Diagnostic value of oligoclonal bands in children: A nationwide population-based cohort study

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PII: S0887-8994(19)30017-7
DOI: https://doi.org/10.1016/j.pediatrneurol.2019.03.002
Reference: PNU 9542

To appear in: Pediatric Neurology

Received Date: 8 January 2019
Revised Date: 4 March 2019
Accepted Date: 6 March 2019


This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
TITLE PAGE

Title: Diagnostic value of oligoclonal bands in children: A nationwide population-based cohort study

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Word counts:
Abstract: 247
Main document: 3,489

Running title: Oligoclonal bands in children

Keywords: oligoclonal bands, children, multiple sclerosis, ADEM, population-based

Disclosure of conflicts of interest:
Dr. Boesen has served on a scientific advisory board for Teva; has received speaker honoraria for lecturing from Novartis and support for congress participation from Teva, Novartis and Roche.
Dr. Jensen, Dr. Rosenberg, Dr. Thomassen, Dr. Børresen, Dr. Jørgensen and Dr. Lydolph report no disclosures.

Dr. Born has received speaker honoraria from Novartis and has served on an advisory board for Biogen.

Dr. Blinkenberg has served on scientific advisory boards for Genzyme, Roche, Biogen, Merck, Novartis and Teva; has received speaker honoraria from Genzyme, Biogen, Merck, Novartis, Teva and Roche; has received consulting honoraria from the Danish Multiple Sclerosis Society, Biogen, Teva, Roche and Merck; and has received funding for travel from Genzyme, Roche and Biogen.

Dr. Sellebjerg has served on scientific advisory boards, been on the steering committees of clinical trials, served as a consultant, received support for congress participation, received speaker honoraria or received research support for his laboratory from Biogen, EMD Serono, Genzyme, Lundbeck, Merck Serono, Novartis and Teva
ABSTRACT

Objective: To evaluate the diagnostic value of cerebrospinal fluid oligoclonal bands (OCBs) in children (<18 years).

Methods: In a nationwide population-based setting, we retrieved data on 2,055 children’s OCB examination including concordant cerebrospinal fluid biomarkers during 1994–2017. Case ascertainment was by review of medical records and diagnostic codes. We used Fisher’s exact test to explore distribution differences of OCB positivity in acquired demyelinating syndromes (ADS) before and after 12 years of age and calculated sensitivity, specificity, positive predictive value, and negative predictive value of OCBs to distinguish ADS from the other diagnostic groups.

Results: Median age at OCB examination was 15.2 years (range=1.8–18.0), and 10% had presence of cerebrospinal fluid OCB. OCB positivity was the highest in ADS (52%), but it was age-dependent: 21% in children with ADS before age 12 years and 68% in children 12–17 years (p<0.0001) due to the higher incidence of multiple sclerosis in the latter. Cerebrospinal fluid OCBs were not predictive of ADS before age 12 years compared with the other diagnostic groups. However, cerebrospinal fluid OCBs in children aged 12–17 years were highly predictive of ADS compared with CNS infections and non-ADS immune-mediated CNS diseases (positive predictive value: 0.89; 95% confidence interval=0.82–0.94; p<0.0001), but negative OCBs were not discriminatory (negative predictive value: p=0.17).

Conclusions: In a clinical setting, cerebrospinal fluid OCB examination may be of higher yield in children aged 12–17 years if there is clinical suspicion of multiple sclerosis, and in such circumstances a positive test supports a diagnosis of multiple sclerosis.
INTRODUCTION

Oligoclonal bands (OCBs) are electrophoretic patterns of immunoglobulins produced by plasma cells, and presence of OCBs is a marker of chronic central nervous system (CNS) inflammation.[1,2] Pediatricians often face the dilemma of whether to examine OCBs in cerebrospinal fluid (CSF). “Positive” OCBs are particularly associated with relapsing acquired demyelinating syndromes (ADS) and are present in up to 81% of children with multiple sclerosis (MS).[3–9] However, MS is rare in children, especially in children younger than 12 years of age.[10] In contrast, the most common ADS in children is acute disseminated encephalomyelitis (ADEM), a largely monophasic disease, but only 0–10% of children with ADEM present with positive OCBs.[1,3,6,11–15] In addition, ADEM can be reliably distinguished from MS based on the clinical presentation (age at onset younger than 12 years, presence of encephalopathy, and polyfocal neurological deficits) as well as magnetic resonance imaging findings including diffuse bilateral T2 white matter lesions and lack of ‘typical MS lesions’ [3,5,15]. Furthermore, only one in four children with CNS infection or immune-mediated CNS diseases presents with positive OCBs.[1] Thus, the diagnostic value of OCBs in children may be limited, particularly in children younger than 12 years of age.

In a nationwide population-based setting, we aimed to evaluate the diagnostic value of OCBs in children and whether the diagnostic value was age dependent. Our hypothesis was that the ADS phenotype differs in children before and after age 12 years; accordingly, the predictive value of OCB positivity differs between children aged 0–11 years and children aged 12–17 years.
METHODS

Source and study population

The source population was all children (<18 years) in Denmark during 1994–2017. The mid-year population of Denmark in the year 2010 was 5,547,683 persons (22% younger than 18 years).[16] We included all children with an OCB analysis before 18 years of age (study population).

Data sources

The Danish Civil Registration System

The Danish Civil Registration System was established in 1968 as a register of residents in Denmark.[17,18] In Denmark, every resident at birth or on immigration receives a unique personal identification number which is used to link all nationwide registers.[19] We used the children’s personal identification number to link biomarker analyses with hospital diagnoses in the National Patient Register.

The National Patient Register

The National Patient Register is a nationwide register with routinely collected administrative and health-related data on all hospital admissions in Denmark since 1977 [20]. Data include date of admission and diagnoses according to the International Classification of Diseases (ICD) version 8 (1977–1993) and ICD-10 (1994 until today). On hospital discharge, physicians code each patient by diagnosis with one primary diagnosis and, if relevant, one or more secondary diagnoses. In Denmark, hospital admissions and outpatient visits are tax funded and free of charge. Patients are coded at each hospital visit, giving patients with chronic disease multiple registrations.[21] Private consultant physicians (e.g., general practitioners, neurologists, and ophthalmologists) and private
hospitals in Denmark play a minor role in the diagnostic procedure and refer children with suspected demyelinating disease to public hospitals.

The National Patient Register has been validated in several studies.[17,21–31] Recently, Boesen et al [32] found that the agreement between a diagnostic code in the National Patient Register and a correct medical record-validated diagnosis was excellent for pediatric multiple sclerosis and acceptable for pediatric optic neuritis and transverse myelitis but unacceptable for acute disseminated encephalomyelitis.

**Diagnostic groups**

**Register-based diagnoses**

We grouped children into diagnostic groups based on hospital diagnoses in the National Patient Register from two months before and one year after the date of OCB examination; however, children with MS anytime during follow-up would be classified as MS because the initial ADS code (e.g. optic neuritis) would then represent onset of MS.[32] Both somatic and psychiatric diagnoses from outpatient hospital visits and admissions were included. ICD-10 codes in each diagnostic group were defined *pre hoc*.

We grouped children into ‘CNS diseases’ and ‘non-CNS diseases’. Further, we divided CNS diseases into the following groups: ADS (e.g. ADEM, MS), non-ADS immune-mediated CNS diseases (e.g. Rasmussen's encephalitis, anti-N-methyl-D-aspartate (NMDA) receptor encephalitis, neurosarcoidosis, opsoclonus myoclonus syndrome, pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections, or vasculitis), CNS infection (e.g. bacterial or viral meningoencephalitis), epilepsy, sleep disorders (e.g. narcolepsy), CNS malignancy, movement disorders (e.g. chorea, dystonia), static encephalopathy (e.g. cerebral palsy), progressive
encephalopathy/mitochondrial disease (e.g. Leigh syndrome), cerebrovascular disease (e.g. stroke), or other CNS diseases (e.g. hydrocephalus, asphyxia). ‘Non-CNS diseases’ were heterogenous and included diagnoses such as pneumonia, febrile seizures, and leukemia.

Medical record-validated ADS during 2008–15

The first author, MS Boesen, and a senior consultant, AP Born, reviewed the medical records in a selected cohort of 321 children with either a non-MS ADS code in the National Patient Register during 2008–15 (optic neuritis [H46], transverse myelitis [G37.3], neuromyelitis optica spectrum disorder [G36.0], and other acquired demyelination including ADEM [G04.0, G04.8, G04.9, G36.8, G36.9, G37.8, G37.9]) or an OCB analysis at Copenhagen University Hospital, Rigshospitalet during 2010–16. We did not include diagnostic ICD-10 codes for MS (G35) in the chart review because the positive predictive value of MS (G35) in the National Patient Register is excellent (93%) for a medical record-validated MS diagnosis.[32] However, we reviewed the medical record in children with MS initially coded with a non-MS diagnostic code (e.g. optic neuritis) during 2008–15.

Taken together, children included in the chart review and children coded as MS (G35) cover all registered cases of ADS during 2008–15 in Denmark.[10,15,33] The medical record-validated diagnoses overruled the ICD-10 codes from the National Patient Register.

Definition of diagnoses for chart review

ADS can be divided into four groups: 1) MS, 2) neuromyelitis optica spectrum disorder, 3) ADEM, and 4) clinically isolated syndromes, which includes transverse myelitis, optic neuritis and other
ADS such as brainstem and hemispheric syndromes.[3] MS was defined by the McDonald criteria at the time of diagnosis, either the 2005 or 2010 revision.[4,34] Neuromyelitis optica spectrum disorder was defined by the Wingerchuk criteria.[35] Optic neuritis was based on the ophthalmologist’s final diagnosis. Transverse myelitis was defined as neurological symptoms verified by a spinal magnetic resonance imaging with corresponding lesions and a brain magnetic resonance imaging not fulfilling the MS McDonald criteria.[4] A diagnosis of ADEM included an abnormal magnetic resonance imaging and neurological symptoms with a varying degree of encephalopathy and neurological deficits.[15] A diagnosis of MS after an ADEM-like first attack was defined by the International Pediatric MS Study Group criteria.[3]

**Biomarkers, analysis method and reference intervals**

**OCB**

OCB data were nationwide and population-based from Denmark. We retrieved data at the following centers in Denmark (catchment area and inclusion period in parentheses): a) Statens Serum Institut (Denmark, 2000–2017); b) Rigshospitalet, University of Copenhagen (Copenhagen, 2005–2017); c) Zealand University Hospital (Zealand, 2006–2017); d) Odense University Hospital (Funen and Southern Jutland, 2000–2017); e) Aarhus University Hospital (Central Jutland, 2011–2017); f) Aalborg University Hospital (Northern Jutland, 1994–2017).

Historically, two methods have been used to analyze OCB: a) high-resolution agarose gel electrophoresis (presumably 4% of OCB analyses) and b) isoelectric focusing combined with immunoglobulin G immunoblotting (presumably 96% of OCB analyses). The latter is considered more sensitive in detecting OCBs, and the concordance between the two methods is 86%.[2] Presence of OCBs was reported if the child had 2 or more bands in the CSF but not in the
corresponding serum.[36] Mirrored OCBs (presence of the same OCBs in both serum and CSF) were considered negative.

**Laboratory data and reference intervals**

We collected laboratory data on the following CSF additional biomarkers when OCBs were examined: OCBs, immunoglobulin G index, leucocytes including differential count, protein and albumin ratio, erythrocytes, and glucose and glucose ratio. It was possible to obtain concordant biomarker data on most CSF analyses.

We used the following reference intervals: a) CSF pleocytosis>4 leucocytes/µL, b) increased CSF protein defined as albumin ratio>6.8 or CSF protein>0.45 g/L, c) increased immunoglobulin G index>0.67, and d) decreased CSF glucose as a glucose ratio<0.6 or CSF glucose<2.5 mM [36].

**Statistical methods**

We calculated the sensitivity as the proportion of children with the disease of interest who had OCBs, and the specificity as the proportion of children without the disease of interest without OCBs. Further, the positive predictive value was the proportion of children with positive OCBs who had the disease of interest, and the negative predictive value was the proportion of children without OCBs who did not have the disease of interest. Using Fisher’s exact test, we compared the proportion with OCBs in the ADS group between age intervals 0–11 years and 12–17 years, and between ADS and infection/non-ADS immune-mediated CNS diseases. We chose age 12.0 years as cut-off since we have shown that ADEM is the most frequent ADS in children younger than 12 years, whereas MS becomes more frequent during puberty.[10,15]
It was not possible to obtain concordant CSF biomarkers (e.g. CSF leucocytes, protein) for all OCB analyses. However, we assume these ‘missing’ biomarkers are unrelated to the diagnostic groups because all biomarkers presumably were available to physicians making the final diagnosis. We consider it unlikely that physicians would order OCBs without also ordering e.g. CSF leucocytes. Therefore, the data are ‘missing completely at random’ and mean and variance in the studied sample are a random sample of the ‘complete’ sample.[37] Missing biomarker data are listed in Table 1 (footnote).

All statistical analyses were performed using SAS, version 9.4 (SAS Institute Inc., Cary, North Carolina).

**Ethics:**

The study was approved by the Danish Data Protection Agency at Rigshospitalet, University of Copenhagen (case number 30-1423/03567) and the Danish Health Data Authority (case number 00003101). The Danish Health and Medicines Authority waived the requirement to obtain patient informed consent to access medical records (case number 3-3013-896/2). The chief physicians of the Danish pediatric, neurological, or biochemistry departments approved access to patient data from their hospitals.
RESULTS

Diagnostic groups including demographic and CSF data

From 1995 through 2017, we identified 2,185 children analyzed for OCBs before 18 years of age. We excluded children without a valid personal identification number (40 children) or CSF erythrocyte count above 500/µl (n=90) regarded as blood contamination. We included 2,055 children with 2,227 OCB analyses (2,198 OCB analyses before 18 years of age). Overall, 255/2,227 (11%) of OCB analyses were positive, and stratifying by age, 69/786 (9%) OCB analyses were positive in children younger than 12 years and 186/1,441 (13%) were positive in children aged 12–17 years.

Demographic and cerebrospinal fluid data

Among the 2,055 children included, 209 (10%) had OCBs in the CSF at the first OCB examination; OCB positivity was more frequent in children with CNS disease (15%) than in children without CNS disease (4%, Table 1).

For children with ADS, the median age at OCB examination was 15.2 years (range=1.8–18.0) and 62% were girls, whereas it was lower in children with other immune-mediated CNS diseases (10.2 years, range=0.7–18.0) and CNS infections (11.0 years, range=0.2–17.8, Table 1).

CSF pleocytosis was present in two-thirds of children with ADS and CNS infections, but the groups differed considerably regarding OCB positivity: 52% were positive in ADS, but only 18% were positive in immune-mediated CNS diseases, 13% in CNS infections and progressive encephalopathy, and 16% in CNS malignancies. In the remaining disease groups, less than 10% had positive OCBs, and only 4% in the non-CNS disease group were positive (5% in children<12 years
and 3% in children aged 12–17 years). In total, 44% of children with ADS had OCB positivity with CSF pleocytosis. However, having positive OCBs with normal CSF leucocytes was seen in 0–14%.

Absence of OCBs with CSF pleocytosis was more common in CNS infections (56%), CNS malignancies (32%) and immune-mediated CNS diseases (31%) and only 19% of children with ADS showed this pattern. Increased immunoglobulin G index showed the same pattern as OCB positivity, but it was less sensitive for ADS with only 38% having increased immunoglobulin G index as compared with 52% having OCBs (Table 1).

Sensitivity, specificity, positive predictive value, and negative predictive value of OCBs to differentiate ADS from other CNS diseases

In children younger than 12 years of age, absence of OCBs was moderately predictive of not having ADS compared with infection/other immune-mediated CNS disease (negative predictive value: 0.70; 95% confidence interval=0.62–0.78, p<0.0001), but positive OCBs were not predictive of ADS compared with either infection/immune-mediated CNS or other CNS diseases (p=0.17, Table 2). In contrast, OCBs in children aged 12–17 years was highly predictive of ADS compared with infection/immune-mediated CNS diseases (positive predictive value: 0.89; 95% confidence interval=0.82–0.94; p<0.0001) and compared with other CNS diseases except infection/immune-mediated CNS diseases (positive predictive value: 0.85; 95% confidence interval=0.78–0.91; p<0.0001). Further, in children aged 12–17 years, absence of OCBs was highly predictive of not having ADS compared with other CNS diseases (negative predictive value: 0.87; 95% confidence interval=0.84–0.90; p<0.0001) and infection/immune-mediated CNS disease compared with other CNS diseases (negative predictive value: 0.84; 95% confidence interval=0.81–0.87; p<0.0001), but
interestingly absence of OCBs was not discriminatory between ADS and infection/immune-mediated CNS in children 12–17 years of age (p=0.23).

**Change in OCB test result over time**

Of the children, 133 (6%) underwent more than one OCB test (range=2–5, Table 3). Among these children, 91 (68%) continued to be negative OCBs, and 20 (15%) remained positive with repeated measurements. However, 22 (17%) children changed OCB status during follow-up; 11 (8%) children went from OCB positive to negative over a mean follow-up of 2.6 years and the majority had either non-ADS immune-mediated CNS disease (n=3), CNS infection (n=2), static encephalopathy (n=2) or ADS (n=2); and 7 (2%) children went from negative to positive OCB and the majority either had ADS (n=3) or immune-mediated CNS disease (n=2).

**OCB positivity differed in children with ADS before age 12 years compared with ADS onset at 12–17 years**

We validated the National Patient Register diagnostic codes of non-MS ADS in the medical records during 2008–15 and included diagnostic codes for MS (*the medical record-validated ADS cohort*). Using this cohort, we identified 125 children with ADS during 2008–15 who all underwent OCB testing, and we subdivided this population into those with onset before age 12 years (n=29) and those with onset during 12–17 years of age (n=96) (Table 4). Only 24% of children with ADS before age 12 years were OCB positive, whereas 68% of children with ADS aged 12–17 years were OCB positive (p<0.0001). None of the 16 children with ADEM before age 12 years was OCB positive, but 22% of children with ADEM at 12–17 years of age were OCB positive. Further,
children with ADS onset at 12–17 years compared with ADS onset at 0–11 years were more likely to have positive OCBs with CSF pleocytosis (54% vs 16%, p= 0.004) and increased immunoglobulin G index (43% vs 20%, p=0.06) and were less likely to have CSF pleocytosis without OCBs (9% vs 53%, p<0.0001).

**OCBs to differentiate ADS from CNS infections/non-ADS immune-mediated CNS disease**

Using the medical record-validated ADS cohort for ADS (n=125) for comparison with children with non-ADS immune-mediated CNS disease or CNS infections (n=106) during 2008–15, OCB positivity was more frequent in ADS (58% vs 15%, p<0.0001) (Table 5). However, when comparing only children with onset before age 12 years, there was no difference between the groups (24% vs 13%, p=0.23), but OCB positivity was more frequent among children with ADS onset aged 12–17 years compared with infection/immune-mediated CNS diseases (p<0.0001). The same pattern was seen for OCBs with CSF pleocytosis (p=0.0002) and increased immunoglobulin G index (p=0.02). In addition, infection/immune-mediated CNS diseases more often had CSF pleocytosis without OCBs (51% vs 18%, p<0.0001), but the difference was not seen in children younger than 12 years at onset (p=0.78). Two-thirds of children had CSF pleocytosis; this was not discriminatory across groups or age intervals.

**DISCUSSION:**

In a nationwide population-based setting, we identified 2,055 children with at least one OCB examination before 18 years of age and found 10% to be OCB positive. In line with previous findings, OCB positivity was seen in 52% of ADS, 18% of immune-mediated CNS diseases, and
13% of CNS infections. However, OCB positivity in ADS was highly age dependent, and only 21% were OCB positive before age 12 years, whereas 68% were OCB positive at age 12–17 years. The difference in OCB positivity by age at onset is presumably because ADEM is more frequent before 12 years of age, and all 16 children with onset of ADEM before age 12 years were OCB negative; in contrast, MS is more frequent in children aged 12–17 years, and 93% of children with MS were OCB positive. These findings underline that "ADS" is not a disease, but a description of an acute event, and that the pathophysiology of ADS differs for the two age intervals. Accordingly, OCB positivity increases in line with the age-related risk of MS, a disease in which chronic intrathecal B-cell activation is a hallmark.

A major challenge in the clinical setting is to distinguish ADS from CNS infections and non-ADS immune-mediated CNS diseases. Albeit with the caveat of being dependent on disease prevalence, we found that OCB analysis is valuable if the physician suspects ADS in children aged 12–17 years and if this is the case, only a positive test has predictive value (positive predictive value: 0.89; 95% confidence interval=0.82–0.94; p<0.0001; negative predictive value: p=0.17). Positive OCBs in children before age 12 years were not discriminatory between ADS and infection/non-ADS immune-mediated CNS diseases (p=0.17), and negative OCBs were only moderately predictive of not having ADS (negative predictive value=0.70).

Our results are likely generalizable to countries with a similar age-specific incidence of pediatric ADS and preference for OCB examination. OCB examination may not provide diagnostic information in children younger than 12 years because the pre-test probability for MS is extremely low. In such circumstances, other markers are more valuable in the diagnostic work-up for differential diagnoses such as CSF leucocytes and brain magnetic resonance imaging. By contrast, OCB examination in children aged 12–17 years may be of diagnostic value because of the higher MS frequency. Nevertheless, we acknowledge that we make inferences at the group level,
whereas decisions of OCB examination are made for individuals and many patient variables may influence the decision to test. This implies that OCB examination may still be indicated in individuals younger than 12 years if the pre-test clinical suspicion of MS is high. Further, whether to undertake OCB examination may also depend on the consequence of misdiagnosing a child, particularly regarding MS. Further, OCBs may facilitate an earlier MS diagnosis by demonstrating dissemination in time using the 2017 McDonald Criteria (also applicable for children <12 years of age), and initiation of prophylactic MS treatment early in pediatric MS is important.[40,41] Taken together, children may present with numerous, rare differential diagnoses and the decision regarding OCB testing needs individual assessment.

A strength of our study is that it was nationwide, population-based, and multi-centered. Denmark has a uniform, publicly financed healthcare system with complete coverage, which minimizes the risk of referral bias. The number of children studied was large and few children were lost to follow-up due to the personal identification number system. We undertook detailed case ascertainment including chart review of 321 children, including validation of the cohort with ADS onset during 2008–15. However, several limitations must be mentioned. 1) We defined most diagnoses using diagnostic codes in the National Patient Register, which may have caused misclassification compared with medical record-validated diagnoses. 2) Diagnostic work-up may not have been equally systematic in all children because we included children from several different centers. 3) Diagnoses (especially in the field of autoimmune encephalitis and ADS) have evolved over time with the introduction of new biomarkers and magnetic resonance imaging criteria, and ICD classifications have not been updated accordingly.[3,15,32,42] Thus, diagnoses and codes may not be comparable over time. 4) Assessing a diagnostic test such as OCBs ideally requires comparison with a criterion standard because medical doctors may be more inclined to diagnose ADS in a child with positive OCBs; consequently, the exposure (OCB) and outcome (diagnosis) may be dependent
(ascertainment bias). 5) It was not possible to obtain concordant biomarker data on all OCB analyses, but we presume the data were available to the physicians who made the final diagnosis. 6) Myelin oligodendrocyte glycoprotein (MOG) antibodies were not examined in most of the ADS cohort. However, anti-MOG antibodies are present in 18–35% of children with ADS at disease onset (who usually do not have CSF OCBs) and may predict a monophasic disease course,[43–45] whereas high, persistent levels of anti-MOG antibodies at the 2-year follow-up are associated with a relapsing non-MS disease course.[43]

OCBs were rare in ADEM and as this is the most common ADS before age 12 years, OCB positivity was not discriminatory between diagnostic groups at this age. However, OCBs were frequent in ADS in children aged 12–17 due to the increasing frequency of MS. In the clinical setting, OCB examination may be of higher yield in children aged 12–17 years if there is clinical suspicion of MS, and in such circumstances a positive test supports a diagnosis of MS.

Acknowledgements

The study was supported by the Danish MS Society (grant numbers A29625 and A31526), Novartis, Teva, and Genzyme.

We thank the following individuals for assistance in data retrieval: Henrik Poulsen, Niels Heegaard, Ruth Frikke-Schmidt, Jan Hellden, Mette Østergaard, Dora Simonsen, Pia Heumann, Uffe Lystbæk, and Simon Lykkeboe. We thank Barbara Olsen for assistance in SAS data management.
References


Table 1 Baseline demographic and cerebrospinal fluid data by diagnostic group during 1994–2017 in Denmark

<table>
<thead>
<tr>
<th>Diagnostic group</th>
<th>Children, n (% total)</th>
<th>Age at onset, y, median (range)</th>
<th>Girls, n (%)</th>
<th>CSF pleocytosis, n (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>OCB positive, n (%)</th>
<th>OCB positive and CSF pleocytosis&lt;sup&gt;c&lt;/sup&gt;</th>
<th>OCB positive and normal CSF leucocytes&lt;sup&gt;a&lt;/sup&gt;</th>
<th>OCB negative and CSF pleocytosis&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IgG index&gt;0.67, n (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>CSF protein increased, n (%)&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ALL DISEASES</strong></td>
<td>2,055 (100%)</td>
<td>14.1 (0.02–18.0)</td>
<td>1,222 (59%)</td>
<td>291 (22%)</td>
<td>209 (10%)</td>
<td>88 (7%)</td>
<td>37 (3%)</td>
<td>203 (15%)</td>
<td>211 (12%)</td>
<td>252 (12%)</td>
</tr>
<tr>
<td><strong>Non-CNS disease</strong></td>
<td>933 (45%)</td>
<td>14.8 (0.02–18.0)</td>
<td>588 (63%)</td>
<td>82 (13%)</td>
<td>37 (4%)</td>
<td>8 (1%)</td>
<td>10 (2%)</td>
<td>74 (11%)</td>
<td>61 (7%)</td>
<td>99 (11%)</td>
</tr>
<tr>
<td><strong>CNS disease</strong> (subgroups below)</td>
<td>1,122 (55%)</td>
<td>13.5 (0.08–18.0)</td>
<td>634 (57%)</td>
<td>209 (31%)</td>
<td>172 (15%)</td>
<td>80 (12%)</td>
<td>27 (4%)</td>
<td>129 (19%)</td>
<td>150 (16%)</td>
<td>153 (14%)</td>
</tr>
<tr>
<td><strong>ADS</strong></td>
<td>211 (10%)</td>
<td>15.2 (1.8–18.0)</td>
<td>131 (62%)</td>
<td>82 (63%)</td>
<td>109 (52%)</td>
<td>57 (44%)</td>
<td>12 (9%)</td>
<td>25 (19%)</td>
<td>69 (38%)</td>
<td>35 (17%)</td>
</tr>
<tr>
<td><strong>Other immune-mediated CNS</strong></td>
<td>61 (3%)</td>
<td>10.2 (0.7–18.0)</td>
<td>38 (62%)</td>
<td>16 (44%)</td>
<td>11 (18%)</td>
<td>5 (14%)</td>
<td>4 (11%)</td>
<td>11 (31%)</td>
<td>6 (12%)</td>
<td>11 (18%)</td>
</tr>
<tr>
<td><strong>CNS infections</strong></td>
<td>143 (7%)</td>
<td>11.0 (0.2–17.8)</td>
<td>67 (46%)</td>
<td>50 (69%)</td>
<td>18 (13%)</td>
<td>10 (14%)</td>
<td>0</td>
<td>40 (56%)</td>
<td>29 (24%)</td>
<td>50 (36%)</td>
</tr>
<tr>
<td><strong>Epilepsy</strong></td>
<td>98 (5%)</td>
<td>11.3 (0.3–17.7)</td>
<td>45 (46%)</td>
<td>13 (22%)</td>
<td>2 (2%)</td>
<td>0</td>
<td>1 (2%)</td>
<td>13 (22%)</td>
<td>11 (13%)</td>
<td>10 (11%)</td>
</tr>
<tr>
<td><strong>Sleep disorders</strong></td>
<td>65 (3%)</td>
<td>15.7 (4.8–17.9)</td>
<td>37 (57%)</td>
<td>2 (5%)</td>
<td>4 (6%)</td>
<td>0</td>
<td>3 (7%)</td>
<td>2 (5%)</td>
<td>3 (5%)</td>
<td>5 (8%)</td>
</tr>
<tr>
<td><strong>Malignant</strong></td>
<td>31 (2%)</td>
<td>11.6 (0.9–17.3)</td>
<td>18 (58%)</td>
<td>7 (32%)</td>
<td>5 (16%)</td>
<td>0</td>
<td>3 (14%)</td>
<td>7 (32%)</td>
<td>1 (4%)</td>
<td>6 (20%)</td>
</tr>
<tr>
<td><strong>Movement disorders</strong></td>
<td>29 (1%)</td>
<td>14.6 (1.3–17.4)</td>
<td>20 (69%)</td>
<td>1 (5%)</td>
<td>1 (3%)</td>
<td>0</td>
<td>0</td>
<td>1 (5%)</td>
<td>1 (4%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td><strong>Static encephalopathy</strong></td>
<td>155 (8%)</td>
<td>5.6 (0.1–17.6)</td>
<td>68 (44%)</td>
<td>8 (10%)</td>
<td>11 (7%)</td>
<td>2 (3%)</td>
<td>3 (4%)</td>
<td>6 (8%)</td>
<td>16 (13%)</td>
<td>15 (10%)</td>
</tr>
<tr>
<td><strong>Cerebrovascular disease</strong></td>
<td>43 (2%)</td>
<td>14.8 (0.1–17.9)</td>
<td>24 (56%)</td>
<td>7 (23%)</td>
<td>2 (5%)</td>
<td>2 (6%)</td>
<td>0</td>
<td>5 (16%)</td>
<td>3 (8%)</td>
<td>4 (10%)</td>
</tr>
<tr>
<td><strong>Progressive encephalopathy</strong></td>
<td>8 (0.4%)</td>
<td>8.8 (0.6–15.5)</td>
<td>3 (38%)</td>
<td>2 (40%)</td>
<td>1 (13%)</td>
<td>0</td>
<td>0</td>
<td>2 (40%)</td>
<td>0</td>
<td>2 (25%)</td>
</tr>
<tr>
<td><strong>Other CNS diseases</strong></td>
<td>278 (14%)</td>
<td>14.7 (0.2–17.9)</td>
<td>183 (66%)</td>
<td>21 (11%)</td>
<td>8 (3%)</td>
<td>4 (2%)</td>
<td>2 (1%)</td>
<td>17 (9%)</td>
<td>11 (0.4%)</td>
<td>14 (5%)</td>
</tr>
</tbody>
</table>

<sup>a</sup>CSF pleocytosis>4 leucocytes. CSF leucocytes were available in 1,331 (65%) of the children's OCB analyses at disease onset

<sup>b</sup>IgG index available in 1,787 (87%)

<sup>c</sup>Increased CSF protein was defined as albumin-ratio>6.8 or CSF protein<0.45 g/L. Data were available in 2,021 children (98%)

<sup>d</sup>Using CSF leucocytes>10 as cutoff for CSF pleocytosis, 22 (3%) had CSF pleocytosis, and 15 (2%) had negative OCBs and CSF pleocytosis

<sup>e</sup>In children<5 years at onset, increased CSF/serum albumin ratio or CSF protein was present in 41/282 (15%).

Abbreviations: ADS, acquired demyelinating syndromes; CSF, cerebrospinal fluid, CNS, central nervous system; OCBs, oligoclonal bands; Ig, immunoglobulin.
Table 2 Diagnostic performance: Sensitivity, specificity, positive and negative predictive value of OCB during 1995–2017

<table>
<thead>
<tr>
<th>Diagnostic group</th>
<th>Children</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Estimate (CI)</td>
<td>p value</td>
<td>Estimate (CI)</td>
<td>p value</td>
</tr>
<tr>
<td><strong>ADS vs</strong></td>
<td>211</td>
<td>0.52 (0.45–0.59)</td>
<td>0.68</td>
<td>0.86 (0.80–0.90)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Infection and immune-mediated CNS disease</strong></td>
<td>204</td>
<td>0.18 (0.08–0.31)</td>
<td>&lt;0.0001</td>
<td>0.85 (0.78–0.91)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Infection or immune (&lt;12y)</strong></td>
<td>51</td>
<td>0.63 (0.55–0.70)</td>
<td>0.002</td>
<td>0.86 (0.77–0.93)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Infection or immune (12–17y)</strong></td>
<td>117</td>
<td>0.18 (0.08–0.31)</td>
<td>&lt;0.0001</td>
<td>0.94 (0.91–0.97)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>ADS (&lt;12y) vs</strong></td>
<td>707</td>
<td>0.52 (0.45–0.59)</td>
<td>0.68</td>
<td>0.95 (0.93–0.97)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>CNS diseases except infection/immune-mediated CNS</strong></td>
<td>291</td>
<td>0.18 (0.08–0.31)</td>
<td>&lt;0.0001</td>
<td>0.94 (0.91–0.97)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Infection/immune-mediated CNS (&lt;12y)</strong></td>
<td>160</td>
<td>0.63 (0.55–0.70)</td>
<td>0.002</td>
<td>0.96 (0.94–0.98)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>CNS disease except ADS</strong></td>
<td>416</td>
<td>0.14 (0.10–0.20)</td>
<td>&lt;0.0001</td>
<td>0.95 (0.93–0.97)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>CNS disease except ADS (&lt;12y)</strong></td>
<td>117</td>
<td>0.15 (0.09–0.22)</td>
<td>&lt;0.0001</td>
<td>0.94 (0.91–0.97)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>CNS disease except ADS (12–17y)</strong></td>
<td>291</td>
<td>0.14 (0.07–0.23)</td>
<td>&lt;0.0001</td>
<td>0.96 (0.94–0.98)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Abbreviations: ADS, acquired demyelinating syndromes; CNS, central nervous system; OCBs, oligoclonal bands.
Table 3: Repeated OCB analyses among 133 children during 1995–2017

<table>
<thead>
<tr>
<th>OCB category</th>
<th>Children with &gt;1 OCB test, n</th>
<th>Time between first OCB analysis and last OCB, y, mean (SD)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remained negative</td>
<td>91</td>
<td>2.0 (3.0)</td>
<td>Epilepsy (n=3), sleep disorder (n=2), malignancy (n=2), movement disorder (n=1), other CNS disease (n=10), static encephalopathy (n=3), cerebrovascular (n=2), ADS (n=20), non-ADS immune-mediated CNS (n=9), CNS infection (n=12), non-CNS disease (n=27)</td>
</tr>
<tr>
<td>Remained positive</td>
<td>20</td>
<td>1.8 (2.3)</td>
<td>Static encephalopathy (n=1), cerebrovascular (n=2), ADS (n=12), non-ADS immune-mediated CNS (n=1), CNS infection (n=2), non-CNS disease (n=2)</td>
</tr>
<tr>
<td>Positive to negative</td>
<td>11</td>
<td>2.6 (3.8)</td>
<td>Malignancy (n=1), progressive encephalopathy/mitochondrial disease (n=1), static encephalopathy (n=2), ADS (n=2), non-ADS immune-mediated CNS (n=3), CNS infection (n=2)</td>
</tr>
<tr>
<td>Negative to positive</td>
<td>7</td>
<td>1.6 (1.5)</td>
<td>Sleep disorder (n=1), other CNS disease (n=1), ADS (n=3), non-ADS immune-mediated CNS (n=2)</td>
</tr>
<tr>
<td>Negative to positive to negative</td>
<td>3</td>
<td>—</td>
<td>Non-CNS (n=1), immune-mediated CNS (n=2)</td>
</tr>
<tr>
<td>Positive to negative to positive</td>
<td>1</td>
<td>—</td>
<td>Static encephalopathy (n=1)</td>
</tr>
<tr>
<td>Total</td>
<td>133 (6% total)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations: ADS, acquired demyelinating syndromes; CNS, central nervous system; OCB, oligoclonal band
Table 4. Medical-record validated ADS by onset before and after 12 years of age during 2008–2015

<table>
<thead>
<tr>
<th>Diagnostic group</th>
<th>Children, n (% total)</th>
<th>Follow-up years, median (range)</th>
<th>OCB positive, n (%)</th>
<th>CSF leucocytes&gt;4, n (%)</th>
<th>OCB positive and CSF pleocytosis</th>
<th>OCB positive and normal CSF leucocytes</th>
<th>OCB negative and CSF pleocytosis</th>
<th>IgG index&gt;0.67, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADS (&lt;12y)</td>
<td>29 (23%)</td>
<td>6.7 (2.9–11.2)</td>
<td>7/29 (24%)</td>
<td>13/19 (68%)</td>
<td>3/19 (16%)</td>
<td>1/19 (5%)</td>
<td>10/19 (53%)</td>
<td>5/25 (20%)</td>
</tr>
<tr>
<td>ADS (12–17y)</td>
<td>96 (77%)</td>
<td>6.4 (3.0–10.8)</td>
<td>65/96 (68%)</td>
<td>43/69 (62%)</td>
<td>37/69 (54%)</td>
<td>9/69 (13%)</td>
<td>6/69 (9%)</td>
<td>38/89 (43%)</td>
</tr>
<tr>
<td>ADEM (&lt;12y)</td>
<td>16 (13%)</td>
<td>6.6 (3.4–8.1)</td>
<td>0/16 (0%)</td>
<td>9/12 (75%)</td>
<td>0/12 (0%)</td>
<td>0/12 (0%)</td>
<td>9/12 (75%)</td>
<td>1/14 (7%)</td>
</tr>
<tr>
<td>ADEM (12–17y)</td>
<td>9 (7%)</td>
<td>7.6 (3.7–10.8)</td>
<td>2/9 (22%)</td>
<td>5/7 (71%)</td>
<td>1/7 (14%)</td>
<td>0/7 (0%)</td>
<td>4/7 (57%)</td>
<td>5/9 (56%)</td>
</tr>
<tr>
<td>CIS (&lt;12y)</td>
<td>11 (9%)</td>
<td>6.1 (3.0–10.8)</td>
<td>5/11 (45%)</td>
<td>3/6 (50%)</td>
<td>2/6 (33%)</td>
<td>1/7 (17%)</td>
<td>1/6 (17%)</td>
<td>3/9 (33%)</td>
</tr>
<tr>
<td>CIS (12y–17y)</td>
<td>23 (18%)</td>
<td>6.2 (3.0–10.6)</td>
<td>4/23 (17%)</td>
<td>5/16 (31%)</td>
<td>4/16 (25%)</td>
<td>0/16 (0%)</td>
<td>1/16 (6%)</td>
<td>4/22 (18%)</td>
</tr>
<tr>
<td>MS (&lt;12y)</td>
<td>1 (1%)</td>
<td>7.9</td>
<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
<td>0/1 (0%)</td>
<td>0/1 (0%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>MS (12y–17y)</td>
<td>61 (48%)</td>
<td>6.5 (3.0–10.7)</td>
<td>57/61 (93%)</td>
<td>33/44 (74%)</td>
<td>31/43 (72%)</td>
<td>8/43 (19%)</td>
<td>1/43 (2%)</td>
<td>29/56 (52%)</td>
</tr>
<tr>
<td>NMOSD (&lt;12y)</td>
<td>1 (1%)</td>
<td>11.2</td>
<td>1/1 (100%)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td>NMOSD (12y–17y)</td>
<td>3 (2%)</td>
<td>4.7 (3.0–5.0)</td>
<td>2/3 (66%)</td>
<td>1/3 (33%)</td>
<td>1/3 (33%)</td>
<td>1/3 (33%)</td>
<td>0/3 (0%)</td>
<td>0/2 (0%)</td>
</tr>
</tbody>
</table>

*P-value < 0.0001 for independence of OCB positivity among ADS (<12y) vs ADS (12–17y) using Fisher’s exact test. Cell numbers comparing the specific ADS were too small for statistical testing.

bP-value = 0.79 for independence of OCB positivity among ADS (<12y) vs ADS (12–17y) using Fisher’s exact test.

cP-value = 0.004 for independence of OCB positivity among ADS (<12y) vs ADS (12–17y) using Fisher’s exact test.

dP-value = 0.68 for independence of OCB positivity among ADS (<12y) vs ADS (12–17y) using Fisher’s exact test.

eP-value < 0.0001 for independence of OCB positivity among ADS (<12y) vs ADS (12–17y) using Fisher’s exact test.

fP-value = 0.06 for independence of OCB positivity among ADS (<12y) vs ADS (12–17y) using Fisher’s exact test.

gDue to the low percentage of children with OCB positivity in CIS (12y–17y), we stratified into the specific monophasic ADS (OCB positive/OCBs total in parentheses): a) CIS and ‘dissemination in space’ but not ‘dissemination in time’ (4/5), b) isolated ON with normal MRI (0/16), and c) TM (0/2).

hChart review was from June to August 2016. From June 2016 to December 2018 diagnostic codes for MS (”G35”) and NMOSD (”G36.0”) were used to assess disease progression from ADEM/CIS to MS/NMOSD including chart review of relevant cases.

Abbreviations: ADEM, acute disseminated encephalomyelitis; CIS, clinically isolated syndromes; CSF, cerebrospinal fluid; Ig, immunoglobulin; MS, multiple sclerosis; NMOSD, neuromyelitis optica spectrum disorders; OCB, oligoclonal bands.
<table>
<thead>
<tr>
<th>Diagnostic group</th>
<th>Children</th>
<th>OCB positive</th>
<th>CSF leucocytes&gt;4</th>
<th>OCB positive and CSF pleocytosis</th>
<th>OCB positive and normal CSF leucocytes</th>
<th>OCB negative and CSF pleocytosis</th>
<th>IgG index&gt;0.67</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (% total)</td>
<td>n/total (%)</td>
<td>p value&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n/total (%)</td>
<td>p value&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n/total (%)</td>
<td>p value&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ADS</td>
<td>125 (54%)</td>
<td>72/125 (58%)</td>
<td>&lt;0.0001</td>
<td>56/88 (64%)</td>
<td>0.74</td>
<td>40/88 (45%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Infection/immune-</td>
<td>106 (46%)</td>
<td>16/106 (15%)</td>
<td>45/67 (67%)</td>
<td>11/67 (16%)</td>
<td>0.74</td>
<td>6/67 (9%)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.78</td>
</tr>
<tr>
<td>mediated CNS</td>
<td>ADS (&lt;12y)</td>
<td>29 (13%)</td>
<td>7/29 (24%)</td>
<td>13/19 (68%)</td>
<td>1.00</td>
<td>3/19 (16%)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Infection/immune-(&lt;12y)</td>
<td>96 (42%)</td>
<td>7/54 (13%)</td>
<td>22/34 (65%)</td>
<td>1.00</td>
<td>6/34 (18%)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>ADS (12–17y)</td>
<td>54 (23%)</td>
<td>65/96 (68%)</td>
<td>43/69 (61%)</td>
<td>0.51</td>
<td>37/69 (54%)</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>Infection/immune-(12–17y)</td>
<td>52 (23%)</td>
<td>9/52 (17%)</td>
<td>23/33 (70%)</td>
<td>5/33 (15%)</td>
<td>1/33 (3%)</td>
<td>18/33 (55%)</td>
</tr>
</tbody>
</table>

<sup>a</sup>P-values are the probability for the observed OCB distributions or some that are more extreme for ADS and infection/immune-mediated CNS disease under the null hypothesis using Fisher’s exact test. Age-stratified analyses are also shown.

Abbreviations: ADS, acquired demyelinating syndromes; CSF, cerebrospinal fluid; Ig, immunoglobulin; OCB, oligoclonal bands,