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Pediatric autoimmune encephalitis in Denmark during 2011–17: A nationwide multicenter population-based cohort study

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ABSTRACT

Background: The incidence of pediatric autoimmune encephalitis (AIE) is unknown. Our aim was to assess the incidence of pediatric AIE in Denmark 2011–17.

Methods: In a nationwide population-based setting, we retrieved data on all children tested for AIE before age 18 years. We reviewed medical records in a) children with AIE antibodies (n = 18) to assess whether children fulfilled the AIE consensus criteria, b) children tested negative for AIE antibodies who were registered with an AIE diagnostic code to estimate the incidence of "antibody negative but probable AIE", and c) a reference cohort (n = 596) to determine the positive predictive value of International Classification of Diseases (ICD) codes used for anti-NMDAR encephalitis.

Results: 375 children were tested for AIE 2011–17 (median age 11.1 years; 54% girls); 18 children (5%) had AIE antibodies (percentage tested positive): CSF GAD_{65}-IgG (3.1%), plasma NMDAR-IgG (2.8%), CSF NMDAR-IgG (1.8%), plasma GAD_{65}-IgG (1.0%), and plasma CASPR2-IgG (0.4%). Five children fulfilled the criteria for probably/definite anti-NMDAR encephalitis (incidence: 0.07/100,000 person-years; 95% CI = 0.03–0.17), and 4 children with anti-GAD_{65} associated AIE (incidence = 0.055/100,000 person-years, 95% CI = 0.021–0.15). The incidence of "antibody negative but probable AIE" was 0.055/100,000 person-years (95% CI = 0.021–0.15). The positive predictive value of ICD diagnostic codes used for anti-NMDAR encephalitis was 8%.

Conclusions: We diagnosed only children with anti-NMDAR, anti-GAD_{65}, and "antibody negative but probable AIE". Before examining AIE antibodies, clinical presentation, paraclinical studies (CSF, EEG, and MRI), and incidence of pediatric AIEs should be considered. Updating the ICD to include AIE codes is warranted.

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Abbreviations

AMPAR1/2	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor 1 and 2
ADEM	Acute disseminated encephalomyelitis
AIE	Autoimmune encephalitis
CSF	Cerebrospinal fluid
CI	Confidence interval
Caspr2	Contactin-associated protein-like 2
DPPX	Dipeptidyl-peptidase–like protein 6
EEG	Electroencephalogram
GAD_{65}	Glutamic acid decarboxylase 65 kilodalton
Ig	Immunoglobulin
IgLON5	IgLON family member 5
ICD	International Classification of Diseases
LGI1	Leucine-rich glioma inactivated 1
MRI	Magnetic resonance imaging
MOG	Myelin oligodendrocyte glycoprotein
MS	Multiple sclerosis
NMDAR	N-methyl-D-aspartate receptor
$GABA_BR$	γ-aminobutyric-acid B receptor
PPV	Positive predictive value
RU	Relative units
VGKC	Voltage-gated potassium channel complex

1. Introduction

Autoimmune encephalitis (AIE) is a rapidly progressive encephalopathy caused by antibody-mediated neuroinflammation.[1,2] It is increasingly recognized in children but diagnosis is challenging before age 18 years owing to a) numerous differential diagnoses, b) low incidence, and c) changes of mood, behavior, and personality, possibly coinciding with puberty.[2] Because AIE can resemble acute psychosis and schizophrenia, children are sometimes referred to psychiatric services, causing concern about misdiagnosis of children with acute psychosis.

In children, encephalitis associated with antibodies against *N*-methyl-D-aspartate receptor (NMDAR), γ-aminobutyric acid A receptor ($GABA_AR$), and possibly glutamic acid decarboxylase 65 kilodalton (GAD_{65}) are the most frequent.[3–6] Anti-NMDAR encephalitis is caused by immunoglobulin G (IgG) anti-GluN1 antibodies.[2] It causes rapid onset (less than 3 months) of psychosis, memory deficits, seizures, and language disintegration with progression to unresponsiveness with catatonic features often associated with abnormal movements and autonomic dysfunction.[2] $GABA_AR$ is a ligand-gated chloride channel that facilitates fast inhibitory synaptic transmission in the CNS, and anti-$GABA_AR$ encephalitis is associated with seizures, alteration of cognition, behavior, consciousness, and abnormal movements.[6] Conversely, anti-GAD_{65} associated neurological disease is caused by autoantibodies against the rate-limiting enzyme for the synthesis of GABA, the major inhibitory neurotransmitter in the CNS.[4] Although controversy regarding the pathogenic effect of anti-GAD_{65} antibodies exists, several neurological phenotypes of anti-GAD_{65} associated neurological disease have been described. These include stiff-person syndrome, limbic encephalitis, or cerebellitis, the latter being a common manifestation of encephalitis in children, and all are associated with very high anti-GAD_{65} titers.[4] Lastly, cases of AIE with no well-characterized antibodies—"antibody negative but probable AIE"—also occur in children.[2]

In a nationwide population-based setting, our aim was to a) determine the pediatric AIE incidence in Denmark during 2011–17 because the incidence is a key component of the pretest probability of AIE in children and should be considered before AIE testing; b) assess the AIE antibody testing pattern; and c) determine the positive predictive value of International Classification of Diseases (ICD) codes for anti-NMDAR encephalitis.

2. Patients and methods

2.1. Study population

We included children who underwent AIE testing before 18 years of age in Denmark during 2011–2017 (Fig. 1, flow chart). Autoimmune antibody examination in Denmark was analyzed at one national reference laboratory (Statens Serum Institute, Copenhagen) during the study period. Denmark has a relatively homogenous Caucasian population with a mid-year population in the year 2014 of 5,667,243 persons (21% younger than 18 years).[7]

2.2. CSF and serum testing, including AIE antibodies

AIE antibodies against the following proteins were tested in serum and/or CSF in the study population: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor 1 and 2 (AMPAR1/2), contactin-associated protein-like 2 (Caspr2), dipeptidyl-peptidase–like protein 6 (DPPX), γ-aminobutyric-acid B receptor ($GABA_BR$), IgLON family member 5 (IgLON5), leucine-rich glioma inactivated 1 (LGI1), GAD_{65}, and NMDAR. Anti-$GABA_AR$ antibodies were not part of the diagnostic AIE test packages used in Denmark during the study period.

The assays for AIE were based on commercially available Biochip mosaics (Euroimmun, Lübeck, Germany) that contain recombinant cell substrates (formalin- or acetone-fixed recombinant HEK293 cells), each expressing a different neural antigen (NMDAR, LGI1, Caspr2, $GABA_BR$, AMPAR1/2, DPPX, or IgLON5). Expression of the individual recombinant protein (autoantigen) has been validated by immunological methods using human or commercially available monospecific animal antibodies. Myelin oligodendrocyte glycoprotein (MOG) antibody testing in Denmark was undertaken only by cell-based assay at either John Radcliffe Hospital (Oxford, United Kingdom), or Statens Serum Institute (Copenhagen, Denmark).[8] Anti-GAD_{65} was measured using radioimmunoassay.

All antibody test results (except anti-GAD_{65}) were reported on an ordinal scale as 'negative', 'grey zone', 'weakly positive', 'moderately positive', or 'strongly positive' based on visual evaluation. Positive test results were not confirmed by confirmatory tests (e.g. live neurons or tissue

immunohistochemistry).[2] For anti-GAD_{65}, antibody testing was reported in relative units (RU)/mL. We reviewed the medical record in children with anti-GAD_{65} levels in serum over 127 relative units (RU)/mL or CSF over 100 RU/mL. We were unable to calculate the index for intrathecal synthesis of IgG against anti-GAD_{65} antibodies (anti-GAD_{65}-specific IgG index) owing to missing data on either anti-GAD_{65} or albumin in serum or CSF.[9] Serum and CSF underwent viral screening including herpes simplex, varicella, Epstein-Barr Virus, cytomegalovirus, and enterovirus.

2.3. Data sources

2.3.1. The Danish Civil Registration System

The Danish Civil Registration System was established in 1968 as a register of residents in Denmark.[10,11] In Denmark, every resident at birth or on immigration receives a unique personal identification number which is linked with all national registers including health registers.[12] We used the children's personal identification number to link biomarker analyses with hospital diagnoses in the National Patient Register.

2.3.2. The National Patient Register

The Danish National Patient Register is a nationwide register with routinely collected administrative and health-related data on all hospital admissions in Denmark since 1977.[13] Data include date of admission and diagnoses according to the ICD-8 (1977–1993) and ICD-10 (1994 until today—ICD-9 was never used). On hospital discharge, physicians code each patient by diagnosis with one primary diagnosis and, if relevant, one or more secondary diagnoses. Since 1994, diagnoses at ambulatory hospital consultations have been included. 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.[14] Private consultant physicians (e.g., general practitioners, neurologists, and ophthalmologists) and private hospitals in Denmark play a minor role in management of acute medical conditions in children; accordingly, children suspected of AIE are referred only to public hospitals.

The National Patient Register has been validated in several studies with acceptable agreement between diagnostic codes and medical record-validated diagnoses.[10,14–25]

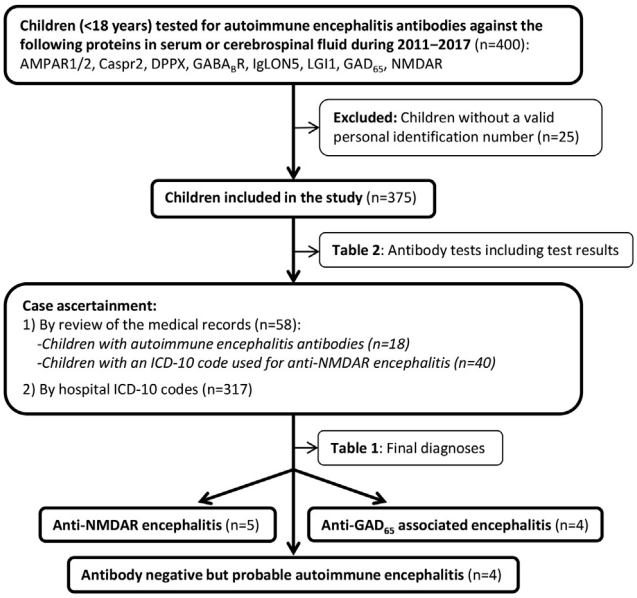

Fig. 1 – Flow chart.

2.4. Diagnostic groups

2.4.1. Medical record-validated case ascertainment

We reviewed the medical record in children tested for AIE antibodies who had a) presence of AIE antibodies (18 children), or b) an ICD-10 code used for medical record-validated anti-NMDAR encephalitis (40 children). As there are no specific codes for AIE in the ICD-10, we identified the codes that children with medical record-validated anti-NMDAR encephalitis in the National Patient Register were given (terms in parentheses): *G04.9* ("encephalitis and encephalomyelitis, unspecified"), *G05.1* ("encephalitis, myelitis and encephalomyelitis in viral diseases classified elsewhere"), *G05.2* ("encephalitis, myelitis and encephalomyelitis in other infectious and parasitic diseases classified elsewhere"), *G05.8* ("encephalitis, myelitis and encephalomyelitis in other diseases classified elsewhere"), and *A86* ("unspecified viral encephalitis"). By applying these codes to the remaining children tested for AIE antibodies, we identified a cohort of 40 children who did not have AIE antibodies but was registered with one of these codes. We reviewed their medical records to estimate the incidence of "antibody negative but probable AIE".[2]

For the chart review we collected data on date of onset, symptoms, treatment, clinical evolution and whether the children fulfilled the diagnostic criteria for AIE proposed by Graus et al.[2] Further, we used a medical record-validated reference cohort of children suspected of neurological disease (n = 596) during 2008–2016 from our previous publications to determine the positive predictive value of ICD-10 codes used for anti-NMDAR encephalitis.[25–27]

2.4.2. Register-based case ascertainment

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 AIE antibody testing. Both somatic and psychiatric diagnoses from hospital admissions and outpatient hospital visits were included. The diagnostic groups and concordant ICD-10 codes were defined *pre hoc*.

We grouped children into 'CNS disease' and 'non-CNS disease'. Further, we divided CNS diseases into the following groups: acquired demyelinating syndromes (e.g. acute disseminated encephalomyelitis [ADEM], multiple sclerosis [MS] as defined in previous papers[25,26,28]), immune-mediated CNS disease (e.g. Rasmussen's Syndrome), CNS infection (e.g. viral or bacterial meningoencephalitis), epilepsy, sleep disorders (e.g. narcolepsy), CNS malignancy, movement disorders (e.g. spinocerebellar ataxia), 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). Medical record-validated diagnoses overruled the register-based diagnoses.

2.5. Statistical methods

Age- and sex-specific incidence rates and 95% confidence interval (CI) were calculated by assuming Poisson distribution. The assumptions for the Poisson regression were met because (1) observations were independent, (2) the number of children at risk of AIE was large (approximately 1.2 million children), and (3) the probability of acquiring AIE was small.

We estimated the positive predictive value (PPV) of presence of IgG anti-NMDAR or anti-GAD_{65} antibodies for medical record-validated anti-NMDAR or anti-GAD_{65} associated encephalitis. Further, we calculated the PPV of ICD-10 codes used for anti-NMDAR encephalitis by applying these codes to a reference cohort of 596 children with medical record-validated diagnoses who were initially referred to hospital due suspected of neurological disease.

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

2.6. Standard protocol approvals, registrations, and patient consents

The study was approved by the Danish Data Protection Agency at Copenhagen University Hospital (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 approved access to patient data from their hospital.

3. Results

We included all children (<18 years) with an AIE antibody test from November 2, 2011 to December 27, 2017. We excluded children without a valid personal identification number (n = 25). In total, 375 children were included in the study (Fig. 1, flow chart).

The median age at AIE antibody testing was 11.0 years (range = 0.2–18.0, Table 1); 201 (54%) were girls. The diagnostic groups most frequently tested for AIE were non-CNS disease (n = 110), other CNS diseases (n = 52), static encephalopathy (n = 52), epilepsy (n = 40), CNS infections (n = 38), non-AIE immune-mediated CNS disease (n = 34), and acquired demyelinating syndromes (n = 25). In total, 22% of children tested for AIE antibodies had a psychiatric hospital admission or ambulatory visit up to two months before the AIE examination.

3.1. Autoimmune antibody test results

Eighteen children (5%) had AIE antibodies in either serum or CSF (proportion and percentage of positive test results in parentheses): plasma GAD_{65}-IgG (3/244 = 1.0%), CSF GAD_{65}-IgG (9/261 = 3.1%), plasma NMDAR-IgG (8/244 = 2.8%), CSF NMDAR-IgG (5/267 = 1.8%), and plasma Caspr2-IgG (1/241 = 0.4%) (Table 2). Antibodies against the following proteins in both plasma and CSF were negative only: AMPAR1/2, DPPX, $GABA_BR$, IgLON5, and LGI1.

The number of children tested for IgG anti-NMDAR antibodies increased considerably from 2012 to 2017, even though the Danish population younger than 18 years remained constant during the same period (Fig. 2). However, the number of children diagnosed with anti-NMDAR encephalitis did not increase accordingly.

3.2. Medical record-validated pediatric autoimmune encephalitis in Denmark during 2011–17

3.2.1. Anti-NMDAR encephalitis

Five children, all girls, had medical record-validated probably/definite anti-NMDAR encephalitis (Table 3), equivalent to an incidence rate of 0.07/100,000 person-years (95% CI = 0.03–0.17; girls: 0.14, 95% CI 0.06–0.34). Two of the girls (40%) were initially admitted to a psychiatric ward due to mood disorders and hallucinations (cases 14 and 17). Only one of the 5 children had an ovarian teratoma (case 3). The EEG was abnormal in 4/5 (80%), including focal and generalized slowing, and delta waves, and all brain MRIs were normal. All five girls with anti-NMDAR encephalitis recovered well on follow-up (one girl without receiving immunosuppressive treatment because the medical doctors did not diagnose her with anti-NMDAR encephalitis). One child had anti-NMDAR antibodies in serum only (case 12), and one child had antibodies in CSF only (case 14). Interestingly, 3 children without anti-NMDAR encephalitis (cases 2, 6, and 15) had weakly positive anti-NMDAR IgG antibodies in serum but not in the CSF, and one of these children had a pontine glioma.

Table 1 – Children tested for autoimmune encephalitis antibodies by sex, age at onset, and discharge diagnoses in the Danish National Patient Register during 2011–17.

Diagnostic groups	Children, n	Female, n (%)	Age at antibody examination, median (range)
Total	375	201 (54)	11.0 (0.2–18.0)
Acquired demyelinating syndromes	25	11 (44)	8.4 (2.5–15.4)
CNS infection	38	17 (45)	10.3 (0.8–16.9)
Immune-mediated CNS disease	34	23 (68)	8.6 (0.9–17.6)
CNS malignancy	5	2 (40)	8.8 (3.8–14.3)
Static encephalopathy	52	26 (50)	9.9 (0.2–17.2)
Cerebrovascular disease	5	4 (80)	5.3 (0.7–7.4)
Movement disorders	9	7 (78)	10.6 (1.8–16.2)
Epilepsy	40	16 (40)	7.4 (0.5–17.9)
Sleep disorders	5	3 (60)	13.3 (6.7–15.2)
Other CNS diseases	52	25 (48)	13.4 (1.6–17.6)
Non-CNS diseases	110	67 (61)	12.8 (0.7–18.0)

Table 2 – Positive and negative autoimmune encephalitis antibody tests in children in Denmark 2011–17.

Biomarker	Analyses	Children<18 y tested	Positive tests, n (%)
CSF			
NMDAR-IgA	163	154	1 (0.6%)
NMDAR-IgG	284	267	5 (1.8%)[d]
NMDAR-IgM	138	130	2 (1.4%)
GAD_{65}-IgG	290	261	9 (3.1%)[b,d]
AMPAR1-IgA[a]	160	152	0
AMPAR1-IgG[a]	224	209	0
AMPAR1-IgM[a]	138	130	0
AMPAR2-IgA[a]	160	152	0
AMPAR2-IgG[a]	224	209	0
AMPAR2-IgM[a]	138	130	0
Caspr2-IgA	138	130	0
Caspr2-IgG	278	263	0
Caspr2-IgM	138	130	0
DPPX-IgG	15	15	0
$GABA_BR$-IgA	138	130	0
$GABA_BR$-IgG	226	211	0
$GABA_BR$-IgM	138	130	0
IgLON5-IgG	14	14	0
LGI1-IgA	138	130	0
LGI1-IgG	278	263	0
LGI1-IgM	138	130	0
Serum			
NMDAR-IgA	155	138	0
NMDAR-IgG	285	244	8 (2.8%)[d]
NMDAR-IgM	124	111	0
GAD_{65}-IgG	293	244	3 (1.0%)[c,d]
AMPAR1-IgA[a]	154	137	0
AMPAR1-IgG[a]	237	201	0
AMPAR1-IgM[a]	124	111	0
AMPAR2-IgA[a]	154	137	0
AMPAR2-IgG[a]	236	201	0
AMPAR2-IgM[a]	124	111	0
Caspr2-IgA	124	111	0
Caspr2-IgG	280	241	1 (0.4%)
Caspr2-IgM	124	111	0
DPPX-IgG	7	7	0
$GABA_BR$-IgA	124	111	0
$GABA_BR$-IgG	237	202	0
$GABA_BR$-IgM	124	111	0
IgLON5-IgG	7	7	0
LGI1-IgA	124	111	0
LGI1-IgG	280	241	0
LGI1-IgM	124	111	0

[a] Initial assays for AMPAR1 and AMPAR2 did not distinguish between the two antibodies, and a negative test would be counted as negative for both AMPAR1 and AMPAR2.
[b] Positive anti-GAD_{65} in cerebrospinal fluid>100 RU/mL.
[c] Positive anti-GAD_{65} in serum>127 RU/mL.
[d] One child with GAD_{65}-IgG and one child with NMDAR-IgG were tested positive twice.

As 8 children had anti-NMDAR IgG antibodies in either serum or CSF, the PPV was 5/8 (0.63; 95% CI = 0.25–0.91) for children with IgG anti-NMDAR antibodies truly having medical record-validated anti-NMDAR encephalitis. All 4 children with anti-NMDAR IgG antibodies in CSF had anti-NMDAR encephalitis (PPV = 1.00, 95% CI = 0.40–1.00), regardless of the level of antibody positivity. Seven children had anti-NMDAR IgG antibodies in serum, but only 4 children had medical record-validated anti-NMDAR encephalitis (PPV = 0.57, 95% CI = 0.18–0.94). However, when excluding the 4 children with "weakly positive" serum antibodies, the PPV increased to 1.00 (95% CI = 0.29–1.00).

3.2.2. *Anti-GAD_{65} associated encephalitis*

Among the 9 children with IgG anti-GAD_{65} antibodies>100 RU/mL in CSF, 4 children had medical record-validated encephalitis (PPV = 0.44, 95% CI = 0.12–0.79 and sensitivity = 1.00; 95% CI= CI=0.40–1.00). CSF levels ranged from 104 to 111 RU/mL. The 4 children presented with limbic encephalitis (case 1); confusion, truncal instability, choreoathetotic movements, and speech dysfunction (case 5); truncal instability and reduced consciousness (case 11); and urinary retention, photophobia and reduced consciousness (case 13). CSF leucocytes were normal in two children (cases 1 and 5), one had 13 CSF leucocytes (case 11), and one had 22 CSF leucocytes (case 13). CSF oligoclonal bands were normal in one child (case 5) but not investigated in the other children. EEG was abnormal in 3 children (cases 1, 11, and 13) and brain MRI was abnormal in one child showing cerebellar atrophy (case 5). None of the children had insulin-dependent diabetes mellitus, but the child with the highest anti-GAD_{65} IgG level in serum (2358 RU/mL) had pediatric acute-onset neuropsychiatric syndrome and was diagnosed with insulin-dependent diabetes mellitus 6 months later (case 9). However, presence of anti-GAD_{65} IgG antibodies>100 RU/mL in the CSF was also discovered in children with upper respiratory tract infection (case 4), Kleine-Levin syndrome (case 10), MS (case 16), and radiologic isolated syndrome (case 18). The incidence rate of anti-GAD_{65} associated encephalitis was 0.055/100,000 person-years (95% CI = 0.021–0.15; boys: 0.081, 95% CI = 0.026–0.25; girls: 0.028, 95% CI = 0.0040–0.20).

3.2.3. *"Antibody negative but probable autoimmune encephalitis"*

After excluding children with medical record-validated anti-NMDAR encephalitis and anti-GAD_{65} associated encephalitis, we found that 40 children were registered in the National Patient Registry with at least one of the ICD-10 codes used for anti-NMDAR encephalitis (ICD-10 codes: G04.9, G05.1, G05.2, G05.8, or A86). After reviewing their medical records, 4 children fulfilled the consensus criteria for "antibody negative but probable autoimmune encephalitis",[2] equivalent to an incidence rate of 0.055/100,000 person-years (95% CI = 0.021–0.15) during 2011–17 (case descriptions in Table 4). All the four children experienced neurological symptoms during or following an infection. One child with anti-Caspr2 antibodies in serum was diagnosed with Kleine-Levin syndrome (case 7—voltage-gated potassium channel complex [VGKC] antibodies were not tested).

3.3. *Diagnostic codes used for medical record-validated anti-NMDAR encephalitis*

In the medical record-validated reference cohort of children with suspected CNS diseases (n = 596 children), we identified 86 children in the Danish National Patient Register registered with ICD-10 codes used for anti-NMDAR encephalitis (G04.9, G05.1, G05.2, G05.8, or A86), and two additional children had anti-NMDAR encephalitis (both diagnosed in year 2010, hence not included in our incidence estimate). The remaining 84 children had the following diagnoses (descending order, only largest

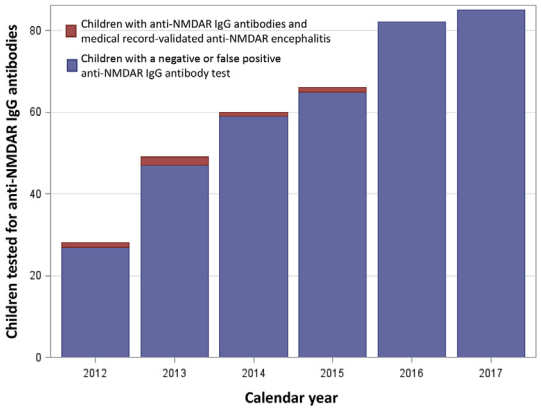

Fig. 2 – Children tested for anti-NMDAR IgG antibodies in relation to children with medical record-validated anti-NMDAR encephalitis during 2012–17.

groups listed): encephalitis (e.g. infection or unknown pathogen, n = 33), unspecified non-neurological disorder (n = 8), ADEM (n = 7), cerebellitis (n = 5), epilepsy (n = 4), and meningitis (n = 4). We calculated the positive predictive value as 7/84 = 0.08 (95% CI = 0.03–0.15)—accordingly, the codes used for anti-NMDAR encephalitis (G04.9, G05.1, G05.2, G05.8, or A86) had a low positive predictive value for medical record-validated anti-NMDAR encephalitis.

3.4. *Anti-MOG antibodies and anti-aquaporin-4 antibodies in children with ADEM*

In the 'demyelinating cohort' consisting of 25 children, 10 children were diagnosed with ADEM. Among these, 8/10 (80%) were tested for anti-aquaporin-4, and 3/10 (33%) children were tested for anti-MOG antibodies. None of the children had either anti-aquaporin-4 or anti-MOG antibodies.

4. Discussion

We found that awareness of AIE in Denmark increased during the study period from 2011 to 2017, with an increasing number of AIE antibody tests being requested but an unchangingly low number of children diagnosed with AIE. Among children tested for AIE antibodies, 22% had a psychiatric hospital visit up to 2 months before the test requisition. Anti-NMDAR and anti-GAD_{65} associated encephalitis were the only AIE identified in our cohort and both were rare. All children with anti-NMDAR encephalitis were girls and one child (20%) had an ovary teratoma. The positive predictive value of anti-NMDAR encephalitis ICD-10 codes was only 8%. None of the children with ADEM had anti-MOG or anti-aquaporin-4 antibodies, although few children with ADEM were tested for anti-MOG antibodies.

In line with previous findings, CSF NMDAR-IgG was highly specific for anti-NMDAR encephalitis with 100% PPV in both CSF and serum, after exclusion of children with "weakly positive" serum IgG anti-NMDAR levels. Interestingly, we identified a girl with probable anti-NMDAR encephalitis who had IgG anti-NMDAR only in plasma but not in CSF (case 12). According to Dalmau et al.,[29] this is a rare finding. Despite not receiving immunosuppressive treatment, she recovered completely. Apart from this, our findings that anti-NMDAR predominantly occurs in girls and that approximately 20% have an ovarian teratoma are in line with the literature.[29] To our knowledge, only one pediatric anti-NMDAR incidence study has been published, showing an incidence of anti-NMDAR encephalitis of 0.085/100,000 (95% CI = 0.64–1.06) among children in the United Kingdom.[30] It seems that anti-NMDAR encephalitis is the most frequent pediatric AIE.[31] Further, the incidence of anti-NMDAR encephalitis in both children and adults in the United States during 1995–2015 was 0.03/100,000, but it was only based on one person with anti-NMDAR encephalitis and no CI was reported.[32] In line, we found that anti-NMDAR was the most frequent pediatric AIE, with an incidence of 0.07/100,000 (95% CI = 0.03–0.17). However, pediatric anti-NMDAR encephalitis (and AIE in general) is exceptional, implying that the pre-test probability of AIE in children is low, and this should be considered before examining AIE antibodies in children. Nevertheless, AIE (and in particular anti-NMDAR encephalitis) should be carefully excluded following herpes simplex infections because AIE occurs in 27% of patients following herpes simplex encephalitis.[33]

In contrast, association of encephalitis to increased CSF anti-GAD_{65} antibodies was more uncertain, and presence of anti-GAD_{65} antibodies may have been coincident with the encephalitis symptoms. CSF anti-GAD_{65} antibodies were only marginally elevated and, if ignoring anti-GAD_{65} levels, none of the four children with anti-GAD_{65} associated encephalitis fulfilled the consensus criteria for "antibody negative but probable AIE".[2] Further, only one child (who had insulin-dependent diabetes mellitus but not encephalitis) had increased serum anti-GAD_{65} levels according to Saiz et al.[4] Studies have explored the association between high levels of anti-GAD_{65} in serum or albumin-corrected anti-GAD_{65} index ratio and stiff-person syndrome and cerebellar ataxia, but the clinical significance of anti-GAD_{65} in children remains unclear.[4,9,34–36] Further, anti-GAD_{65} antibodies may be markers of underlying autoimmune processes, and these patients may have other, unexplored autoimmune antibodies. Importantly, children tested negative for the currently available AIE antibody panels may still benefit from immune-targeted therapies.[31]

Despite appropriate microbiological evaluation, a bacterial or viral pathogen has not been identified in at least 50% of children with encephalitis, and ADEM may represent as many as 15% of these cases.[37] In these children, evaluation of anti-MOG antibodies is important because it may stratify subsequent risk for relapse and MS.[38–40] However, although rare, anti-NMDAR, anti-$GABA_AR$ and anti-GAD_{65} associated encephalitis should also be considered.[5,6] Further, other differential diagnoses, such as drug abuse, neuroleptic malignant syndrome, late onset autism, Kleine-Levin syndrome, and metabolic disorders, should be excluded.[41] We have the following three suggestions for clinical application of our results:

1) Anti-NMDAR encephalitis versus acute onset psychiatric disease: We show that the incidence of pediatric AIE including anti-NMDAR encephalitis is low and—although also rare—is manifold lower than childhood onset psychiatric conditions such as schizophrenia.[42] However, correct diagnosis of anti-NMDAR encephalitis and adequate

Table 3 – Review of children with autoimmune encephalitis antibodies in Denmark 2011–17.

Case	Negative biomarkers (class)[a]	Positive biomarkers (value)	History, paraclinical findings, and treatment	IDDM	Cancer	Diagnosis	Fulfillment of AIE criteria
1	CSF: AMPAR1/2 (IgA, IgG, IgM), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), LGI1 (IgA, IgG, IgM), NMDAR (IgA, IgG, IgM) Serum: AMPAR1/2 (IgA, IgG, IgM), AQP4 (IgG), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), LGI1 (IgA, IgG, IgM), NMDAR1 (IgG)	GAD_{65}-IgG in CSF (104 RU/mL), GAD_{65}-IgG in serum (1025 RU/mL)	8-year-old boy presented with a generalized tonic-clonic seizure. In the year before the admission his behavior had been increasingly impulsive, aggressive and hypermotoric (particularly at night). Further, he had had problems with sleep and concentration, anxiety, difficulty socializing with peers, weight gain (10 kg), and episodes of memory loss. During hospital admission, his EEG was abnormal with sharp waves and 3-4 Hz slow waves in the right temporal region; brain MRI and CSF examination were normal, except GAD_{65}-IgG in CSF of 104 RU/mL, and GAD_{65}-IgG in serum was 1025 RU/mL (CSF OCBs were not tested). He was treated with oxcarbamazepine, levetiracetam, clobazam, immunoglobulins, plasma exchange, and rituximab. His symptoms gradually remitted with complete remission 30 months after onset.	No	No	Anti-GAD_{65} associated encephalitis	Yes, working memory deficits, seizures, and psychiatric symptoms. Normal MRI (bad corporation), CSF pleocytosis, abnormal EEG and anti-GAD_{65} IgG in plasma and CSF
2	CSF: AMPAR1/2 (IgG), AQP4 (IgG), Caspr2 (IgG) DPPX (IgG), $GABA_BR$ (IgG), IgLON5 (IgG) LGI1 (IgG), MOG (IgG), NMDAR (IgG) OCBs, IgG index Serum: AQP4 (IgG), $GABA_BR$ (IgG), GAD_{65} (IgG), MOG (IgG)	NMDAR-IgG serum ("weakly positive")	12-year-old girl presented with right ON. Her brain MRI showed edema of the optic nerve and her CSF examination was normal. She received high-dose steroids. Seven months later she experienced a left ON, and her CSF examination was normal (including negative anti-MOG, anti-AQP4 and anti-NMDAR IgG), but she had anti-NMDAR IgG antibodies in serum. MRI of the brain and spinal cord was normal, and she was treated with high-dose steroids.	No	No	Optic neuritis (recidivating)	No
3	CSF: AMPAR1/2 (IgA, IgG, IgM), Caspr2 (IgA, IgG, IgM), GABAR1 (IgA, IgG, IgM), GAD_{65} (IgG), LGI1 (IgA, IgG, IgM), OCBs, IgG index Serum: AMPAR1/2 (IgA, IgG, IgM), AQP4 (IgG), Caspr2 (IgA, IgG, IgM), $GABA_BR1$ (IgA, IgG, IgM), GAD_{65} (IgG), LGI1 (IgA, IgG, IgM)	NMDAR-IgG CSF ("strongly positive"), NMDAR-IgG serum ("moderately positive")	15-year-old girl was admitted to hospital with abdominal filling (later diagnosed as ovary teratoma, grade 1). The month before admission she experienced gradual progression of episodes with confusion, problems with concentration and sleep, memory loss, aggressive and emotional behavior, and paranoid delusions. She had started to repeat herself and exhibited speech latency. She felt she was "beside herself". Routine CSF examination (including CSF OCBs) and brain MRI two months after onset of symptoms were normal. However, EEG was abnormal but without paroxysmal activity, and she had anti-NMDAR IgG antibodies in plasma and CSF. She received high-dose steroids, immunoglobulins and rituximab; 30 months after onset, she has recovered well and attends high school.	No	Yes (ovary teratoma)	Definite anti-NMDAR encephalitis	Yes, rapid onset of psychiatric behavior, cognitive dysfunction, seizures, speech dysfunction, decreased level of consciousness, abnormal EEG, ovary teratoma and NMDAR IgG antibodies in CSF

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Table 3 – (*continued*)

Case	Negative biomarkers (class)[a]	Positive biomarkers (value)	History, paraclinical findings, and treatment	IDDM	Cancer	Diagnosis	Fulfillment of AIE criteria
4	CSF: AMPAR1/2 (IgA, IgG, IgM), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), LGI1 (IgA, IgG, IgM), NMDAR (IgG), OCBs IgG-index Serum: GAD_{65} (IgG)	GAD_{65}-IgG in CSF (123 RU/mL)	15-year-old girl experienced sore throat, headaches, cervical lymph node enlargement, and recidivating abdominal muscle cramps during febrile episodes. She had no seizures, and her neurological exam and mental status were normal. Brain MRI and routine CSF examination including CSF OCBs were normal one month after onset of symptoms, but GAD_{65}-IgG in CSF was 123 RU/mL. She received antibiotics, but no immunosuppressive treatment.	No	No	Upper respiratory tract infection	No
5	CSF: AMPAR1/2 (IgA, IgG, IgM), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), LGI1 (IgA, IgG, IgM), NMDAR (IgG), OCBs, IgG-index	GAD_{65}-IgG in CSF (111 RU/mL)	15-year-old girl with Smith Magenis Syndrome was admitted to hospital with staphylococcus aureus pyelonephritis. During this episode she became restless, confused, agitated with choreoathetotic movements; her language was reduced and she had truncal instability. EEG and routine CSF examination were normal, including no CSF OCBs and normal IgG index. However, brain MRI showed cerebellar atrophy which was not seen on MRI 18 months earlier, and GAD_{65}-IgG in CSF was 111 RU/mL. She received high-dose steroids and immunoglobulins. Two years later, she has cerebellar sequelae with atactic speech.	No	No	Possible anti-GAD_{65} associated encephalitis	Possibly, she was confused, agitated with choreoathetotic movements, speech dysfunction, and anti-GAD_{65} IgG antibodies in CSF
6	CSF: AMPAR1/2 (IgG), Caspr2 (IgG), $GABA_BR$ (IgG), GAD_{65} (IgG), LGI1 (IgG), NMDAR (IgG) Serum: AMPAR1/2 (IgG), Caspr2 (IgG), $GABA_BR$ (IgG), GAD_{65} (IgG), LGI1 (IgG)	NMDAR-IgG serum ("weakly positive")	16-year-old girl with schizophrenia, suicide attempts, chronic headache and nausea. No encephalopathy. Brain BRI showed mild hydrocephalus and aqueductal stenosis, and anti-NMDAR IgG antibodies in serum but not in the CSF.	No	No	Schizophrenia, hydrocephalus due to congenital aqueductal stenosis, chronic headache	No
7	CSF: AMPAR1/2 (IgG), Caspr2 (IgG), $GABA_BR$ (IgG), GAD_{65} (IgG), LGI1 (IgG), NMDAR (IgG), OCBs, IgG-index Serum: AMPAR1/2 (IgG), $GABA_BR$ (IgG), GAD_{65} (IgG), LGI1 (IgG), NMDAR (IgG) *VGKC antibodies were not tested	Caspr2-IgG serum ("weakly positive")	13-year-old girl experienced repeated episodes of 7–10 days with confusion, excessive sleep, executive dysfunction, emotional instability, difficulty finding words, and autonomic dysfunction (headache, cold extremities, and lipothymia). Brain MRI and PET were normal, but EEG showed low–frequency activity 3–5 Hz in the left frontal and temporal region. Ictal PET showed increased blood flow in various brain regions. Routine CSF examination (including GAD_{65}-IgG, NMDAR-IgG and Caspr2-IgG) two months after onset was normal, but Caspr2-IgG in plasma was weakly positive. She was treated with oxcarbamazepine. On follow-up, she gradually improved with longer between episodes and shorter duration of episodes.	No	No	Kleine-Levin syndrome	No

8	CSF: AMPAR1/2 (IgG), Caspr2 (IgG), $GABA_BR$ (IgG), GAD_{65} (IgG), LGI1 (IgG) Serum: AMPAR1/2 (IgA, IgG, IgM), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), GAD_{65} (IgG), LGI1 (IgA, IgG, IgM)	NMDAR-IgG CSF ("strongly positive"), NMDAR-IgG serum ("moderately positive"), positive OCBs	10-year-old girl was admitted with generalized tonic-clonic seizures. During admission she experienced episodes with aggressive and emotional behavior, psychotic hallucinations, hyperventilation, abnormal postures (opistotonus and hypertonic lower extremities), and speech latency. Brain MRI was normal, but EEG showed 2–3 Hz activity in right temporal and frontal region. CSF examination 5 days post-admission revealed lymphocytic pleocytosis with 30 leucocytes and positive OCBs, and 12 days post-admission autoimmune screening revealed positive NMDAR-IgG in CSF and plasma. She was treated with high-dose steroids, immunoglobulins, plasma exchange, mycophenolatmofetil, rituximab, and valproic acid. She has complete remission 2 years later.	No	No	Definite NMDAR encephalitis	Yes, rapid onset of psychiatric behavior, cognitive dysfunction, seizures, speech dysfunction, movement disorder, decreased level of consciousness, abnormal EEG, and positive NMDAR-IgG in CSF
9	CSF: AMPAR1/2 (IgA, IgG, IgM), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), GAD_{65} (IgG), LGI1 (IgA, IgG, IgM), NMDAR (IgG), OCBs, IgG-index Serum: AMPAR1/2 (IgA, IgG, IgM), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), LGI1 (IgA, IgG, IgM), NMDAR (IgG)	GAD_{65}-IgG in serum (1311 RU/mL)	8-year-old girl, previously healthy, developed over a few weeks obsessive-compulsive symptoms with phobia for bacteria (resulting in excessive hand washing), daily anxiety episodes and urinary incontinence. She presented in psychiatry without clothes, wearing only a towel. Brain CT and routine CSF examination (including IgG index, OCBs, and GAD_{65}-IgG) were normal, but her serum GAD_{65}-IgG was 1311 RU/mL. She was not tested for PANS (lyso-GM1, dopamine receptor 1 and 2, tubulin, and CaMKII activity) or anti-streptolysin antibodies. She recovered completely over 6 weeks without immunosuppressive treatment. After 6 months she developed polydipsia and polyuria and was diagnosed with IDDM. At that time her serum GAD_{65}-IgG was 2358 RU/mL.	Yes	No	Transient obsessive-compulsive disorder or PANS	No
10	CSF: AMPAR1/2 (IgA, IgG, IgM), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), LGI1 (IgA, IgG, IgM), NMDAR (IgG) Serum: AMPAR1/2 (IgA, IgG, IgM), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), GAD_{65} (IgG), LGI1 (IgA, IgG, IgM), NMDAR (IgG)	GAD_{65}-IgG in CSF (113 RU/mL)	13-year-old boy presented with repeated episodes of excessive sleep. His routine CSF examination and brain MRI were normal, but EEG showed 3–5 Hz activity in the temporal and frontal region, and GAD_{65}-IgG in the CSF was 113 RU/mL (normal GAD_{65}-IgG in serum). On first admission he received high-dose steroids and immunoglobulins.	No	Yes, normal abdominal ultrasound	Kleine-Levin syndrome	No
11	CSF: AMPAR1/2 (IgA, IgG, IgM), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), LGI1 (IgA, IgG, IgM), NMDAR (IgG)	GAD_{65}-IgG in CSF (109 RU/mL)	5-year-old boy experienced truncal instability and reduced consciousness during an episode of chickenpox. CSF examination showed mild pleocytosis (13 leucocytes), normal CSF varicella titers, and GAD_{65}-IgG in the CSF of 109 RU/mL. EEG showed abnormal background activity, but no paroxysms. His brain MRI was normal. He was treated with acyclovir. No follow-up case notes.	No	No	Possible anti-GAD_{65} associated encephalitis	Possibly, decreased level of consciousness, truncal instability, CSF pleocytosis and increased anti-GAD_{65} antibody levels in CSF

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Table 3 – (*continued*)

Case	Negative biomarkers (class)[a]	Positive biomarkers (value)	History, paraclinical findings, and treatment	IDDM	Cancer	Diagnosis	Fulfillment of AIE criteria
12	CSF: AMPAR1/2 (IgG), Caspr2 (IgG), $GABA_BR$ (IgG), GAD_{65} (IgG), LGI1 (IgG), NMDAR (IgG) Serum: AMPAR1/2 (IgG), Caspr2 (IgG), $GABA_BR$ (IgG), GAD_{65} (IgG), LGI1 (IgG)	NMDAR-IgG in serum (positive–no level available)	14-year-old girl presented with subacute onset of altered behavior, confusion, cognitive dysfunction, expressive aphasia, memory loss, irritability, reduced consciousness, feeling of being 'beside herself', and increased sweating. Her EEG showed focal abnormal 1–2 Hz activity in left frontal and temporal region. CSF examination revealed lymphocytic pleocytosis (26 leucocytes), but brain MRI including angio sequence and SPECT were normal. Twenty-one days after onset, she had anti-NMDAR IgG antibodies in serum but not in CSF. IgG index was increased, and CSF OCBs were not evaluated. She received oxcarbamazepine and valproic acid, but no immunosuppressive treatment. Six months later, she had seemingly recovered completely.	No	No	Probable anti-NMDAR encephalitis	Yes, rapid onset of psychiatric behavior, cognitive dysfunction, seizures, speech dysfunction, decreased level of consciousness, abnormal EEG, CSF pleocytosis and anti-NMDAR IgG antibodies in plasma
13	CSF: AMPAR1/2 (IgA, IgG, IgM), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), LGI1 (IgA, IgG, IgM), NMDAR (IgG) Serum: AMPAR1/2 (IgA, IgG, IgM), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), GAD_{65} (IgG), LGI1 (IgA, IgG, IgM), NMDAR (IgG)	GAD_{65}-IgG in CSF (109 RU/mL)	2-year-old boy presented with fever, urinary retention, encephalopathy, anorexia, mydriasis, and photophobia. Viral and bacterial investigations were negative. CSF examination on Day 3 revealed lymphocytic pleocytosis (22 leucocytes) and GAD_{65}-IgG was 109, but only 25 in serum; brain MRI was normal. EEG showed diffuse 1-2 Hz activity, but no focal or paroxysmal activity. He was treated with antibiotics, but no immunosuppressive treatment. Normal CSF examination including GAD_{65}-IgG on Day 12. Complete recovery 2 months after disease onset.	No	No	Possible anti-GAD_{65} associated encephalitis	Possibly, decreased level of consciousness, abnormal EEG, CSF pleocytosis, increased anti-GAD_{65}-IgG in CSF
14	CSF: AMPAR1/2 (IgA, IgG, IgM), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), GAD_{65} (IgG), LGI1 (IgA, IgG, IgM), IgG-index Serum: AMPAR1/2 (IgA, IgG, IgM), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), GAD_{65} (IgG), LGI1 (IgA, IgG, IgM), NMDAR (IgG)	NMDAR-IgG in CSF ("strongly positive"), CSF OCBs	17-year-old girl was admitted to psychiatry due to altered behavior (flirtatious and impulsive), episode of reduced level of consciousness, executive dysfunction, speech latency, emotional instability, sleep problems, catatonia and opistotonus, auditive and visual hallucinations, automatisms, and self-harming behavior. She suddenly developed generalized tonic seizures and was transferred to a somatic ward. CSF examination one month after onset of symptoms revealed pleocytosis (29 leucocytes), OCBs, and NMDAR-IgG, but her NMDAR-IgG was negative in plasma. EEG under remifentanil sedation showed diffuse 2–5 Hz activity and delta waves. The following analyses were normal: MRI of the brain and pelvis, PET/CT of neck, thorax, abdomen and pelvis, ultrasound of pelvis, and bone marrow biopsy. She received high-dose steroids and immunoglobulins. She had complete remission one year later.	No	Yes, normal diagnostic workup	Definite anti-NMDAR encephalitis	Yes, rapid onset of psychiatric behavior, cognitive dysfunction, seizures, speech dysfunction, decreased level of consciousness, CSF pleocytosis, CSF OCBs, and anti-NMDAR IgG antibodies in CSF

15	CSF: AMPAR1/2 (IgA, IgG, IgM), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), GAD_{65} (IgG), LGI1 (IgA, IgG, IgM), NMDAR (IgG), OCBs, IgG-index Serum: AMPAR1/2 (IgA, IgG, IgM), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), GAD_{65} (IgG), LGI1 (IgA, IgG, IgM)	NMDAR-IgG in serum ("weakly positive")	8-year-old right-handed girl presented in psychiatry because for one month she had experienced increasing aggressive behavior, concentration difficulty, reduced use of right hand (she started using left hand for writing), right head tilt, reduced eye movements to the left, auditive hallucinations, headache, unsteady gait, and nausea and vomiting. Her brain MRI showed infiltrating pontine glioma. CSF examination showed normal leucocytes, no OCBs or NMDAR-IgG in the CSF, but presence of plasma NMDAR-IgG antibodies. She died 7 months later.	No	Yes, pontine glioma	Pontine glioma	No
16	CSF: AMPAR1/2 (IgA, IgG, IgM), AQP4 (IgG), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), LGI1 (IgA, IgG, IgM), MOG (IgG), NMDAR (IgG), IgG index Serum: AMPAR1/2 (IgA, IgG, IgM), AQP4 (IgG), Caspr2 (IgA, IgG, IgM), GAD_{65} (IgG), $GABA_BR$ (IgA, IgG, IgM), LGI1 (IgA, IgG, IgM), MOG (IgG), NMDAR (IgG)	GAD_{65}-IgG CSF (115 RU/mL), CSF OCBs	13-year-old boy experienced 2 ADEM-like episodes and 2 non-encephalopathic clinical relapses. CSF examination initially revealed negative OCBs, but 6 months after the initial ADEM episode he had CSF OCBs and GAD_{65}-IgG of 116 RU/mL in the CSF. Anti-MOG and AQP4 antibodies were negative. Brain MRI initially showed multiple contrast-enhancing lesions, but follow-up MRIs revealed juxtacortical, infratentorial and spinal WM lesions. He was initially treated with high-dose steroids, immunoglobulins, plasma exchange and rituximab, but later received natalizumab.	No	No	Multiple sclerosis	No
17	None	NMDAR-IgG CSF ("moderately positive"), NMDAR-IgG serum ("moderately positive"), CSF OCBs, increased IgG index	14-year-old girl was admitted to psychiatry due to auditory hallucinations, anxiety, sexual behavior, fatigue, emotional episode, and cognitive dysfunction, and regression to childish behavior. Three months later she experienced generalized tonic-clonic seizures. CSF examination revealed lymphocytic pleocytosis (58 leucocytes), OCBs, and increased IgG index. Her brain MRI and EEG were normal. NMDAR-IgG was positive in both CSF and plasma. She was treated with acyclovir, high-dose intravenous steroids and immunoglobulins. Four years later she had complete recovered, and NMDAR-IgG was negative in both CSF and serum.	No	No	Definite anti-NMDAR encephalitis	Yes, rapid onset of psychiatric behavior, cognitive dysfunction, seizures, decreased level of consciousness, CSF pleocytosis, OCBs, and anti-NMDAR IgG antibodies in both plasma and CSF
18	CSF: AMPAR1/2 (IgA, IgG, IgM), Caspr2 (IgA, IgG, IgM), $GABA_BR$ (IgA, IgG, IgM), LGI1 (IgA, IgG, IgM), NMDAR (IgG), OCBs, IgG index	GAD_{65}-IgG in CSF (105 RU/mL)	13-year-old girl presented with right-sided headache and transient sensory disturbance in right side of face and right hand. CSF examination was normal including CSF OCBs, but CSF GAD_{65}-IgG was 105 RU/mL. MRI showed small WM lesions localized in the subcortical and juxtacortical region. No treatment.	No	No	Migraine with aura and radiologic isolated syndrome	No

Abbreviations: AQP4; aquaporin-4 antibodies; Hz, hertz; Ig, immunoglobulin; IDDM, insulin-dependent diabetes mellitus; OCBs, oligoclonal bands; ON, optic neuritis; PANS, pediatric acute-onset neuropsychiatric syndrome; PET, positron emission tomography; RU, relative units; SPECT, single-photon emission computed tomography; VGKC, voltage-gated potassium channel complex; WM, white matter.

[a] Only anti-NMDAR IgG are included because anti-NMDAR IgA and IgM are non-specific for NMDAR encephalitis.

Table 4 – Review of children with "antibody negative but probable autoimmune encephalitis" in Denmark 2011–17.

Case	History, paraclinical findings, and treatment
1	15-year-old girl presented with nausea, vomiting, diarrhea, fever, photophobia, confusion, aggressive behavior, ataxia, and memory deficits. Serum cytomegalovirus titers for IgG and IgM were positive. CSF examination revealed 439 leucocytes (91% monocytes), protein 0.86 mg/L, negative OCBs and cultures/titers for herpes simplex, varicella, enterovirus, EBV, borrelia and mycoplasma. AIE antibodies were negative. EEG showed 1.5–2.5 Hz activity in the fronto-temporal region, but no paroxysmal activity. Brain MRI showed discrete abnormalities in mesencephalon and decreased diffusion in vermis cerebellum. Normal abdominal ultrasound. She was treated with acyclovir, penicillin, corticosteroids and immunoglobulins. She improved markedly after immunosuppressive treatment.
2	12-year-old girl presented with involuntary movements, reduced consciousness and mutism after upper respiratory tract infection. Her CSF examination revealed CSF pleocytosis (27 leucocytes), normal IgG index, but presence of OCBs. Brain MRI showed cerebellar edema. Normal diagnostic work-up including bacterial and viral cultures/titers, urinary metabolic screening, EEG, muscle biopsy, and abdominal ultrasound. She received corticosteroids, plasma exchange, immunoglobulins and antibiotics. Six months later she had sequelae with gait and speech ataxia and used walking aids.
3	3-year-old boy presented with confusion and behavioral change after an upper respiratory tract infection. CSF examination showed low-grade CSF pleocytosis (9 leucocytes), normal protein, and negative viral titers. Brain MRI showed diffusion restriction in cerebellum and posterior corpus callosum. He received high-dose steroid and immunoglobulins.
4	7-year-old boy presented with behavioral change, fatigue, mutism, and left facial palsy following a respiratory tract infection. CSF examination showed CSF pleocytosis (22 leucocytes) and normal IgG index. Brain MRI showed cortical and subcortical lesions in the frontal lobe, insula, and occipital lobe compatible with encephalitis, and EEG showed slow frequency and multifocal spikes. All viral and bacterial cultures were negative except PCR for mycoplasma pneumonia in tracheal secretions. He was intubated and treated with antibiotics, corticosteroids, immunoglobulins, plasma exchange, cyclophosphamide, and anti-epileptics. His symptoms remitted gradually, but two years after the encephalitis episode he had epilepsy and learning difficulties.

immunosuppressive treatment are key to optimizing patient outcome.[29] As anti-NMDAR encephalitis can present with mood disorders and hallucinations, resembling acute psychosis and schizophrenia, we suggest the following points should increase suspicion of anti-NMDAR encephalitis in children presenting to psychiatry (percent with the feature among persons with anti-NMDAR encephalitis): a) onset within weeks to months; b) few "psychiatric" risk factors; c) language disintegration, d) decreased consciousness (97%); e) epileptic seizures (84%);[43] f) abnormal postures or movements, mostly orofacial and limb dyskinesias;[29] g) abnormal EEG (91–100%) showing non-specific, slow, and disorganized activity sometimes with electrographic seizures, or possibly delta brushes;[43–45] h) signs of CNS inflammation (85%), usually CSF lymphocytic pleocytosis (91%); presence of CSF oligoclonal bands (60%) or increased IgG index;[45] and i) abnormal brain MRI showing mild and transient FLAIR or contrast-enhancing abnormalities (31–55%).[43,45,46]

2) Low pre-test probability of AIE in children: Large numbers of AIE antibody tests are ordered in clinical practice with few positive test results, indicating that either there are other auto-antibodies of clinical significance than those commonly tested for or physicians too often suspect pediatric AIE. In children, most VGKC complex antibodies do not bind to LGI1 or Caspr2, and detection of anti-VGKC complex antibodies in children is of limited diagnostic value.[47] Further, antibodies against intracellular antigens (e.g. ANNA1, ANNA2, MA2, CRMP5, PCA1, amphiphysin, SOX1, and SOX2) are rare in children.[5] In our pediatric cohort, only IgG anti-NMDAR and anti-GAD_{65} antibodies improved diagnostics (anti-NMDAR IgA and IgM are non-specific and not associated with encephalitis).[48] Accordingly, the low incidence of AIE (and hereby low pre-test probability) should be considered in the diagnostic work-up in children. However, if the pre-test probability of AIE is higher, e.g. if the child presents with a typical history of anti-NMDAR encephalitis, IgG anti-NMDAR should be tested in both CSF and plasma because up to 14% of persons with anti-NMDAR encephalitis have antibodies in CSF but not in the serum;[49] in our cohort, one child with anti-NMDAR encephalitis had antibodies in CSF only and another had antibodies in serum only.

3) Need for specific ICD codes for AIE: There is not a specific code for AIE in the ICD-9 or ICD-10. With the increasing knowledge of AIE, there is a need to include specific codes for AIE in the ICD. We propose that there should be a code for 'autoimmune encephalitis' with subgroups for each specific type of AIE. Further, there should be a code for "antibody negative but probable autoimmune encephalitis". This will enable better surveillance and facilitate research in the area.

The strengths of the study are that it is nationwide, population-based, and multicentered with inclusion of a large sample size of tested individuals with review of the medical records of relevant cases. All AIE tests were analyzed at only one reference laboratory. However, the present study must be viewed in the light of several limitations: a) Children with transient psychiatric symptoms or 'post-infectious' encephalitis may have had AIE antibodies, but this may not have been revealed due to the retrospective study design and inclusion of children from several different centers; b) Confirmatory tests were not used to confirm positive test results and particularly children with 'weakly positive' anti-NMDAR IgG antibodies are prone to false positives; however, nearly all AIE tests were negative, and use of confirmatory tests would not have impacted our main conclusions; c) We were unable to calculate the anti-GAD_{65} IgG index in children with anti-GAD_{65} associated encephalitis; d) It would have been optimal to review all medical records in children tested for AIE antibodies to explore the proportion of children with presumed seronegative AIE. Instead, we identified this group by reviewing the medical record of children tested for AIE antibodies who were coded with one of the codes used for medical record-validated NMDAR encephalitis; and e) $GABA_AR$ antibodies were not part of the AIE diagnostic package during the study period.

5. Conclusions

We diagnosed only children with anti-NMDAR, anti-GAD_{65}, and "antibody negative but probable autoimmune encephalitis". Before examining AIE antibodies, clinical presentation, paraclinical studies (CSF, EEG, and MRI), and incidence of pediatric AIEs should be considered. Updating the ICD to include AIE codes is warranted. Future studies should include assays with greater sensitivity in order to detect disease-causing bacteria and viruses, such as the multiplex polymerase chain reaction system, and explore new biomarkers in "antibody negative but probable autoimmune encephalitis".[2] Further, it needs to be established whether anti-GAD_{65} IgG antibodies are associated with encephalitis in children, because the cases we defined as having anti-GAD_{65} associated encephalitis had only marginally increased CSF levels of anti-GAD_{65}.

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Conflicts of interests

Dr Boesen has served on scientific advisory board for Teva; has received speaker honoraria for lecturing from Novartis and support for congress participation from Teva, Novartis and Roche.

Drs Børresen, Lydolph and Blaabjerg report no disclosures.

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

Contributors' statement page

Dr Boesen conceptualized and designed the study, collected data, carried out the initial analyses, and drafted the initial manuscript.

Drs Børresen, Born, and Blaabjerg conceptualized and designed the study, and reviewed and revised the manuscript.

Mr Lydolph collected data and reviewed and revised the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpn.2019.03.007.

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