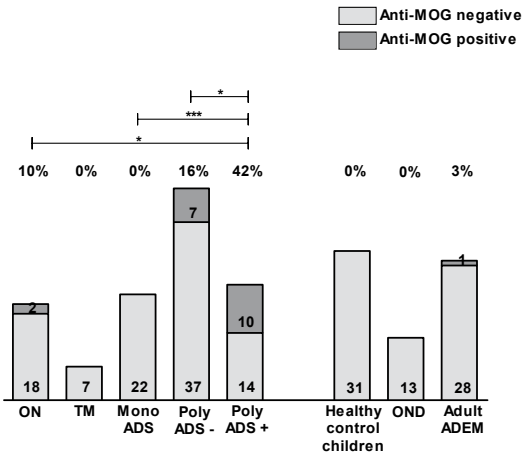

Figure 7.1. MOG-antibody presence in the ADS patients divided by clinical presentation, healthy pediatric controls, OND and adult ADEM patients
* $p<0.05$ *** $p\leq0.001$
ON= optic neuritis, TM= transverse myelitis, Mono ADS= monofocal ADS, Poly ADS - = polyfocal ADS without encephalopathy, Poly ADS + = polyfocal ADS with encephalopathy, ADS= acquired demyelinating syndrome, ADEM= acute disseminated encephalomyelitis, OND= other neurological disorders.

7.2 shows the disease course of the 8 seropositive children with clinical or radiological relapsing disease.

Clinical recovery of the MOG-antibody positive patients following the primary event was satisfactory in all except one patient (Table 7.2). The patients with incomplete recovery had only mild residual symptoms, like fatigue, attention or behavioral deficits or mild persisting visual loss.

In only one adult patient with ADEM we were able to detect MOG-antibodies (3% versus 42% of the children with polyADS +; Pearson Chi-Square, $p<0.001$). This man was 37 years old at disease-onset. The sample was obtained within 1 month after onset and he had a monophasic disease.

In 11 children follow-up samples were tested, 9 patients were anti-MOG seronegative at onset and remained negative (including 6 children with MS, one with mono ADS, one with ON, one with poly ADS +). A second sample was obtained in 2 anti-MOG seropositive children (patients 4 and 7 in Table 7.2). In both patients MOG-antibodies remained detectable, respectively 3 months and 7 months after last relapse.

Table 7.2. Clinical presentation and disease course of MOG-antibody positive children

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

Table 7.3. Characteristics of MOG-antibody positive and negative pediatric patients

	MOG + patients (*n*=19)	MOG – patients (*n*=98)	*p*-value
Female, *n* (%)	9 (47)	52 (53)	0.5
Mean age, years (range)	7.1 (1.2 – 17.1)	11.4 (0.5 – 17.5)	<0.001
Mean time disease-onset – sampling, years (range)	2.5 (7 d – 11.7 y)	0.9 (0 d – 13.5 y)	0.1
Sampling < 3 months, *n* (%)	13 (72)	60 (61)	0.3
Mean follow-up time, years (range)	5.4 (2 m – 15.4 y)	4.6 (1 m – 19.4 y)	0.5

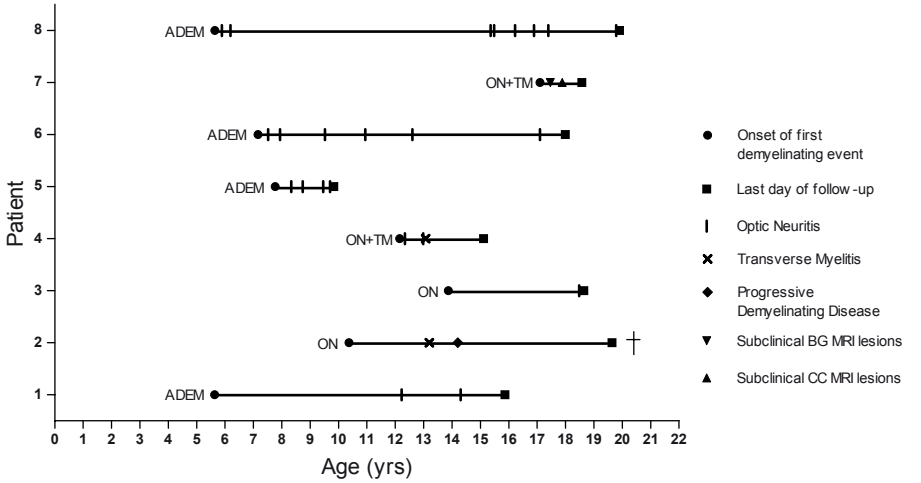

Figure 7.2. Disease courses of the 8 MOG-antibody positive children with a clinical relapsing disease or radiological disease activity
ADEM= acute disseminated encephalomyelitis, ON= optic neuritis, TM= transverse myelitis, BG= basal ganglia, CC= corpus callosum, MRI= magnetic resonance imaging.

DISCUSSION

It is now widely accepted that antibodies to MOG are specific for demyelinating CNS diseases in children.[10-14 16] However, this biomarker appears to lack sensitivity for the whole group of patients. In this study we investigated MOG seropositivity amongst the spectrum of distinct clinical presentations, rather than the association between the antibodies and a subsequent diagnosis of MS or ADEM. We here observed that antibodies were almost exclusively detected in children with a polyfocal disease-onset. MOG-antibodies were especially present in children with a polyfocal disease-onset plus encephalopathy (42% positivity in this group fulfilling ADEM according to the IPMSSG definitions[22]) when compared to all other patient groups. Also a significant part of the children with a polyfocal onset but without encephalopathy tested positive (16%). This group fulfilled the IPMSSG criteria for ADEM, except for the lack of encephalopathy at onset.

Of the total set of 19 anti-MOG seropositive patients, only 4 did not have a typical ADEM presentation. One patient had recurrent ON. This is in line with a previous study showing that MOG-antibodies in pediatric patients with ON are predominantly detected in children with recurrent ON contrary to monophasic ON and ON as part of a clinically isolated syndrome.[17] Three girls had a NMO or NMO spectrum disorder, but without detectable AQP4-antibodies, confirming previous studies showing that a subgroup of AQP4-antibody negative NMO patients do have MOG-antibodies.[18-20]

An interesting observation is that the four children in this cohort with a clear ADEM onset followed by multiple episodes of ON only, all tested anti-MOG seropositive. As this phenomenon has already been described by Huppke et al, we suggest that this may represent a newly identified disease entity.[25]

None of the children with MS in our cohort were anti-MOG seropositive, which is in contrast to former studies.[9-12 14 16 18 20] It has been discussed that if these antibodies are present in MS patients, the titers are lower in comparison to ADEM patients.[11 13 14] We have chosen a rather stringent cut-off in our study. Another explanation may be a different application of the diagnostic criteria for pediatric MS. In the current study children with 2 non-ADEM episodes were diagnosed with MS, whereas in another study only a second non-ADEM attack or clinically silent new lesions on MRI were enough for MS diagnosis.[14] According to the recently published revised IPMSSG criteria a diagnosis of MS can be made after one non-encephalopatic clinical event that is associated with new MRI lesions that fulfill 2012 revised McDonald criteria. Applying these criteria, the diagnoses of MS did not change.[26]

It is still unclear whether MOG-antibodies have demyelinating activity or whether they represent an epiphenomenon of myelin destruction. Some studies showed that these antibodies may remain detectable during the disease course[10 12 17], whereas two longitudinal studies showed that MOG-antibodies in some ADEM patients disappear over time.[11 14] We did not obtain sequential samples routinely. But in 2 children with a NMO-like disease the antibodies remained detectable. Furthermore in 4 of the 19 MOG positive patients, the sample was obtained during remission instead of during the active stage of the disease. Despite the small numbers in our study, this may counteract the observation that antibodies are only present as a kick-off of the disease or reflect the presence of a chronic active disease.[8 14] As MOG-antibodies were virtually absent in adult ADEM patients, it is unlikely that these antibodies merely reflect fulminant white matter damage.

This study is the first to describe the presence of MOG-antibodies in an unbiased cohort encompassing all clinical ADS subtypes in children. ADS is a heterogeneous group of clinical phenotypes and diagnosis can be inaccurate, partly because substantial clinical overlap between the subgroups can exist. Here we zoomed in on the clinical features at disease-onset of MOG-antibody positive patients. Most of these seropositive patients had an ADEM-like disease and none developed MS. In our study the presence of MOG-antibodies in children with a first attack of CNS demyelination strongly pleads against future diagnosis of MS. We expect that the future value of testing MOG-antibodies in a clinical setting will depend on international collaboration on assay standardization and on consensus about the proper cut-off values. Such studies are underway.

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CHAPTER 8

General discussion

Acquired inflammatory demyelinating diseases of the central nervous system (CNS) represent a spectrum of disorders of which multiple sclerosis (MS) is the most common. This thesis focuses on the more uncommon demyelinating variants, called acquired demyelinating syndromes (ADS) in children, which include acute disseminated encephalomyelitis (ADEM) and neuromyelitis optica (NMO). It is important for clinicians to be aware of these uncommon syndromes because they represent the key differential diagnoses of MS in children and adults. The main aim of this thesis is to extend insight into and improve diagnosis of these syndromes by describing their clinical features.

In this chapter the most important findings are pooled and discussed in relation to other studies and in relation to adult MS. The challenges and steps to take for future research are discussed.

Main findings

The incidence of ADS in the Netherlands is 0.66/ 100,000 children/ year.

Most children with ADS have a polyfocal disease-onset.

The incidence of ADS is higher in Dutch children of non-European ancestry.

Full motor recovery after ADEM is better when the disease occurs at a younger age.

19% of children with ADEM have a relapsing demyelinating disease, but only 6% of children with ADEM receive a diagnosis of MS during follow-up. This indicates that not every patient with a relapsing disease after ADEM should be diagnosed as MS.

MRI is a necessary diagnostic tool in ADS patients, and Callen MS-ADEM MRI criteria can reliably distinguish ADEM from MS at a first event.

Fatigue occurs more often in children with MS than in children who suffered from a monophasic ADS.

AQP4-antibodies are present in 74% of patients with relapsing NMO, but are absent in monophasic NMO patients.

Antibodies against MOG can identify a subset of young children with ADS (< 10 years) with an ADEM-like phenotype, and without a subsequent diagnosis of MS. MOG antibodies are rarely detected in adults with ADEM.

DEMOGRAPHIC FEATURES OF ADS IN CHILDREN

ADS is very rare in children. Before we initiated this research project in the Netherlands, incidence numbers were not available. Four years later we could report an incidence of 0.66/100,000 per year (**chapter 2**). This is comparable to the reported incidence in Canada (0.9/100,000) and more recently in the British Isles (0.98/100,000).[1 2] Of course these numbers reflect the minimum incidence in a country, because children with ADS may be missed. In all surveillance studies, reporting rates were high. Report forms were received from 85% of all Dutch pediatricians, which agrees with the 80% reporting rate in Canada and 94% in the British Isles.[1 2]

The differences between adult and pediatric MS have been summarized in **chapter 1** (Introduction, Table 1.5). Children seem to have a more aggressive inflammatory disease, reflected by a higher relapse rate and a greater lesion load on magnetic resonance imaging (MRI), compared to adults.[3] In our cohort, 75% of patients with a relapsing disease had their second relapse within 2 years after onset. Half of the ADS children had a polyfocal disease-onset (**chapter 2**). This is in contrast to adults, who tend to present more often with a monofocal disease-onset.[4 5] Children seem to recover better than adults after a demyelinating event.[3] We were able to confirm this in our ADEM cohort, because adults with ADEM were more likely to have a severe disease course and less favorable recovery (**chapter 3**).

MS in adults is considered to typically occur in women and Caucasian individuals of Northern European descent.[6 7] In our pediatric cohort a slight female preponderance was found across all ADS groups, except in the children with optic neuritis. However, the female:male ratio was about equal when we compared ADS patients younger than 10 years old to ADS patients older than 10 years. Of the 20 children who already developed MS, the ratio was exactly 1:1. These children were all older than 10 years at the time of their first attack (**chapter 2**). Our findings on gender distributions agree with the findings in the Canadian cohort[1], but not with those described in other studies who found an increased female:male ratio in children older than 10 years.[2 8-11] It is hypothesized that this gender effect and also the higher incidence of MS in postpuberty occurs because of factors related to puberty, like hormonal (especially estrogen) changes. Another hypothesis is that there could also be some gender-specific genetic influence on environmental risk factors and immunological reactivity.[8 10 12]

Our study demonstrates that children of non-European descent are especially vulnerable for developing MS compared to children of European descent (**chapter 2**).[8 9 13] This increased susceptibility has also been observed in pediatric MS studies from other countries, but has not been reported in adults of similar ancestry. One reason for this difference between pediatric and adult onset MS may be just the reflection of the demographic change in the Western world, instead of a relevant difference between adult- and pediatric-onset MS. During the sixties and seventies of the previous century, people of non-Caucasian ancestry from countries with low MS prevalence settled in countries with a high prevalence of MS. Their children may miss potential protective factors, putting them at risk of developing MS at a younger age.[8 13 9] Possibly in the near future we will observe a similar shift in ethnic background in the adult MS population, as has recently been demonstrated in the United States.[14 15] There are several theories about the higher incidence of MS among people of certain ethnicity. Most cited is the idea that people with a darker skin tone living in temperate climates are more likely to be vitamin D deficient and as a consequence have a higher risk of MS. Interestingly, a higher MS incidence in the Hispanic population has not been observed.[9 14 15] Furthermore, in a prospective study it was shown that a higher risk of MS is associated with lower vitamin D levels in Caucasians, but not in blacks and Hispanics, who

actually had lower 25-OH vitamin D levels.[16] Based on these findings it is suggested that genetic variations or gene-environment interactions could contribute to an increased MS risk in certain populations.

DISTINGUISHING PEDIATRIC ADS SUBTYPES: A DIAGNOSTIC DILEMMA

As a result of growing awareness, ADS in children is now increasingly being identified. But the challenge remains to assign the appropriate and reliable diagnosis as early as possible in the disease course. The currently available consensus criteria are a 'best guess' until better differentiating factors are identified. The 2007 International Pediatric MS Study Group (IPMSSG) criteria were available when performing the studies described in this thesis. The criteria were recently modified as cited in **chapter 1** (Table 1.1).[17 18] According to the 2007 IPMSSG criteria a diagnosis is predominantly based on clinical features, thus leaving out MRI criteria.[17] In most cases the clinical presentations of optic neuritis (ON), transverse myelitis (TM) or NMO hardly yield any diagnostic problems. Differentiating the clinical presentations of ADEM and a first episode of MS may cause difficulty, as both can have a similar polyfocal clinical presentation. The main distinguishing feature is proposed to be encephalopathy.[17] As discussed in **chapter 1**, this feature cannot reliably distinguish the diagnosis in all children. A diagnosis of encephalopathy remains difficult and subjective. The symptoms can be transient, or simply relate to a young child who is ill.[19] Therefore we proposed to divide all children with ADS in the following subgroups based on clinical presentation (**chapter 2**):

- optic neuritis
- transverse myelitis
- neuromyelitis optica
- monofocal onset
- polyfocal onset with encephalopathy
- polyfocal onset without encephalopathy.

In that way we avoid the term ADEM, which in our opinion cannot be properly defined based on clinical presentation alone and hence can be confusing. As a consequence, the group of children with a polyfocal onset without encephalopathy includes children originally diagnosed with a first onset of MS or ADEM.

This classification is not only suitable for research, but also for clinical diagnosis. Because all these subtypes of ADS can be a first episode of MS, they are all treated the same in the acute setting (i.e. with methylprednisolone). All these children deserve long-term follow-up even if they underwent complete recovery and did not experience further attacks. This clinical management advice is supported by the results described in **chapter 3**. ADEM is generally considered a monophasic disease with a benign disease course[20], but we found that only 58% of children had full motor recovery at follow-up. In addition 33% of children

reported cognitive impairment and/or behavioral change after suffering from ADEM. Our results have been validated by a recent study, including children at least 2 years after ADEM onset. This report also showed that a small subset of patients may have behavioral and internalizing problems (24%) and cognitive impairment (16%) whereas subtle deficits were more frequently observed.[21] The combined results indicate that ADEM has a less 'benign' disease course as was previously assumed.[22]

We showed that even after long follow-up, it can be challenging to classify a patient as suffering from one of the conventional ADS disease entities. Although it is important to diagnose MS as soon as possible, one has to be very careful to diagnose MS after a first episode of ADEM. It was generally assumed that up to 30% of all children with a first event typical of ADEM would have a final diagnosis of MS (as is shown in Table 8.1).[10] According to the 2007 IPMSSG criteria a first episode of ADEM cannot count as the first episode of MS. This assumption, although rather prudent, is important as an incorrect MS diagnosis in a relapsing ADEM patient should be avoided. This means that after a first episode of ADEM, two new non-ADEM attacks (or a non-ADEM attack followed by clinically silent lesion accrual) are necessary for the diagnosis of MS. As we showed in **chapter 3** some children go on to suffer from relapses after a first presentation of ADEM, but they cannot be diagnosed as having MS. Nineteen percent of ADEM patients experience one or more relapses, but only 6% of patients subsequently meet the criteria for the diagnosis of MS. The (repeated) absence of oligoclonal bands in the cerebrospinal fluid or the absence of typical MS lesions on cranial MRI with resolution of lesions and no new T2-lesions on follow-up MRI scans, made a diagnosis of MS unlikely in the other 13% of patients. The percentage of patients with a definite final diagnosis of MS after ADEM is thereby much lower than previously assumed. After critical review of previous published literature and applying the 2007 IPMSSG criteria, we found similar findings as in our study (presented in Table 8.1). More recently, published prospective cohorts employing the 2007 IPMSSG criteria, also showed that only 0-8% of ADEM patients are finally diagnosed with MS.[23 24 9]

Table 8.1. Relapsing ADEM patients described in studies before 2007

	Patients with at least one relapse after ADEM (%)	Patients with at least two relapses after ADEM (%)
Dale et al. 2000 [26]	20	6
Hynson et al. 2001 [27]	13	6
Tenembaum et al. 2002 [28]	10	0
Mikaeloff et al. 2004 [29]	29	14
Mikaeloff et al. 2007 [30]	18	8

The 2007 IPMSSG criteria advise that MS diagnosis can only be made after 2 relapses when the first event fulfils ADEM criteria[17]

The modified 2013 IPMSSG criteria eliminated the requirement that only the second non-ADEM relapse can be MS defining.[18] They now state that one non-ADEM attack (thus without encephalopathy), at least three months after the first attack, with new MRI lesions fulfilling the revised radiological McDonald criteria for dissemination in space (DIS; Table 8.2)[25], is enough to diagnose MS. Whether these adapted criteria will cause false-positive MS diagnoses, especially in younger children, needs to be studied in prospective cohorts.[18]

It is still debated how to best classify the children with relapsing demyelination that do not meet the diagnostic criteria for MS. Future research must reveal whether these are part of the anti-aquaporin 4 (AQP4) associated disorders or anti-myelin oligodendrocyte glycoprotein (MOG) associated disorders, as will be discussed later in this chapter.

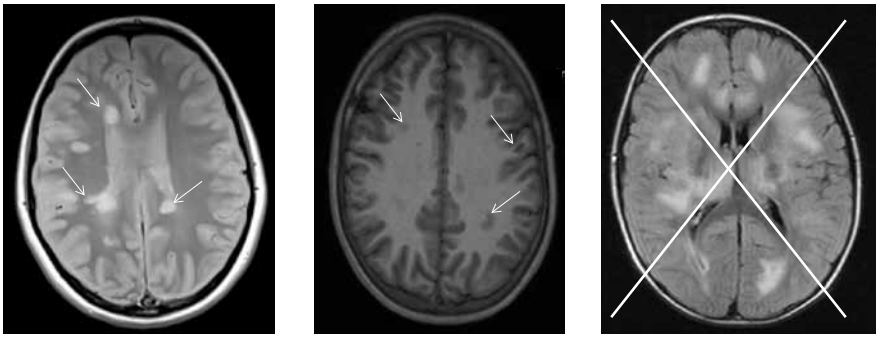
Table 8.2. 2010 McDonald criteria for MS (derived from Polman et al. Ann Neurol. 2011)[25]

Clinical presentation	Additional data needed for MS diagnosis
≥ 2 attacks; objective clinical evidence of ≥ 2 lesions or 1 lesion with reasonable historical evidence of a prior attack	None
≥ 2 attacks; objective clinical evidence of 1 lesion	*Dissemination in space (DIS)*, demonstrated by: - ≥ 1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS* Or - A further clinical attack implicating a different CNS site
1 attack; objective clinical evidence of ≥ 2 lesions	*Dissemination in time (DIT)*, demonstrated by: - Simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing lesions at any time Or - A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan Or - A second clinical attack
1 attack; objective clinical evidence of 1 lesion	*DIS*, demonstrated by - ≥ 1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS* Or - A second clinical attack implicating a different CNS site And *DIT*, demonstrated by - Simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing lesions at any time Or - A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan Or - A second clinical attack

* MS typical regions of the CNS are: periventricular, juxtacortical, infratentorial and spinal cord. Symptomatic lesions are excluded from consideration in subjects with brainstem or spinal cord syndromes. Gadolinium-enhancing lesions are not required.

MRI AS AN IMPORTANT DIAGNOSTIC TOOL IN PEDIATRIC ADS

In our view, ADEM and MS cannot be diagnosed based on clinical presentation alone. The identification of MRI features that are specific for pediatric MS helped in establishing a more reliable diagnosis of MS in children.[31-33] Of course, the MRI criteria can only be used when there is no better explanation for the clinical presentation than pediatric ADS by exclusion of other diseases.[34] As ADEM is the most challenging differential diagnosis of MS, it is important to recognize the MRI features of these diseases. In **chapter 4** we showed that the Callen MS-ADEM MRI criteria are most discriminative for the distinction between ADEM and a first episode of MS (Figure 8.1).[33] Recently new, more simplified, MRI criteria became available. The presence of at least one T1-weighted hypointense lesion, and at least one T2-weighted periventricular lesion was predictive for MS diagnosis (with a sensitivity of 84% and specificity of 93%) in a group of children with ADS.[35] The applicability of these MRI criteria is confirmed in the Dutch pediatric ADS prospective cohort, revealing a sensitivity of 93.3% and a specificity of 86.7%.[36]



≥ 2 periventricular lesions (on T2-weighted or FLAIR images)

Presence of black holes (hypointense to gray matter on T1-weighted images with a signal intensity similar to CSF)

Absence of diffuse bilateral lesion pattern (on T2-weighted or FLAIR images)

Figure 8.1. The Callen MS-ADEM MRI criteria (2 out of 3)

The role of MRI is also emphasized by the 2010 modifications of the adult MS McDonald criteria. These criteria have been simplified and the use of imaging became more important. In some cases, MS diagnosis (fulfilling dissemination in space and time) can be made by a single MRI scan.[25] The usefulness of these criteria in children with MS has already been established.[37-40] However, the authors advise against the use of the dissemination in space and time criteria on the initial MRI in children, especially in the younger children (< 12 years old) and children with an ADEM-like onset. In these children the predictive value is lower because children with monophasic ADEM may demonstrate multiple variably enhancing

lesions (although most often larger and more confluent), typically located in the juxtacortical white matter, infratentorial space and spinal cord.[25 37 40] We recommend using the 2010 McDonald criteria only in children older than 12 years with first attack symptoms typical of MS.

FUNCTIONAL OUTCOME OF PEDIATRIC MS

Fatigue is one of the most reported, and often most disabling, symptoms of MS. A pilot study (**chapter 5**) indicated that children with MS were more fatigued than healthy controls and other children with monophasic ADS. They also experienced a worse health-related quality of life in the domains of locomotor functioning, cognition and interaction with peers. Though two of the ten MS children showed signs of a depressive disorder, there was no difference between the groups overall. Despite the small sample size, the strong points of our study were the inclusion of healthy peers as well as monophasic pediatric patients as control groups and the use of a validated questionnaire for children in the Netherlands.

In general, the role of fatigue and depression in pediatric MS is not clear and only studied in cohorts tested for cognitive dysfunction or in studies that lacked an age and demographic matched control group.[41-45] However we conclude that, in addition to the well-known cognitive impairment, fatigue and depression also occur in children with MS. Fatigue and depression influence quality of life, as it affects personal development, school performance and interaction with peers. Therefore the impact of these features in such a critical period of development can even be more severe than in adults with MS. It is thus essential to evaluate and monitor these features, and for example start early treatment or offer support at school.[46]

Studies on fatigue and depression in pediatric MS are hampered by standardized tests. In adult MS studies the Modified Fatigue Impact Scale or the Fatigue Severity Scale have been used[47], but validation and established cut-off values for use in children are lacking. A standardized assessment is further impeded by the broad age range of the study population. Studies are also biased by the fact that children who feel depressed or fatigued are less likely to participate in (self-report) questionnaire studies. Furthermore multiple factors, including family or social environment and developmental stage, play a role in the perception of fatigue and mood disturbances.[48] The IPMSSG recently proposed a standardized test battery of 45 minutes which can be used to evaluate cognitive dysfunction. They also recommend evaluating fatigue, depression and quality of life in children with MS, but to date cannot advise which tests are useful for these purposes.[3]

Future studies need to be longitudinal and correlated with parameters like disability, time since disease-onset, relapses, disease-modifying therapy and MRI parameters like normal appearing white matter disruption, brain volume and grey matter pathology.[49-51] Interventions, for example a multidisciplinary approach coordinated by a rehabilitation specialist, need to be evaluated.

GENETIC AND ENVIRONMENTAL RISK FACTORS IN PEDIATRIC MS

It is possible that children with MS have a heightened genetic load or an altered immune response, because they already develop a disease which is very rare in a young population when exposed to nearly the same environmental factors as their peers.[52]

The increased susceptibility of adults with a HLA-DRB 1*15 genotype to develop MS also pertains to pediatric-onset MS.[53] This effect is mainly attributable to the children of European ancestry.[24 54] About half of the children with MS have at least one HLA-DRB1*15 allele.[24 53 54] In our population we found that 74% of patients with at least one HLA-DRB1*15 allele developed a recurrent disease, in contrast to 39% of patients without HLA-DRB1*15 (p=0.01; unpublished results). Multiple GWAS (genome-wide association studies) have identified non-HLA genes as candidates for risk of MS in adults. The effects of these common genetic variations are much weaker though.[55]

Other studies confirmed the consistent finding in adult MS that of all the common viruses a child is exposed to, only a significant association between a remote Epstein-Barr virus (EBV) infection and pediatric MS is found.[56 57] The difference in EBV seropositivity between children with MS compared to healthy peers is even more striking in the pediatric cohorts, given the high community seroprevalence in adults. [24 56 58 59] The role for EBV in the disease pathogenesis is uncertain, as it may be trigger, cause or consequence. Theories include the latent infection and transformation of B cells, 'molecular mimicry' (T cells specific for EBV peptide sequences are cross-reactive against a peptide of myelin basic protein) or an altered immune response (for example an activation and expansion of autoreactive T and B cells during primary EBV infection).[52 60 61]

The fact that not all children with MS are EBV seropositive emphasizes on one hand the difficulty and uncertainty of MS diagnosis in this population (thus longer follow-up is necessary to reevaluate the correct diagnosis), and on the other hand that an EBV infection may not be mandatory to develop MS. Whether other infectious agents have a role in the development of MS is not yet clear. For instance, Chlamydia pneumoniae IgM antibodies were more often present in children with MS, but the prevalence was only 29% (compared to 2% in controls).[62] Interestingly, some viruses may have a protective role. One study showed that a previous infection with cytomegalovirus (CMV) decreased the risk of developing pediatric MS.[57] Herpes simplex virus (HSV)-1 infection decreased the risk of MS in children who carry a HLA-DRB1*1501/1503 allele. In those who are HLA-DRB1 negative an increased MS risk was observed.[57] A possible protective role of some past infections suggests that timing of infections during childhood and a complex interplay is relevant in MS development. Furthermore, the data described above suggest a gene-environment interaction. There may also be an interaction between EBV and HLA-DRB1. Pediatric MS patients and controls positive for seroconversion against EBV had the same levels of antibody to EBNA-1, but the titers were higher in HLA-DRB1 positive individuals. The authors of this study conclude that the humoral response to EBV might be influenced by genotype.[63]

The protective role of CMV in risk of MS in adults has not yet been proven.[64] Several infectious agents have been reported to be associated with MS, but were later disproven, except for the possible role of human herpesvirus (HHV-6).[65 66]

In adults as well as in children, higher serum vitamin D levels are associated with lower risk of a subsequent MS diagnosis and lower relapse rate.[24 16 67 68] Vitamin D is involved in the modulation of innate and adaptive immune responses and has anti-inflammatory properties.[69] In adult MS the vitamin D receptor gene plays a role in MS risk, and the vitamin D response element region of HLA-DRB*1501 was associated with MS.[70] But these factors have not been studied in pediatric MS yet.

Associations between vitamin D status and antibody levels to common viruses, including EBV, CMV and HSV-2, were found in children with MS. Serum vitamin D sufficiency was associated with higher CMV antibody levels in MS and CIS patients, but lower levels in controls.[67]

In the prospective cohort we have commenced to address environmental factors involved in the cause and development of MS. In a group of 61 Dutch children with ADS, 21 had serological evidence of a remote EBV infection (antibodies against EBV nuclear antigen). This was more frequent in the MS patients (78% of MS patients versus 16% of other ADS patients, $p<0.0001$). Of 92 ADS children, more children with MS had 25-OH vitamin D levels below the average of 63 nmol/l in healthy Dutch children (69% of MS patients versus 41% of other ADS patients, $p=0.02$; unpublished results).

NMO AND AN EXPANDING SPECTRUM OF AQP4-ANTIBODY ASSOCIATED DISORDERS

Historically, Devic's disease or 'classical' NMO was considered a monophasic variant of MS, characterized by ON and TM, with a very severe disease course. Relapsing variants of Devic's disease were called MS. The discovery of disease-specific autoantibodies directed against AQP4, led to enhanced understanding of the disease. NMO is now considered distinct from MS and several AQP4-antibody associated disorders have been discovered, which are considered part of the NMO spectrum. These include relapsing or bilateral ON and (relapsing) longitudinally extensive transverse myelitis (LETM), initially described in adults by Wingerchuk et al. (**chapter 1**).[71] But this spectrum is expanding and other patients who are seropositive for AQP4-antibodies are now included. This includes patients with more limited phenotypes such as short-segment TM, monophasic unilateral ON with severe visual impairment, brainstem symptoms like intractable hiccups or nausea and vomiting with evidence of periaquaductal medullary lesion on MRI.[25 71-74] These AQP4-antibody associated disorders must be considered in the continuum of NMO. Whether the presence of AQP4-antibodies alone is sufficient to diagnose a NMO spectrum disorder is still under debate.[75 76]

Since the discovery of the AQP4-antibody, it is generally accepted that most NMO patients have a relapsing disease course. This is confirmed in our adult NMO population. However, this observation may be biased, because most patients are referred to our center after a second attack or when AQP4-antibodies were detected. An important finding in our study was that AQP4-antibodies were not present in patients with a monophasic disease (**chapter 6**). Although other studies confirmed that AQP4-antibody positive patients had significantly more often a relapsing disease, they could not make such a strict distinction as we did. This may likely be due to differences in follow-up time between the studies.[77-80] Also children with AQP4-antibodies are more likely to have a relapsing disease.[81]

The AQP4-antibody positive patients are more often female, as is described in our and other studies. Other features include a non-Caucasian ethnicity, more severe clinical attacks, higher spinal cord lesion load, greater incidence of a gadolinium-enhancing spinal cord lesion, more often brainstem lesions, and decreased incidence of simultaneous ON and TM at first episode or bilateral ON. Recovery of visual acuity and motor symptoms is worse at follow-up.[78 80 82 83] Interestingly, these disease features of AQP4-antibody positive NMO are in line with the features described in relapsing NMO patients before the antibody was detected.[84] The AQP4-antibody presence predicts relapse and conversion to NMO also in patients with spectrum disorders.[85-87] Future research must also focus on other clinical characteristics of relapsing and monophasic NMO. For example MRI characteristics of both disease entities must be identified.[88]

We hypothesized that monophasic NMO, in the absence of AQP4-antibodies, is a variant distinct from recurrent NMO. One theory is that monophasic NMO is more often post-infectious.[89] It is then likely that this disease is more similar to (monophasic) ADEM. Another theory is that AQP4-antibody positive NMO could express a more widespread or intense autoimmune response, as there is a frequent association with coexisting autoimmunity. These patients more often have other (non-organ specific) autoantibodies or other autoimmune diseases, like systemic lupus erythematosus, Sjögren syndrome or myasthenia gravis, compared to patients with AQP4-antibody negative NMO.[90] Thus the AQP4-antibody positive NMO patients could either be more prone to autoimmune diseases, or the presence of the AQP4-antibodies in this group of patients may just be an indication of CNS involvement of a systemic autoimmune disease.[91] Another possibility is that the current assays are not sensitive enough to detect antibodies in the monophasic patients. This problem may be circumvented in future studies by the increasing improvement of the sensitivity of the assays.[92 80] Of course, it may also be possible that there is another, undiscovered autoantigen in these AQP4-antibody negative patients.

Pediatric NMO is mostly similar to adult NMO, although brain abnormalities on MRI are quite frequent and these are often symptomatic.[93] The number of pediatric NMO patients in the Netherlands was yet too small to include in the study on AQP4-antibodies described in this thesis, but the prevalence of AQP4-antibodies in this group is comparable to the adult

NMO cohort. In a group of 18 children (three with monophasic NMO, one with recurrent NMO and 14 with NMO spectrum disorders including bilateral ON, relapsing ON and LETM), four children had AQP4-antibodies: one patient presented with monophasic NMO, one with recurrent NMO, and two children with LETM.

MOG-ANTIBODY ASSOCIATED DISORDERS

MOG is one of the most serious candidate autoantigens in demyelinating diseases, given its location on the outer surface of the myelin sheath and because numerous animal studies have shown that an immune reaction against MOG induces demyelination.[94] An interesting observation is that antibodies directed against MOG are especially present in children with demyelinating diseases and are very rare in adult patients.[95-100] In our pediatric ADS population, these MOG-antibodies could in particular be detected in children with a polyfocal disease-onset, especially with encephalopathy at onset, at a young age and who are unlikely to develop MS (**chapter 7**). These clinical features indicate a more ADEM-like presentation. However, controversies about how to classify the group of MOG-antibody positive patients remain. The MOG-antibody is highly specific for identifying CNS demyelinating diseases, but sensitivities differ depending on the patients tested (up to 50% in pediatric ADEM cases). A general finding is that the frequency of these antibodies is much higher in the youngest patients compared to adult patients.[101] Other results from previous studies show discrepancies and cannot be compared, especially because different assay techniques were used and thus different MOG epitopes may have been recognized.[101] It is now clear that only cell-based assays, recognizing MOG in its native form, are useful to identify relevant patient groups. The problem of the cell-based assays is the complexity of the cell surface. As different cell lines and different protocols are used, it may be possible that other co-expressed antigens are recognized giving rise to false-positive results.[94 101]

It is also unclear why these antibodies are barely detectable in adults in contrast to children with demyelinating diseases. Several hypotheses have been suggested. For example it may be due to distinct pathogenesis of ADEM versus MS, as ADEM is more likely a post-infectious (or post-vaccination) disease and molecular mimicry may play a role.[20 94] However, to our knowledge not all children had a post-infectious disease and in other cohorts, these antibodies could also be detected in a few children with MS.[95-97 99 100] Furthermore because the antibody response is IgG instead of IgM, a mature antibody response is more likely than an acute reaction to cell damage.[96 99 100] EBV is a likely causative agent in demyelinating disease, thus a cross-reaction between EBV and myelin proteins has been suggested. However this hypothesis is refuted by findings in a study demonstrating that there is no relation between MOG-antibodies and the antibody response to EBV.[102] It is also hypothesized that these antibodies are present at initiation of the disease.[94 103] This may be more likely because the time between initiation of MS is much closer to the disease

presentation in children as it is in adults. Accordingly, these antibodies should disappear during disease course, but this is contradicted by longitudinal studies showing that these antibodies persist, with fluctuations, in small groups of children with MS.[97 100] A final theory is that the antibodies are not per se pathogenic but represent an epiphenomenon subsequent to cell damage.[96] As the lesion load visible on MRI is larger in ADEM than in MS, this may reflect more extensive demyelination. But also this hypothesis is challenged by the fact that antibodies can be present in children without white matter lesions on MRI. Therefore, the role of MOG-antibodies in the pathophysiology of ADS remains elusive.

VARIANTS OF MS OR DISTINCT DISEASES?

MS is generally considered to be an autoimmune disease.[104 105] The definition of an autoimmune disease includes the immune response to self-antigens. In MS at least some patients respond to therapies like plasma exchange, indicating that antibody-dependent mechanisms play a role in disease pathogenesis in these patients. However the causative antigen-specific immune responses remain to be elucidated.

MS, in adults as well as in children, is a very heterogeneous disease in clinical presentation, disease course and disability accumulation. Different levels of inflammation, demyelination and axonal loss have been described. It is likely that MS is an entity consisting of distinct diseases.[4]

Identification of autoantigens in demyelinating diseases could be helpful to serve as a diagnostic biomarker for diagnosis, prognosis and treatment. Two antigens have already been detected in small subgroups of patients: AQP4 in patients with NMO and related variants and MOG in a subgroup of children with ADEM-like disease. Evidence exists that autoantibodies against AQP4 are pathogenic.[106] Although there are strong suggestions for pathogenicity of MOG-antibodies as well, this has not been proven with transfer experiments yet.[94] The discovery of these antibodies showed that NMO and ADEM, which were previously considered variants of MS, might in fact be distinct disorders. However, the discovery of the antibodies also raised new questions: what about the patients with AQP4-antibody negative recurrent ON, recurrent TM and LETM, who do neither fulfill criteria for NMO spectrum diseases, but nor for MS? Do they still need to be considered as MS variants? Or are they different diseases?

For example we identified a group of patients in our ADEM cohort who experience recurrent ON after ADEM, but could not be diagnosed as MS (**chapter 3**). Similarly, such a cohort has recently been described by others.[107] These patients are likely to have MOG-antibodies. Four out of eight children with ADEM followed by recurrent ON in our cohort were evaluated for MOG-antibodies and turned out to be seropositive as well. Furthermore, other studies showed that MOG-antibodies could be detected in subgroups of patients with NMO or NMO spectrum disorder phenotype (like recurrent ON or TM) who were seronegative

for AQP4-antibodies.[108-111] Again the MOG-antibodies were especially present in pediatric patients. This illustrates the clinical overlap between the different disorders (represented in Figure 8.2), but also the diagnostic uncertainties. It is generally accepted that the clinical presentation of ADEM in children is diverse and they can present with (recurrent or severe bilateral) ON, or (extensive) TM.[22] Thus the patients with AQP4-antibody negative NMO and MOG-antibodies in serum can as well have a diagnosis of ADEM.[101] In part, this diagnostic inaccuracy can be solved by grouping the patients based on clinical characteristics, thus phenotype instead of diagnosis, as is mentioned before. Shared clinical characteristics in the antibody positive patients may help identify the appropriate groups.

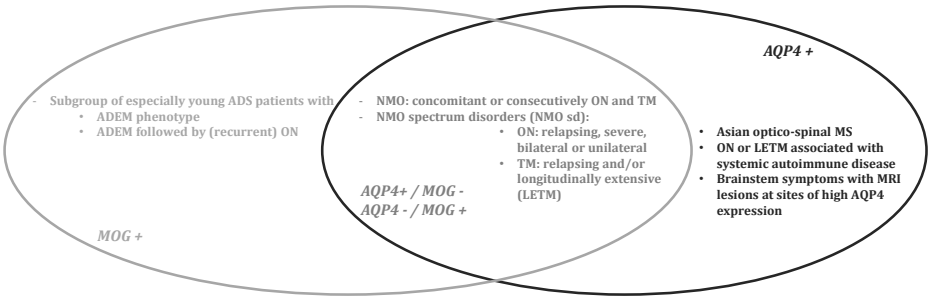


Figure 8.2. The confluent spectrum of AQP4- and MOG-antibody associated demyelinating diseases

It is still a puzzle how to characterize the patients in whom autoantibodies are detectable in the whole spectrum of acquired demyelinating syndromes. With emerging treatment opportunities it is currently unknown what the best treatment is for these patients, in case it is agreed that these patients do not have MS. It has been suggested that antibody-depleting or immune-suppressive therapies are most useful. At least in NMO it has been shown that these therapies are effective, unlike disease-modifying MS treatments which may have no or adverse effects.[106] However, contrary to AQP4-antibodies, MOG-antibodies do not inevitably predict a relapsing disease. Thus long-term treatment is not necessarily required in all anti-MOG positive patients.

The identification of new disease-specific diagnostic and prognostic markers that can further distinguish different acquired demyelinating disease variants depends on the development of better assays and a more reliable characterization of patients.

FUTURE RESEARCH

Research on prognostic factors starts with identifying patients at risk. This is challenged by the diagnostic delay in many children with ADS due to the rarity of ADS. Thus not all children can be included in the studies at first event. The group of pediatric ADS patients is heterogeneous

Future directions

Long-term follow-up of the current Dutch ADS cohort will determine the incidence of MS in Dutch children, and identify the clinical factors associated with the risk of MS diagnosis after a first demyelinating event.

Characteristics of ADS and prognostic factors for pediatric MS should be studied and compared in children of pre- and postpubertal age.

Large international collaborative studies are needed to identify novel genetic and environmental risk factors for disease-onset and progression in MS. These may be best identified in children, as the clinical presentation of MS in children is much closer to the time of the suspected exposure, and the interval to neurological progression is longer, compared to adults with MS.

As children seem to recover better after a demyelinating attack than adults, it has to be determined what causes this difference.

Future studies, especially evaluating treatment effect, should include evaluation of cognitive disability, fatigue and depression. Useful standardized tests for these purposes need to be developed.

The differences between monophasic and relapsing NMO need to be further elucidated: patients with monophasic NMO should be screened for novel autoantibodies.

The role of MOG-antibodies in cause or effect of demyelinating diseases needs to be determined.

Large collaborative studies must focus on detecting novel autoantibodies for the different ADS groups.

Therapies with proven efficacy and favorable side effect profiles in adults are currently being planned for evaluation in clinical trials that include children with MS, to obtain pharmacokinetic, safety and efficacy data in children. Furthermore, a single long-term drug safety registry must be used in all countries for all children with MS.

in clinical presentation, outcome and disease course, which challenges appropriate classification. To date, there are no definitive tests to differentiate variants properly. Pathological confirmation can reliably solve this diagnostic inaccuracy, but as the disease manifests in quite inaccessible parts of the body, pathological studies are not feasible.

Current research mainly focuses on the recognition of pediatric ADS and MS and description of the clinical features of the diseases. An important goal was to increase awareness of the disorders and subsequently this may lead to inclusion of more children at a first event. In well-defined cohorts it was already possible to replicate what was already known about adult MS.

Now that we realize that pediatric MS exists and we roughly know in what way pediatric MS is similar or different from adult MS, it is time to try to find new clues in the puzzle of this disease. Future studies must focus on finding novel risk factors, which can be identified in the pediatric MS population for the first time. It has to be determined to which extent the pathophysiology is similar or distinct in adults and children and whether there are

differences in inflammation and axonal injury. As children seem to have better recovery after an attack, it is important to elucidate the mechanisms by which this recovery is achieved, for example remyelination or better brain plasticity including reorganization of functional pathways. The longer time to neurological progression provides a longer time to study the risk factors that may be associated with the second, probably neurodegenerative stage of the disease. So studying similarities and differences between adult- and pediatric-onset MS, may reveal clues of the biological background of MS in general. It is likely that this early onset of the disease in children is caused by different mechanisms or interactions between numerous risk factors, the immune system and the CNS tissue.[52 112]

There are many different environmental variables that may be associated with MS, making it difficult to find the specific cause or trigger for MS. Many children are exposed to them whilst never developing MS. Prospective studies of a large variety of features in children with ADS and MS, and comparison with healthy peers, may identify new disease risk factors in the future. This can only be done in nationwide and international collaborations: large numbers of patients are needed to achieve sufficient statistical power to study these variables.

Preliminary genetic studies in children with MS show that the genetic vulnerability is comparable with adults. In the future large international collaborations may provide the opportunity to perform exome or full-genome sequencing in order to discover novel risk genes for pediatric MS or ADS.

In addition to comparing adults and children with demyelinating diseases, there is one other important issue to address. Multiple studies already show that disease characteristics differ in younger and older children. Thus in studies focusing on demographic, clinical, MRI and immunological aspects of MS in children, the cohort should be divided into children of pre- and postpubertal age. Again, this can only be done in study populations including a sufficient number of children.

Future research will also focus on disease-modifying therapies. To date, children are treated with the same first line medication as adults, which are extensively studied and considered safe, but are moderately effective in adults. The right dose and long-term safety in children are not yet established. Case series in children show the same efficacy and safety profile as in adults (**Chapter 1**, Table 1.4). Side effects and associated complications may be different for children because of developmental differences between children and adults. Especially the more intense second line treatments can only be given after careful consideration of the side effects. New medications are currently tested in trials and some are soon to be approved in adult MS. These drugs need to be tested in children as well, also because this is mandated by regulatory authorities in the US and Europe. Trials in children are challenged by the rarity of the disease, a lack of previous experience and data including inexperience with relevant outcome measures and ethical considerations. The IPMSSG aims for a common design for international clinical trials, and guidelines for outcome measures are currently established.[3]

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CHAPTER 9

Summary / Samenvatting
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Dankwoord

SUMMARY

Multiple sclerosis (MS) is an acquired inflammatory demyelinating disease of the central nervous system (CNS) and typically occurs in young adults. Increasingly, the occurrence of the disease is being recognized in children. Other, less well-known, acquired inflammatory demyelinating diseases in adults and children exist, like acute disseminated encephalomyelitis (ADEM) and neuromyelitis optica (NMO). In children all the subgroups of these disorders together are termed acquired demyelinating syndromes (ADS). Knowledge of these uncommon variants is important, because they represent the differential diagnoses of MS. Furthermore, understanding the way in which these diseases are similar or distinct from (adult) MS can enhance understanding of MS in general. This thesis describes the clinical phenotype of different acquired demyelinating syndromes, with special emphasis on ADS and MS in children, and presents disease-specific characteristics that serve to improve its diagnostic criteria.

Chapter 1 summarizes the current knowledge about the different ADS subtypes in children, including epidemiology, prognostic and risk factors of MS in children, prognosis and treatment. Next to this, NMO and aquaporin-4 (AQP4) antibody associated disorders in children and adults are described.

One of the goals of this research project was to define the incidence of ADS in the Netherlands. In **chapter 2** the first clinical data of the PROUD*kids* study (PRedicting the OUtcome of a Demyelinating event in children) are summarized. Eighty-six children with a diagnosis of ADS were included between January 2007 and December 2010. We found an annual incidence of 0.66/100,000. A polyfocal disease-onset without encephalopathy was most common (in 30% of patients), followed by a polyfocal onset with encephalopathy (24%), optic neuritis (ON, 22%), a monofocal onset (16%), transverse myelitis (TM, 3%), and NMO (3%). The patients with a monofocal disease-onset were older than both groups of patients with a polyfocal onset. There was a slight female preponderance in all groups except in the patients with ON, with no differences in female-male distribution between the groups. Almost one third of patients had a non-European ancestry. Almost a quarter of patients reported familial autoimmune diseases (most often maternally transmitted), especially patients with a relapsing disease. To date, 23% of ADS patients already received a subsequent MS diagnosis.

ADEM is the most common acquired demyelinating syndrome in children, and is considered to be very rare in adults. There are only few studies that describe the clinical characteristics of ADEM in adults, and it is difficult to compare previous studies because of the different definitions used. In **chapter 3** we described the clinical presentation, outcome and disease course of ADEM, and compared these features between 92 children and 25 adults. Clinical presentation was similar in both groups, with pyramidal signs and encephalopathy as the most common symptoms. Ataxia was more frequent in children. Magnetic

resonance imaging (MRI) typically showed ill-defined and large white matter lesions, but in adults periventricular lesions were more common. Adults seem to have a more severe disease course, with longer duration of hospitalization, and more frequent intensive care unit admission. Although ADEM is considered a disease with generally a good recovery, the outcome was also worse in adult patients. Death due to the disease is uncommon, however more frequent in adults (*n*=3). Only 15% of adults had complete motor recovery, compared to 58% of children. About 20% of all patients had a relapsing disease, but only 6% had a final diagnosis of MS, which is lower than previously assumed. Interestingly in seven patients the initial ADEM event was followed by one or several episodes of optic neuritis. This may represent a distinct disease entity.

It is difficult to distinguish the different ADS subtypes based on clinical presentation alone. MRI can be a useful tool to diagnose ADS and distinguish these subtypes at onset. Several sets of MRI criteria for children with MS or other ADS became available: Barkhof criteria, KIDMUS criteria, Callen MS-ADEM criteria, and Callen diagnostic MS criteria. In **chapter 4** we investigated which of these sets are the most useful for distinguishing MS from ADEM, which is the most challenging differential diagnosis of MS at onset in children. Children who had an MRI scan recorded within 2 months of their initial clinical attack were included. Twenty-one ADEM patients who had remained relapse-free for at least 2 years were compared with 28 patients with a definitive clinical diagnosis of MS. We concluded that the Callen MS-ADEM criteria are the most useful for distinguishing a first attack of MS from monophasic ADEM, with 75% sensitivity and 95% specificity. These criteria include at least two of the following characteristics: the presence of at least two periventricular lesions, the presence of black holes and/or the absence of a diffuse bilateral lesion pattern.

On the subject of disease sequelae of MS in children, to date, most studies have focused on cognitive impairment. Fatigue and depression are known to be significant symptoms of MS in adults, and can have a major impact on quality of life. We studied these features in a group of children with MS (*n*=10), and compared them to patients with monophasic ADS (*n*=22) and healthy children (**chapter 5**). Four MS patients suffered from severe fatigue, in contrast to only one child with a monophasic disease. The MS children as a group had higher scores on the subscales 'subjective fatigue' and 'physical activity', compared to both other groups. Two MS patients were likely to have a depressive disorder. Fatigue and depression were correlated in the MS group. The MS patients experienced a lower health-related quality of life, as was shown on three subscales of the quality of life test ('locomotor functioning', 'cognitive functioning' and 'interaction with peers'). Our preliminary results indicate that fatigue and depression occur in children with MS as well, with impact on health-related quality of life. As these are 'hidden' disabilities, the treating physician must be aware of these features and intervene when necessary.

The prevalence of antibodies directed against AQP4 was investigated in a group of 273 patients with inflammatory demyelinating diseases of the CNS and the results are presented

in **chapter 6**. Patients with NMO could be distinguished from the other patients with 99% certainty. The assay had a sensitivity of 56% to detect all NMO patients and of 74% to detect the patients with relapsing NMO. An interesting finding was the absence of antibodies in the monophasic NMO patients. In some patients with a relapsing disease longitudinal samples were tested revealing that the antibodies remained present during follow-up. We hypothesize that monophasic NMO is a different disease than relapsing NMO.

In the search of disease-specific biomarkers, antibodies directed against myelin oligodendrocyte glycoprotein (MOG) are most promising. These antibodies could be detected in 16% of all children with ADS, in none of the healthy control children or children with other neurological diseases, and in only one adult ADEM patient (**chapter 7**). To date, it is unclear how to classify the subgroup of patients in whom these antibodies can be detected. We showed that they are especially present in young children with an ADEM-like phenotype, including children with a polyfocal disease-onset with encephalopathy (in 42% of patients in this subgroup), or a subgroup of patients who presented with a polyfocal clinical onset but without encephalopathy. Furthermore the antibodies were detectable in subgroups of patients with AQP4-antibody negative NMO or NMO spectrum diseases. We also described a newly recognized subgroup comprised of patients with ADEM at onset, followed by recurrent ON. MOG-antibodies were absent in children with MS diagnosis.

In **chapter 8** we discussed our main findings, as well as recommendations for future research.

SAMENVATTING

Multiple sclerose (MS) is een verworven inflammatoire demyeliniserende aandoening van het centrale zenuwstelsel (CZS) en presenteert zich vooral op jongvolwassen leeftijd. Echter MS kan ook al bij kinderen voorkomen en door toegenomen belangstelling hiervoor wordt deze ziekte bij kinderen dan ook vaker herkend. Er bestaan ook andere, mindere bekende, verworven inflammatoire demyeliniserende ziekten die bij volwassenen en kinderen kunnen voorkomen. Voorbeelden zijn acute disseminated encephalomyelitis (ADEM) en neuromyelitis optica (NMO). Bij kinderen worden al deze subgroepen van aandoeningen samen ook wel aangeduid als 'acquired demyelinating syndromes' (ADS). Het is belangrijk om deze zeldzame ziekten te kennen en herkennen, omdat zij vaak de differentiële diagnose van MS vormen. Ook is het belangrijk om te begrijpen hoe deze varianten vergelijkbaar of verschillend zijn van MS (bij volwassenen), omdat dit het begrip van de ziekte MS in het algemeen kan verbeteren. In dit proefschrift wordt het klinisch beeld van deze verschillende verworven demyeliniserende syndromen beschreven, waarbij de nadruk is gelegd op ADS en MS bij kinderen. Er wordt ingegaan op ziekte-specifieke kenmerken die het stellen van de verschillende diagnoses kunnen verbeteren.

In **hoofdstuk 1** wordt samengevat wat er al bekend is over ADS op de kinderleeftijd, zoals de huidige kennis van de epidemiologie, prognostische en risicofactoren voor het krijgen van MS, de prognose en behandeling. Verder wordt er ingegaan op een andere variant, namelijk NMO en aquaporine-4 (AQP4) antistof geassocieerde aandoeningen bij kinderen en volwassenen.

Een van de doelen van dit onderzoek was het bepalen van de incidentie van ADS in Nederland. De eerste klinische data verkregen uit de PROUD*kids* studie (PRedicting the OUtcome of a Demyelinating event in children) zijn samengevat in **hoofdstuk 2.** Van januari 2007 tot december 2010 werden 86 kinderen met een diagnose van ADS geïncludeerd in het onderzoek. Hieruit volgt een incidentie van 0,66/100.000 kinderen per jaar in Nederland. De meeste kinderen hadden een polyfocaal begin van hun ziekte zonder encefalopathie (30%), gevolgd door een polyfocale ziektepresentatie met encefalopathie (24%), neuritis optica (NO, 22%), een monofocale presentatie (16%), myelitis transversa (MT, 3%), en neuromyelitis optica (3%). De patiënten die een monofocaal begin van hun ziekte hadden, waren ouder dan beide groepen patiënten met een polyfocaal begin van de ziekte. In bijna alle subgroepen (behalve in de groep patiënten met NO) kwamen iets meer meisjes dan jongens voor, echter er was geen verschil in de verdeling van meisjes en jongens tussen de groepen. Bijna een derde van de patiënten had een niet-Europese afkomst. Bij bijna een kwart van de patiënten kwamen in de familie auto-immuunziekten voor, meestal via de moeder overgedragen, en dan vooral in de groep patiënten met een recidiverende ziekte. Tot op heden kreeg 23% van alle patiënten met ADS de diagnose MS.

ADEM is zeldzaam, maar op de kinderleeftijd de meest frequent voorkomende subgroep van ADS. Daarom wordt ADEM ook vaak beschouwd als een ziekte van de kinderleeftijd. Maar het kan, zij het nog veel minder vaak, ook op volwassen leeftijd voorkomen. Slechts een beperkt aantal studies beschrijven de klinische karakteristieken van ADEM bij volwassenen. Het is moeilijk om deze onderzoeken met elkaar en met onderzoeken naar ADEM bij kinderen te vergelijken, vanwege de verschillende definities die werden gebruikt. We hebben de klinische presentatie, het herstel en ziektebeloop van ADEM beschreven in **hoofdstuk 3** en deze kenmerken vergeleken tussen 92 kinderen en 25 volwassenen. De klinische presentatie was min of meer hetzelfde tussen beide groepen, met piramidebaanverschijnselen en encefalopathie als meest voorkomende symptomen. Ataxie was frequenter bij kinderen. Op MRI werden vooral matig begrensde en grote witte stof afwijkingen gezien in beide groepen, maar bij volwassenen kwamen vaker periventriculaire laesies voor. Het ziektebeloop lijkt ernstiger te zijn bij volwassenen, aangezien de duur van het verblijf in het ziekenhuis langer was, en ze vaker op de intensive care unit moesten worden opgenomen. In het algemeen wordt gedacht dat het herstel na ADEM goed is, maar bij volwassen patiënten was dit slechter dan bij kinderen. Overlijden als gevolg van de ziekte komt maar zelden voor, echter vaker bij volwassen patiënten (n=3). Slechts 15% van de volwassenen hadden een volledig motorisch herstel, in tegenstelling tot 58% van de kinderen. Ongeveer 20% van alle patiënten hadden een recidiverende ziekte, maar slecht 6% kreeg een uiteindelijke diagnose van MS. Dit is een lager percentage dan in het verleden werd aangenomen. Verder was er een opvallende groep van zeven patiënten die na de initiële episode van ADEM een of meer recidieven kregen van NO. Mogelijk moeten deze patiënten als een aparte groep worden beschouwd.

Het is lastig om de verschillende ADS subgroepen van elkaar te onderscheiden op basis van alleen de klinische symptomen. MRI zou dus een nuttig hulpmiddel kunnen zijn om de diagnose ADS te stellen en de verschillende subtypen te onderscheiden bij de eerste presentatie van de ziekte. Tot op heden zijn er diverse sets MRI criteria voor kinderen met MS of andere ADS beschikbaar: Barkhof criteria, KIDMUS criteria, Callen MS-ADEM criteria, en Callen diagnostic MS criteria. In **hoofdstuk 4** hebben we onderzocht welke van deze sets het best bruikbaar is om het onderscheid tussen MS en ADEM te maken bij het debuut van de ziekte, aangezien ADEM de meest lastige differentiële diagnose van MS is bij het debuut op de kinderleeftijd. Kinderen waarvan de MRI scan was verricht binnen 2 maanden na de eerste klinische episode werden geïncludeerd. We vergeleken 21 kinderen met ADEM die in ieder geval 2 jaar geen nieuwe aanval meer hadden gehad, met 28 kinderen met MS als definitieve klinische diagnose. De Callen MS-ADEM criteria bleken het meest geschikt om het onderscheid te maken tussen een eerste aanval van MS en een monofasische ADEM (sensitiviteit 75% en specificiteit 95%). Hiervoor moet aan minimaal 2 van de volgende karakteristieken worden voldaan: de aanwezigheid van minimaal twee periventriculaire

laesies, de aanwezigheid van black holes, en/of de afwezigheid van een diffuse bilaterale verdeling van de laesies.

Als het gaat om de gevolgen van MS bij kinderen, hebben tot op heden de meeste studies zich gericht op cognitieve achteruitgang. Vermoeidheid en depressie zijn echter ook bekende en belangrijke symptomen van MS op de volwassen leeftijd, en deze ziekteverschijnselen kunnen een grote impact op de kwaliteit van leven hebben. We onderzochten of vermoeidheid en depressie ook bij kinderen voorkomen in een kleine groep kinderen met MS (*n*=10), monofasische ADEM (*n*=22) en gezonde kinderen (**hoofdstuk 5**). Vier MS patiënten leden aan ernstige vermoeidheid, in tegenstelling tot slechts één kind met een monofasische ziekte. De kinderen met MS als groep hadden hogere scores op de subschalen 'subjectieve vermoeidheid' en 'fysieke activiteit', in vergelijking met beide andere groepen. Twee kinderen zouden kunnen worden gediagnosticeerd met een depressieve aandoening. Vermoeidheid en depressie waren gecorreleerd in de groep met MS patiënten. De MS patienten ervoeren een slechtere gezondheid-gerelateerde kwaliteit van leven, wat kon worden aangetoond op drie subschalen van de kwaliteit van leven schaal (functioneren van het bewegingsapparaat, cognitief functioneren en interactie met leeftijdsgenoten). Deze eerste resultaten tonen dat vermoeidheid en depressie ook bij kinderen met MS voorkomen, met impact op de gezondheid-gerelateerde kwaliteit van leven. Aangezien het hier om 'verborgen' beperkingen gaat, moet de behandelend arts zelf zich bewust zijn van het voorkomen van deze ziektekenmerken en ingrijpen indien nodig.

De prevalentie van antistoffen gericht tegen AQP4 werd onderzocht in een groep van 273 patiënten met inflammatoire demyeliniserende aandoeningen van het CZS (**hoofdstuk 6**). Patiënten met NMO konden met 99% zekerheid worden onderscheiden van de andere patiënten met demyeliniserende aandoeningen. De assay had een sensitiviteit van 56% in de gehele groep van NMO patiënten, en van 74% in alleen de groep patiënten met een recidiverende NMO. Een interessante bevinding was de afwezigheid van antistoffen in de groep monofasische NMO patiënten. Van sommige patiënten met een recidiverende ziekte konden longitudinaal verkregen samples worden onderzocht, en deze toonden aan dat de antistoffen aanwezig bleven gedurende het beloop. Onze hypothese is dat monofasische NMO een andere ziekte is dan recidiverende NMO.

Antistoffen gericht tegen myelin oligodendrocyte glycoprotein (MOG) zijn een interessante kandidaat om te gebruiken als ziekte-specifieke biomarker voor ADS. Deze antistoffen waren aanwezig bij 16% van alle kinderen met ADS, bij geen van de gezonde kinderen of kinderen met andere neurologische ziekten, en bij slechts één volwassen patiënt met ADEM (**hoofdstuk 7**). Tot op heden is nog onbekend hoe de subgroep van patiënten bij wie deze antistoffen aanwezig zijn geclassificeerd moeten worden. In onze studie bleken de antistoffen vooral aantoonbaar bij jonge kinderen met een ADEM-achtig fenotype, zoals kinderen met een polyfocale presentatie met encefalopathie (bij 42% van de patiënten in deze groep), of een subgroep van patiënten met een polyfocale presentatie zonder encefalopathie. Verder

zijn de antistoffen aanwezig bij patiënten met AQP4-antistof negatieve NMO of NMO spectrum ziekten. Een interessante groep met MOG-antistoffen bevat de patiënten met ADEM bij debuut, gevolgd door recidiverende NO. De MOG-antistoffen waren niet aantoonbaar in de groep kinderen met MS.

In **hoofdstuk 8** worden de belangrijkste bevindingen bediscussieerd, en aanbevelingen voor toekomstig onderzoek gedaan.

ABBREVIATIONS

ADEM	Acute disseminated encephalomyelitis
ADS	Acquired demyelinating syndrome
AQP4	Aquaporin-4
CBA	Cell-based assay
CDI	Child depression inventory
CIS	Clinically isolated syndrome
CIS	Checklist individual strength
CNS	Central nervous system
CSF	Cerebrospinal fluid
DIS	Dissemination in space
DIT	Dissemination in time
EAE	Experimental autoimmune encephalomyelitis
EBNA	EBV nuclear antigen
EBV	Epstein-Barr virus
EDSS	Expanded disability severity scale
FACS	Fluorescence-activated cell sorting
FLAIR	Fluid-attenuated inversion recovery
GWAS	Genome-wide association study
HLA	Human leukocyte antigen
HRQoL	Health-related quality of life
ICU	Intensive care unit
IgG or IgM	Immunoglobulin G or M
IPMSSG	International Pediatric MS Study Group
LETM	Longitudinally extensive transverse myelitis
MFI	Mean fluorescence intensity
MOG	Myelin oligodendrocyte glycoprotein
Mono ADS	Monofocal ADS
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NMO	Neuromyelitis optica
NMOsd(s)	NMO spectrum disorder(s)
NSCK	Nederlands Signalerings Centrum Kindergeneeskunde (Dutch pediatric surveillance unit)
OCB	Oligoclonal bands
ON	Optic neuritis
OND	Other neurological diseases
Poly ADS +	Polyfocal ADS with encephalopathy

Poly ADS -	Polyfocal ADS without encephalopathy
SD	Standard deviation
SNP	Single nucleotide polymorphism
TACQOL	TNO-AZL child quality of life
TM	Transverse myelitis

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ABOUT THE AUTHOR

Immy Ketelslegers was born on May 19th 1981 in Leunen and raised in Blerick. She graduated in 1998 from the Collegium Marianum in Venlo. She proceeded to study Health Sciences at the University of Maastricht. In 1999 she commenced Medicine at the Erasmus University Rotterdam. Before starting her internships she performed one year of research at the clinical neurophysiology section (supervisor: Dr. G.H. Visser). After obtaining her medical degree in April 2006 she worked as a medical doctor at the department of Neurology at the Albert Schweitzer Hospital in Dordrecht (supervisor: Dr. H. Kerkhoff). One year later she started her PhD research at the MS Center ErasMS under the supervision of Prof. R.Q. Hintzen. The results of this research are described in this thesis. She coordinated the Dutch multicenter study PROUD*kids* (PRedicting the OUtcome of a Demyelinating event in children). From 2011 onward she works as a resident in neurology at the Erasmus University Medical Center in Rotterdam (head: Prof. P.A.E. Sillevis Smitt).

PhD PORTFOLIO

1. PHD TRAINING

	Workload (ECTS)
Courses	
- Statistics:	
Introduction to Data-Analysis; NIHES (2007)	0.7
Classical Methods for Data-analysis; NIHES (2007)	5.7
The basic introduction course on SPSS; MolMed (2010)	0.6
Introductory Course on Statistics & Survival Analysis; MolMed (2010)	0.4
Genetic Analysis in Clinical Research; NIHES (2009)	1.9
- SNP's and human diseases; MolMed (2009)	2.0
- English Biomedical Writing and Communication (2009)	4.0
- English Advanced, Centre for British English, Rotterdam (2009)	0.7
Seminars and workshops	
- Basic immunology, Dept. of Immunology, Rotterdam (2008)	0.5
- Teaching courses ECTRIMS (2008 t/m 2013)	0.6
Oral presentations	
- Surveillance of acquired demyelinating syndromes of the CNS in children; NSCK research meeting, TNO Leiden (2008, 2009)	1.0
- ADS in children; NvKN voorjaarsvergadering (2008)	0.4
- AQP4-antibodies in NMO and spectrum disorders; NVN wetenschappelijke vergadering (2008)	0.4
- MS in children; ErasMS, Rotterdam (2008, 2012)	1.0
- PROUD*kids* study; Benefietconcert and Kinder MS dag (2009)	0.3
- PROUD*kids* study; International pediatric MS Meeting (2009)	0.8
- NMO and spectrum disorders; International pediatric MS Meeting (2009)	0.8
- AQP4-antibodies in NMO and spectrum disorders; ECTRIMS (2009)	1.0
- Future perspectives of children with ADEM and MS; Dutch MS symposium (2010)	0.6
- Prognosis after ADS; Interdisciplinary MS Expert Workshop (2011)	1.0
- MOG-antibodies in children with ADS; ECTRIMS (2011)	1.0
Poster presentations	
- ECTRIMS (2008, 2009, 2x 2010, 2011)	
- EPNS (2009)	
- Meeting of the Dutch MS Research foundation (2009, 2010)	
(Inter)national conferences	
- Congress of the European Committees for Treatment and Research in Multiple Sclerosis (ECTRIMS); Montréal (2008), Düsseldorf (2009), Göteborg (2010), Amsterdam (2011), Lyon (2012), Kopenhagen (2013)	6.0
- Meeting of the Dutch MS Research foundation; Hasselt (2007), Groningen (2009), Alphen a/d Rijn (2010)	1.0
- International pediatric MS meeting; München (2008), Rotterdam (2009)	1.0
- Conference of the 'Nederlandse Vereniging voor Kinderneurologie (NvKN); Nijmegen (2008), Rotterdam (2009)	0.5

- Conference of the 'Nederlandse Vereniging voor Neurologie (NVN); Garderen (2008)	0.2
- Congress of the European Paediatric Neurology Society (EPNS); Harrogate (2009)	1.0
- Dutch MS symposium; Utrecht (2009, 2010)	1.0
- Conference Course Dutch Society of Neuroradiology; Groningen (2010)	0.5
- Interdisciplinary MS Expert Workshop; Göttingen (2011)	0.5
Other meetings attended	
- Several investigator meetings (Fingolimod, Atacicept, Ocrelizumab)	1.0
- Weekly lectures, Neurology department	1.0
2. TEACHING	
Lecturing	
- Workshop 'MS in children'; Dutch MS symposium (2009)	1.0
- Lecture 'Acquired demyelinating syndromes'; Research Master programme Infection & Immunity (MolMed), Rotterdam (2011)	1.0
Supervising Master thesis	
- I.E.R. Visser	6.0
3. OTHER	
Travel Grants	
- ECTRIMS Montréal (2008), Düsseldorf (2009), Göteborg (2010)	
Other publications	
- Editing Booklet 'Naar school met Multiple Sclerose; een handboek voor leerkrachten'. Stichting MS Research, ErasMS.	
- Chapters (1, 3 and 7) in Book 'Multiple Sclerose Lexicon Deel 5: Multiple Sclerose en kinderneurologie'. Academic Pharmaceutical Productions bv, Utrecht. ISBN 978 90 5761 102 5.	
- Diagnostic and treatment guideline for children with acquired demyelinating syndromes. www.nvkn.nl.	
Other activities	
- Organizing "Kinder MS dagen"; Efteling (2010), Dolfinarium (2011).	0.5
- Co-investigator in clinical MS trials (Fingolimod, Natalizumab, Atacicept)	2.0
Total ECTS	49.6

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A book is completed only when it is finished by the reader.

(Colum McCann, Let the great world spin)