Original Article

Diagnostic Value of Oligoclonal Bands in Children: A Nationwide Population-Based Cohort Study

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ABSTRACT

Objective: We evaluated the diagnostic value of cerebrospinal fluid oligoclonal bands in individuals less than 18 years of age.
Methods: In a nationwide population-based setting, we retrieved data on 2055 children's oligoclonal band examination, including concordant cerebrospinal fluid biomarkers, during 1994 to 2017. Case ascertainment was by review of medical records and diagnostic codes. We used Fisher's exact test to explore distribution differences of oligoclonal band positivity in acquired demyelinating syndromes (ADS) before and after age 12 years and calculated the sensitivity, specificity, positive predictive value, and negative predictive value of oligoclonal bands to distinguish ADS from the other diagnostic groups.
Results: Median age at oligoclonal band examination was 15.2 years (range = 1.8 to 18.0), and 10% had presence of cerebrospinal fluid oligoclonal bands. Oligoclonal band 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 aged 12 through 17 years ($P < 0.0001$) owing to the higher incidence of multiple sclerosis in the latter. Cerebrospinal fluid oligoclonal bands were not predictive of ADS before age 12 years compared with the other diagnostic groups. However, cerebrospinal fluid oligoclonal bands in children aged 12 through 17 years were highly predictive of ADS compared with central nervous system infections and non—ADS immune-mediated central nervous system diseases (positive predictive value: 0.89; 95% confidence interval = 0.82 to 0.94; $P < 0.0001$), but negative oligoclonal bands were not discriminatory (negative predictive value: $P = 0.17$).
Conclusions: In a clinical setting, cerebrospinal fluid oligoclonal band examination may be of higher yield in children aged 12 through 17 years if there is clinical suspicion of multiple sclerosis, and in such circumstances a positive test supports a diagnosis of multiple sclerosis.

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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 the 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.[10] In contrast, the most common ADS in children is acute disseminated encephalomyelitis (ADEM), a largely monophasic disease, but only 0% to 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 present with positive OCBs.[1] Thus the diagnostic value of OCBs in children may be limited, particularly in children younger than 12 years.

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 zero to 11 years and children aged 12 to 17 years.

Methods

Source and study population

The *source population* was all children (less than 18 years of age) in Denmark during 1994 to 2017. The midyear 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 age 18 years (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 to 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 MS and acceptable for pediatric optic neuritis and transverse myelitis, but unacceptable for ADEM.

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." Furthermore, we divided CNS diseases into the following groups: ADS (e.g., ADEM, MS), non-ADS immune-mediated CNS diseases (e.g., Rasmussen encephalitis, anti-*N*-methyl-D-aspartate 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 or mitochondrial disease (e.g., Leigh syndrome), cerebrovascular disease (e.g., stroke), or other CNS diseases (e.g., hydrocephalus, asphyxia). "Non-CNS diseases" were heterogeneous and included diagnoses such as pneumonia, febrile seizures, and leukemia.

Medical-record-validated ADS during 2008 to 2015

The first author, M.S.B., and a senior consultant, A.P.B., 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 to 2015 (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 to 2016. 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 to 2015.

Taken together, children included in the chart review and children coded as MS (G35) presumably cover all registered cases of ADS during 2008 to 2015 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 the following four groups: (1) MS, (2) neuromyelitis optica spectrum disorder, (3) ADEM, and (4) clinically isolated syndromes, which include transverse myelitis, optic neuritis, and other ADSs 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 2015 International

Panel for NMO Diagnosis 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): (1) Statens Serum Institut (Denmark, 2000 to 2017); (2) Rigshospitalet, University of Copenhagen (Copenhagen, 2005 to 2017); (3) Zealand University Hospital (Zealand, 2006 to 2017); (4) Odense University Hospital (Funen and Southern Jutland, 2000 to 2017) (5) Aarhus University Hospital (Central Jutland, 2011 to 2017), and (5) Aalborg University Hospital (Northern Jutland, 1994 to 2017).

Historically, the following two methods have been used to analyze OCB: (1) high-resolution agarose gel electrophoresis (presumably 4% of OCB analyses) and (2) 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 two 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 concordant CSF biomarkers when OCBs were examined: OCBs, immunoglobulin G index, CSF leukocytes (including differential count), CSF protein, albumin ratio, CSF erythrocytes, CSF glucose, and glucose ratio. It was possible to obtain concordant biomarker data on most CSF analyses.

We used the following reference intervals: (1) CSF pleocytosis greater than 4 leukocytes/μL, (2) increased CSF protein defined as albumin ratio greater than 6.8 or CSF protein greater than 0.45 g/L, (3) increased immunoglobulin G index greater than 0.67, and (4) decreased CSF glucose as a glucose ratio less than 0.6 or CSF glucose less than 2.5 mM.[36]

Statistical methods

We calculated sensitivity as the proportion of children with the disease of interest who had OCBs and specificity as the proportion of children without the disease of interest without OCBs. Furthermore, positive predictive value was the proportion of children with positive OCBs who had the disease of interest and 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 zero through 11 years and 12 through 17 years and between ADS and infection or non-ADS immune-mediated CNS diseases. We chose age 12.0 years as cutoff because 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 leukocytes, protein) for all OCB analyses. However,

we assume that 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, for example, CSF leukocytes. 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, NC, USA).

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 2185 children analyzed for OCBs before age 18 years. We excluded children without a valid personal identification number (40 children) or CSF erythrocyte count above 500/μL (90 children), regarding as blood contamination. We included 2055 children with 2227 OCB analyses (2198 OCB analyses before age 18 years). Overall, 255 of 2227 (11%) OCB analyses were positive, and stratifying by age, 69 of 786 (9%) OCB analyses were positive in children younger than 12 years and 186 of 1441 (13%) were positive in children aged 12 through 17 years.

Demographic and cerebrospinal fluid data

Among the 2055 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 to 18.0) and 62% were girls, whereas it was lower in children with other immune-mediated CNS diseases (10.2 years, range = 0.7 to 18.0) and CNS infections (11.0 years, range = 0.2 to 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 younger than 12 years and 3% in children aged 12 through 17 years). In total, 44% of children with ADS had OCB positivity *with* CSF pleocytosis. However, having positive OCBs *with* normal CSF leukocytes was seen in 0% to 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 when compared with 52% having OCBs (Table 1).

TABLE 1.
Baseline Demographic and Cerebrospinal Fluid Data by Diagnostic Group During 1994 to 2017 in Denmark

Diagnostic Group	Children, n (% Total)	Age at Onset, y, Median (Range)	Girls, n (%)	CSF Pleocytosis, n (%)*	OCB Positive, n (%)	OCB Positive and CSF Pleocytosis*	OCB Positive and Normal CSF Leukocytes*	OCB Negative and CSF Pleocytosis*	IgG Index > 0.67, n (%)†	CSF Protein Increased, n (%)‡
All diseases	2055 (100%)	14.1 (0.02-18.0)	1222 (59%)	291 (22%)	209 (10%)	88 (7%)	37 (3%)	203 (15%)	211 (12%)	252 (12%)
Non-CNS disease	933 (45%)	14.8 (0.02-18.0)	588 (63%)	82 (13%)§	37 (4%)	8 (1%)	10 (2%)	74 (11%)§	61 (7%)	99 (11%)‖
CNS disease (subgroups below)	1122 (55%)	13.5 (0.08-18.0)	634 (57%)	209 (31%)	172 (15%)	80 (12%)	27 (4%)	129 (19%)	150 (16%)	153 (14%)
ADS	211 (10%)	15.2 (1.8-18.0)	131 (62%)	82 (63%)	109 (52%)	57 (44%)	12 (9%)	25 (19%)	69 (38%)	35 (17%)
Other immune-mediated CNS	61 (3%)	10.2 (0.7-18.0)	38 (62%)	16 (44%)	11 (18%)	5 (14%)	4 (11%)	11 (31%)	6 (12%)	11 (18%)
CNS infections	143 (7%)	11.0 (0.2-17.8)	67 (46%)	50 (69%)	18 (13%)	10 (14%)	0	40 (56%)	29 (24%)	50 (36%)
Epilepsy	98 (5%)	11.3 (0.3-17.7)	45 (46%)	13 (22%)	2 (2%)	0	1 (2%)	13 (22%)	11 (13%)	10 (11%)
Sleep disorders	65 (3%)	15.7 (4.8-17.9)	37 (57%)	2 (5%)	4 (6%)	0	3 (7%)	2 (5%)	3 (5%)	5 (8%)
Malignant	31 (2%)	11.6 (0.9-17.3)	18 (58%)	7 (32%)	5 (16%)	0	3 (14%)	7 (32%)	1 (4%)	6 (20%)
Movement disorders	29 (1%)	14.6 (1.3-17.4)	20 (69%)	1 (5%)	1 (3%)	0	0	1 (5%)	1 (4%)	1 (3%)
Static encephalopathy	155 (8%)	5.6 (0.1-17.6)	68 (44%)	8 (10%)	11 (7%)	2 (3%)	3 (4%)	6 (8%)	16 (13%)	15 (10%)
Cerebrovascular disease	43 (2%)	14.8 (0.1-17.9)	24 (56%)	7 (23%)	2 (5%)	2 (6%)	0	5 (16%)	3 (8%)	4 (10%)
Progressive encephalopathy	8 (0.4%)	8.8 (0.6-15.5)	3 (38%)	2 (40%)	1 (13%)	0	0	2 (40%)	0	2 (25%)
Other CNS diseases	278 (14%)	14.7 (0.2-17.9)	183 (66%)	21 (11%)	8 (3%)	4 (2%)	2 (1%)	17 (9%)	11 (0.4%)	14 (5%)

Abbreviations:
ADS = Acquired demyelinating syndromes
CSF = Cerebrospinal fluid
CNS = Central nervous system
Ig = Immunoglobulin
OCBs = Oligoclonal bands
* CSF pleocytosis > 4 leukocytes. CSF leukocytes were available in 1331 (65%) of the children's OCB analyses at disease onset.
† IgG index was available in 1787 (87%).
‡ Increased CSF protein was defined as albumin ratio > 6.8 or CSF protein < 0.45 g/L. Data were available in 2021 children (98%).
§ Using CSF leukocytes > 10 as cutoff for CSF pleocytosis, 22 (3%) had CSF pleocytosis and 15 (2%) had negative OCBs and CSF pleocytosis.
‖ In children < five years at onset, increased CSF-serum albumin ratio or CSF protein was present in 41 of 282 (15%).

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

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

Change in OCB test result over time

Of the children, 133 (6%) underwent more than one OCB test (range = 2 to 5, Table 3). Among these children, 91 (68%) continued to be tested negative for 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 non-ADS immune-mediated CNS disease (n = 3), CNS infection (n = 2), static encephalopathy (n = 2), or ADS (n = 2); seven (2%)

children went from negative to positive OCB status 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 to 17 years

We validated the National Patient Register diagnostic codes of non-MS ADS in the medical records during 2008 to 2015 (*the Medical record-validated ADS during 2008–2015*) and included diagnostic codes for MS. Using this cohort, we identified 125 children with ADS during 2008 to 2015 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 age 12 through 17 years (n = 96) (Table 4). Only 24% of children with ADS before age 12 years were OCB positive, whereas 68% of children with ADS at age 12 to 17 years were OCB positive ($P < 0.0001$). None of the 16 children with ADEM before age 12 years were OCB positive, but 22% of children with ADEM at age 12 to 17 years were OCB positive. Furthermore, children with ADS onset at 12 to 17 years compared with ADS onset at zero to 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 or 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 to 2015, 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 age 12 to 17 years compared with

TABLE 2.
Diagnostic Performance: Sensitivity, Specificity, and Positive and Negative Predictive Values of Oligoclonal Bands During 1995 to 2017

Diagnostic Group	Children	Sensitivity		Specificity		Positive Predictive Value		Negative Predictive Value	
	N	Estimate (95% CI)	P Value	Estimate (95% CI)	P Value	Estimate (95% CI)	P Value	Estimate (95% CI)	P Value
ADS versus	211	0.52 (0.45-0.59)	0.68	0.86 (0.80-0.90)	<0.0001	0.79 (0.71-0.85)	<0.0001	0.63 (0.57-0.69)	<0.0001
Infection and immune-mediated CNS disease	204								
ADS (<12 y) versus	51	0.18 (0.08-0.31)	<0.0001	0.85 (0.78-0.91)	<0.0001	0.35 (0.17-0.56)	0.17	0.70 (0.62-0.78)	<0.0001
Infection or immune (<12 y)	117								
ADS (12-17 y) versus	160	0.63 (0.55-0.70)	0.002	0.86 (0.77-0.93)	<0.0001	0.89 (0.82-0.94)	<0.0001	0.56 (0.47-0.64)	0.23
Infection or immune (12-17 y)	87								
ADS versus	211	0.52 (0.45-0.59)	0.68	0.95 (0.93-0.97)	<0.0001	0.76 (0.68-0.83)	<0.0001	0.87 (0.84-0.89)	<0.0001
CNS diseases except infection/immune-mediated CNS	707								
ADS (<12 y) versus	51	0.18 (0.08-0.31)	<0.0001	0.94 (0.91-0.97)	<0.0001	0.35 (0.17-0.57)	0.17	0.87 (0.82-0.90)	<0.0001
CNS diseases except infection/immune-mediated CNS (<12 y)	291								
ADS (12-17 y) versus	160	0.63 (0.55-0.70)	0.002	0.96 (0.94-0.98)	<0.0001	0.85 (0.78-0.91)	<0.0001	0.87 (0.84-0.90)	<0.0001
CNS diseases except infection/immune-mediated CNS (12-17 y)	416								
Infection/immune-mediated CNS versus	204	0.14 (0.10-0.20)	<0.0001	0.95 (0.93-0.97)	<0.0001	0.46 (0.33-0.59)	0.61	0.79 (0.76-0.82)	<0.0001
CNS disease except ADS	707								
Infection/immune-mediated CNS (<12 y) versus	117	0.15 (0.09-0.22)	<0.0001	0.94 (0.91-0.97)	<0.0001	0.50 (0.32-0.68)	1.00	0.73 (0.68-0.78)	<0.0001
CNS disease except ADS (<12 y)	291								
Infection/immune-mediated CNS (12-17 y) versus	87	0.14 (0.07-0.23)	<0.0001	0.96 (0.94-0.98)	<0.0001	0.41 (0.23-0.61)	0.46	0.84 (0.81-0.87)	<0.0001
CNS disease except ADS (12-17 y)	416								

Abbreviations:
ADS = Acquired demyelinating syndromes
CNS = Central nervous system

infection or 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 or 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 2055 children with at least one OCB examination before age 18 years 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.[1] 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 to 17 years. The difference in OCB positivity by age at onset is presumably because ADEM is more frequent before age 12 years, 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 to 17 years, and 93% of children with MS were OCB positive.[10,15] 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.[15,38] Accordingly, OCB positivity

TABLE 3.
Repeated OCB Analyses Among 133 Children During 1995 to 2017

OCB Category	Children With >1 OCB Test, n	Time Between First and Last OCB Analysis, y, Mean (S.D.)	Diagnosis
Remained negative	91	2.0 (3.0)	Epilepsy (n = 3), sleep disorder (n = 2), malignancy (n = 2), movement disorder (n = 1), other CNS diseases (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)
Remained positive	20	1.8 (2.3)	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)
Positive to negative	11	2.6 (3.8)	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)
Negative to positive	7	1.6 (1.5)	Sleep disorder (n = 1), other CNS diseases (n = 1), ADS (n = 3), non-ADS immune-mediated CNS (n = 2)
Negative to positive to negative	3	—	Non-CNS (n = 1), immune-mediated CNS (n = 2)
Positive to negative to positive	1	—	Static encephalopathy (n = 1)
TOTAL	133 (6% total)	—	—

Abbreviations:
ADS = Acquired demyelinating syndromes
CNS = Central nervous system
OCB = Oligoclonal band

TABLE 4.
Medical-Record-Validated ADS by Onset Before and After Age 12 Years During 2008 to 2015

Diagnostic Group	Children, n (% Total)	Follow-up y, Median (Range)	OCB Positive, n (%)	CSF Leukocytes > 4, n (%)	OCB Positive and CSF Pleocytosis	OCB Positive and Normal CSF Leukocytes	OCB Negative and CSF Pleocytosis	IgG Index > 0.67, n (%)
ADS (<12 y)	29 (23%)	6.7 (2.9-11.2)	7/29 (24%)*	13/19 (68%)†	3/19 (16%)‡	1/19 (5%)§	10/19 (53%)‖	5/25 (20%)¶
ADS (12-17 y)	96 (77%)	6.4 (3.0-10.8)	65/96 (68%)*	43/69 (62%)†	37/69 (54%)‡	9/69 (13%)§	6/69 (9%)‖	38/89 (43%)¶
ADEM (<12 y)**	16 (13%)	6.6 (3.4–8.1)	0/16 (0%)	9/12 (75%)	0/12 (0%)	0/12 (0%)	9/12 (75%)	1/14 (7%)
ADEM (12-17 y)**	9 (7%)	7.6 (3.7-10.8)	2/9 (22%)	5/7 (71%)	1/7 (14%)	0/7 (0%)	4/7 (57%)	5/9 (56%)
CIS (<12 y)**	11 (9%)	6.1 (3.0-10.8)	5/11 (45%)	3/6 (50%)	2/6 (33%)	1/7 (17%)	1/6 (17%)	3/9 (33%)
CIS (12-17 y)**	23 (18%)	6.2 (3.0-10.6)	4/23 (17%)#	5/16 (31%)	4/16 (25%)	0/16 (0%)	1/16 (6%)	4/22 (18%)
MS (<12 y)	1 (1%)	7.9	1/1 (100%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
MS (12-17 y)	61 (48%)	6.5 (3.0-10.7)	57/61 (93%)	33/44 (74%)	31/43 (72%)	8/43 (19%)	1/43 (2%)	29/56 (52%)
NMOSD (<12 y)	1 (1%)	11.2	1/1 (100%)	—	—	—	—	1/1 (100%)
NMOSD (12-17 y)	3 (2%)	4.7 (3.0-5.0)	2/3 (66%)	1/3 (33%)	1/3 (33%)	1/3 (33%)	0/3 (0%)	0/2 (0%)

Abbreviations:
ADEM = Acute disseminated encephalomyelitis
CIS = Clinically isolated syndromes (non-ADEM)
CSF = Cerebrospinal fluid
Ig = Immunoglobulin
MRI = Magnetic resonance imaging
MS = Multiple sclerosis
NMOSD = Neuromyelitis optica spectrum disorders
OCB = Oligoclonal band
ON = Optic neuritis
TM = Transverse myelitis
* P value < 0.0001 for independence of OCB positivity among ADS (<12 y) versus ADS (12-17 y) using Fisher's exact test. Cell numbers comparing the specific ADS were too small for statistical testing.
† P value = 0.79 for independence of OCB positivity among ADS (<12 y) versus ADS (12-17 y) using Fisher's exact test.
‡ P value = 0.004 for independence of OCB positivity among ADS (<12 y) versus ADS (12-17 y) using Fisher's exact test.
§ P value = 0.68 for independence of OCB positivity among ADS (<12 y) versus ADS (12-17 y) using Fisher's exact test.
‖ P value < 0.0001 for independence of OCB positivity among ADS (<12 y) versus ADS (12-17 y) using Fisher's exact test.
¶ P value = 0.06 for independence of OCB positivity among ADS (<12 y) versus ADS (12-17 y) using Fisher's exact test.
# Due to the low percentage of children with OCB positivity in CIS (12-17 y), we stratified into the specific monophasic ADS (OCB positive/OCB total in parentheses): CIS and "dissemination in space" but not "dissemination in time" (4/5), isolated ON with normal MRI (0/16), and TM (0/2).
** Chart 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.

increases in line with the age-related risk of MS, a disease in which chronic intrathecal B-cell activation is a hallmark.[39]

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 to 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 to 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 or 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.[10,15] OCB examination may not provide diagnostic information in children younger than 12 years because the pretest

TABLE 5.
Medical-Record Validated ADS Cohort Compared With Infection/Non-ADS Immune CNS Diseases During 2008 to 2015 by Onset Before and After Age 12 Years

Diagnostic Group	Children	OCB Positive		CSF Leukocytes > 4		OCB Positive and CSF Pleocytosis		OCB Positive and Normal CSF Leukocytes		OCB Negative and CSF Pleocytosis		IgG Index > 0.67	
	n (% Total)	n/Total (%)	P Value*	n/Total (%)	P Value*	n/Total (%)	P Value*	n/Total (%)	P Value*	n/Total (%)	P Value*	n/Total (%)	P Value*
ADS	125 (54%)	72/125 (58%)	<0.0001	56/88 (64%)	0.74	40/88 (45%)	0.0001	10/88 (11%)	0.07	16/88 (18%)	<0.0001	43/114 (38%)	0.01
Infection/immune-mediated CNS	106 (46%)	16/106 (15%)		45/67 (67%)		11/67 (16%)		2/67 (3%)		34/67 (51%)		21/100 (21%)	
ADS (<12 y)	29 (13%)	7/29 (24%)	0.23	13/19 (68%)	1.00	3/19 (16%)	1.00	1/19 (5%)	1.00	10/19 (53%)	0.78	5/25 (20%)	1.00
Infection/immune (<12 y)	96 (42%)	7/54 (13%)		22/34 (65%)		6/34 (18%)		1/34 (3%)		16/34 (47%)		10/50 (20%)	
ADS (12-17 y)	54 (23%)	65/96 (68%)	<0.0001	43/69 (61%)	0.51	37/69 (54%)	0.0002	9/69 (13%)	0.16	6/69 (9%)	<0.0001	38/89 (43%)	0.02
Infection/immune (12-17 y)	52 (23%)	9/52 (17%)		23/33 (70%)		5/33 (15%)		1/33 (3%)		18/33 (55%)		11/50 (22%)	

Abbreviations:
ADS = Acquired demyelinating syndromes
CSF = Cerebrospinal fluid
Ig = Immunoglobulin
OCB = Oligoclonal band
* 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.

probability for MS is extremely low.[10] In such circumstances, other markers are more valuable in the diagnostic evaluation for differential diagnoses such as CSF leukocytes and brain magnetic resonance imaging. By contrast, OCB examination in children aged 12 to 17 years may be of diagnostic value because of the higher MS frequency.[10] 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 fact implies that OCB examination may still be indicated in individuals younger than 12 years if the pretest clinical suspicion of MS is high. Furthermore, whether to undertake OCB examination may also depend on the consequence of misdiagnosing a child, particularly regarding MS. Moreover, OCBs may facilitate an earlier MS diagnosis by demonstrating dissemination in time using the 2017 McDonald criteria (also applicable for children less than 12 years 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 multicentered. Denmark has a uniform, publicly financed health care 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 to 2015. 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 evaluation 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% to 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 two-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 to 17 years due to the increasing frequency of MS. In the clinical setting, OCB examination may be of higher yield in children aged 12 to 17 years if there is clinical suspicion of MS, and in such circumstances a positive test supports a diagnosis of MS.

Acknowledgments

References

1. Sinclair AJ, Wienholt L, Tantsis E, Brilot F, Dale RC. Clinical association of intrathecal and mirrored oligoclonal bands in paediatric neurology. Dev Med Child Neurol. 2013;55:71–75.
2. Fortini AS, Sanders EL, Weinshenker BG, Katzmann JA. Cerebrospinal fluid oligoclonal bands in the diagnosis of multiple sclerosis. Isoelectric focusing with IgG immunoblotting compared with high-resolution agarose gel electrophoresis and cerebrospinal fluid IgG index. Am J Clin Pathol. 2003;120: 672–675.
3. Krupp LB, Tardieu M, Amato MP, et al. International pediatric multiple sclerosis study group criteria for pediatric multiple sclerosis and immune-mediated central nervous system demyelinating disorders: revisions to the 2007 definitions. Mult Scler. 2013;19:1261–1267.
4. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol. 2011;69: 292–302.
5. Sadaka Y, Verhey LH, Shroff MM, et al. 2010 McDonald criteria for diagnosing pediatric multiple sclerosis. Ann Neurol. 2012;72:211–223.
6. Alper G, Heyman R, Wang L. Multiple sclerosis and acute disseminated encephalomyelitis diagnosed in children after long-term follow-up: comparison of presenting features. Dev Med Child Neurol. 2009;51:480–486.
7. Pohl D, Rostasy K, Reiber H, Hanefeld F. CSF characteristics in early-onset multiple sclerosis. Neurology. 2004;63:1966–1967.
8. Chabas D, Ness J, Belman A, et al. Younger children with MS have a distinct CSF inflammatory profile at disease onset. Neurology. 2010;74:399–405.
9. Heussinger N, Kontopantelis E, Gburek-Augustat J, et al. Oligoclonal bands predict multiple sclerosis in children with optic neuritis. Ann Neurol. 2015;77: 1076–1082.
10. Boesen MS, Magyari M, Koch-Henriksen N, et al. Pediatric-onset multiple sclerosis and other acquired demyelinating syndromes of the central nervous system in Denmark during 1977-2015: a nationwide population-based incidence study. Mult Scler J. 2018;24:1077–1086.
11. Mikaeloff Y, Caridade G, Husson B, Suissa S, Tardieu M, Neuropediatric KIDSEP Study Group of the French Neuropediatric Society. Acute disseminated encephalomyelitis cohort study: prognostic factors for relapse. Eur J Paediatr Neurol. 2007;11:90–95.
12. Tenembaum S, Chamoles N, Fejerman N. Acute disseminated encephalomy-elitis: a long-term follow-up study of 84 pediatric patients. Neurology. 2002;59:1224–1231.
13. Dale RC, Pillai SC. Early relapse risk after a first CNS inflammatory demyelination episode: examining international consensus definitions. Dev Med Child Neurol. 2007;49:887–893.
14. Atzori M, Battistella PA, Perini P, et al. Clinical and diagnostic aspects of multiple sclerosis and acute monophasic encephalomyelitis in pediatric patients: a single centre prospective study. Mult Scler Houndmills Basingstoke Engl. 2009;15:363–370.
15. Boesen MS, Magyari M, Koch-Henriksen N, et al. Implications of the Interna-tional paediatric multiple sclerosis study group consensus criteria for paedi-atric acute disseminated encephalomyelitis: a nationwide validation study. Dev Med Child Neurol. 2018;60:1123–1131.
16. Statistikbanken [Internet]. Available at: https://www.statistikbanken.dk/statbank5a/default.asp?w=1920. Accessed May 17, 2018.
17. Schmidt M, Pedersen L, Sørensen HT. The Danish Civil Registration System as a tool in epidemiology. Eur J Epidemiol. 2014;29:541–549.
18. Pedersen CB. The Danish Civil Registration System. Scand J Public Health. 2011;39(7 Suppl):22–25.
19. Thygesen LC, Daasnes C, Thaulow I, Brønnum-Hansen H. Introduction to Danish (nationwide) registers on health and social issues: structure, access, legislation, and archiving. Scand J Public Health. 2011;39(7 Suppl):12–16.
20. Lynge E, Sandegaard JL, Rebolj M. The Danish National Patient Register. Scand J Public Health. 2011;39(7 Suppl):30–33.
21. Andersen TF, Madsen M, Jørgensen J, Mellemkjoer L, Olsen JH. The Danish National Hospital Register. A valuable source of data for modern health sciences. Dan Med Bull. 1999;46:263–268.
22. Ben Ghezala I, Arendt JFB, Erichsen R, et al. Positive predictive value of the diagnosis coding for vitamin B12 deficiency anemia in the Danish National Patient Register. Clin Epidemiol. 2012;4:333–338.
23. Bork CS, Al-Zuhairi KS, Hansen SM, Delekta J, Joensen AM. Accuracy of angina pectoris and acute coronary syndrome in the Danish National Patient Register. Dan Med J. 2017;64(5).
24. Christensen J, Vestergaard M, Olsen J, Sidenius P. Validation of epilepsy diagnoses in the Danish National Hospital Register. Epilepsy Res. 2007;75: 162–170.
25. Djurhuus BD, Skythe A, Faber CE. Validation of the cholesteatoma diagnosis in the Danish National Hospital Register. Dan Med Bull. 2010;57:A4159.

26. Dydensborg Sander S, Størdal K, Plato Hansen T, et al. Validation of celiac disease diagnoses recorded in the Danish National Patient Register using duodenal biopsies, celiac disease-specific antibodies, and human leukocyte-antigen genotypes. Clin Epidemiol. 2016;8:789–799.

27. Hansen DL, Overgaard UM, Pedersen L, Frederiksen H. Positive predictive value of diagnosis coding for hemolytic anemias in the Danish National Patient Register. Clin Epidemiol. 2016;8:241–252.

28. Lasota AN, Overvad K, Eriksen HH, Tjønneland A, Schmidt EB, Grønholdt M-LM. Validity of peripheral arterial disease diagnoses in the Danish National Patient Registry. Eur J Vasc Endovasc Surg. 2017;53:679–685.

29. Lühdorf P, Overvad K, Schmidt EB, Johnsen SP, Bach FW. Predictive value of stroke discharge diagnoses in the Danish National Patient Register. Scand J Public Health. 2017;45:630–636.

30. Mason K, Thygesen LC, Stenager E, Brønnum-Hansen H, Koch-Henriksen N. Evaluating the use and limitations of the Danish National Patient Register in register-based research using an example of multiple sclerosis. Acta Neurol Scand. 2012;125:213–217.

31. Wermuth L, Lassen CF, Himmerslev L, Olsen J, Ritz B. Validation of hospital register-based diagnosis of Parkinson's disease. Dan Med J. 2012;59:A4391.

32. Boesen MS, Magyari M, Born AP, Thygesen LC. Pediatric acquired demyelinating syndromes: a nationwide validation study of the Danish National Patient Register. Clin Epidemiol. 2018;2018:391–399.

33. Boesen MS, Jensen PEH, Magyari M, et al. Increased cerebrospinal fluid chitinase 3-like 1 and neurofilament light chain in pediatric acquired demyelinating syndromes. Mult Scler Relat Disord. 2018;24:175–183.

34. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol. 2005;58:840–846.

35. Wingerchuk DM, Banwell B, Bennett JL, et al. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology. 2015;85:177–189.

36. Deisenhammer F, Bartos A, Egg R, et al. Guidelines on routine cerebrospinal fluid analysis. Report from an EFNS task force. Eur J Neurol. 2006;13: 913–922.

37. Bhaskaran K, Smeeth L. What is the difference between missing completely at random and missing at random? Int J Epidemiol. 2014;43:1336–1339.

38. Renoux C, Vukusic S, Mikaeloff Y, et al. Natural history of multiple sclerosis with childhood onset. N Engl J Med. 2007;356:2603–2613.

39. Bar-Or A, Fawaz L, Fan B, et al. Abnormal B-cell cytokine responses a trigger of T-cell-mediated disease in MS? Ann Neurol. 2010;67:452–461.

40. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. 2018;17:162–173.

41. Safety and Efficacy of Fingolimod in Pediatric Patients With Multiple Sclerosis - Full Text View - ClinicalTrials.gov [Internet]. Available at: https://clinicaltrials. gov/ct2/show/NCT01892722. Accessed June 30, 2018.

42. Graus F, Titulaer MJ, Balu R, et al. A clinical approach to diagnosis of autoimmune encephalitis. Lancet Neurol. 2016;15:391–404.

43. Hennes E-M, Baumann M, Schanda K, et al. Prognostic relevance of MOG antibodies in children with an acquired demyelinating syndrome. Neurology. 2017;89:900–908.

44. Ketelslegers IA, Van Pelt DE, Bryde S, et al. Anti-MOG antibodies plead against MS diagnosis in an Acquired Demyelinating Syndromes cohort. Mult Scler Houndmills Basingstoke Engl. 2015;21:1513–1520.

45. Hacohen Y, Mankad K, Chong WK, et al. Diagnostic algorithm for relapsing acquired demyelinating syndromes in children. Neurology. 2017;89:269–278.