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Antineuronal antibodies in cerebrospinal fluid and serum of 104 patients with psychotic disorders compared to 104 individually matched healthy controls

Rose Jeppesen [a,b], Anna Christine Nilsson [c,d], Nina Vindegaard Sørensen [a,b], Sonja Orlovska-Waast [a], Rune Haubo Bojesen Christensen [a], Michael Eriksen Benros [a,b,*]

[a] Copenhagen Research Center for Mental Health – CORE, Mental Health Centre, Copenhagen, Copenhagen University Hospital, Copenhagen, Denmark
[b] Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[c] Department of Clinical Immunology, Odense University Hospital, Odense, Denmark
[d] Department of Clinical Research, University of Southern Denmark, Odense, Denmark

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ABSTRACT

Background: Antineuronal antibodies can cause psychotic symptoms, particularly NMDAR antibodies; however, studies on the prevalence of antineuronal antibodies in cerebrospinal fluid (CSF) and serum of patients with psychotic disorders compared to matched healthy controls are sparse.
Methods: We included 104 patients with a first-time diagnosis of a psychotic disorder within one year prior to inclusion (50 % outpatients) and 104 individually matched healthy controls, all without any known immunological conditions. CSF and serum were tested for IgG antibodies (Abs) against NMDAR NR1-subunit, GAD65, LGI1, CASPR2, AMPAR1, AMPAR2 and GABAb-receptor B1/B2 using commercial fixed cell-based assays (CBAs) (Euroimmun). Positive samples were retested with CBA twice, and tested with tissue-based assays (TBA). Primary outcomes were the presence of any of the seven anti-neuronal antibodies in CSF or serum. Secondarily, we analyzed the prevalence of each autoantibody.
Results: No antineuronal IgG antibodies were consistently found in any CSF sample and NMDAR-antibodies were not consistently present in any of the 208 participants, neither in CSF nor serum. CASPR2-Abs were consistently found in the serum of one patient and one control, and one healthy control, without diabetes, was seropositive for GAD65-Abs. CASPR2 borderline seropositivity was additionally found in one patient and two controls. All samples positive on CBA were negative on TBA.
Conclusions: We found no significant differences between patients and controls. Antineuronal IgG antibodies are very rare when screening a broad group of individuals with recent-onset psychotic disorders without other indications of autoimmune encephalitis. Thus, much larger studies are needed to conclude on potential contrasts in prevalence compared to healthy controls.

1. Introduction

The etiology of psychotic disorders is still vastly unknown. Many pathways have been implicated to play a role in the development of psychotic disorders, and lately the immune system has increasingly been suggested to be involved, including a possible role of antineuronal antibodies. In anti-*N*-methyl-D-aspartate receptor (NMDAR) autoimmune encephalitis, 40 % of patients are first evaluated by a psychiatrist, and virtually all patients with anti-NMDAR autoimmune encephalitis present with psychiatric symptoms, in particular psychotic symptoms such as hallucinations (43 %) and delusions (26 %) (Herken and Prüss, 2017). Although most patients with anti-NMDAR autoimmune encephalitis at some point develop neurological symptoms, a few cases of autoimmune encephalitis have isolated psychiatric symptoms (Murashko et al., 2021), and thus the concept of autoimmune psychosis has been proposed by a broad collaboration of experts in the field (Pollak et al., 2020). As prompt and early treatment of autoimmune encephalitis has been found to improve functional outcome, timely diagnosis hereof is important, and thus, further knowledge on presence of antineuronal

* Corresponding author at: Mental Health Centre Copenhagen, Copenhagen University Hospital, Gentofte Hospital, Gentofte Hospitalsvej 15, 4th floor, 2900 Hellerup, Denmark.
E-mail address: benros@dadlnet.dk (M.E. Benros).

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Abbreviations

CSF	Cerebrospinal fluid
CBA	Cell-based assay
TBA	Tissue-based assay
PANSS	Positive and Negative Symptom Score
SAPS/SANS	Scale for Assessment of Positive/Negative Symptoms
MMSE	Mini-Mental State Examination
TMT A	Trail Making Test A
BACS	Brief Assessment of Cognition in Schizophrenia
NES	Neurological Evaluation Scale
NMDAR	*N*-methyl-D-aspartate receptor
GAD65	Glutamic acid decarboxylase 65
LGI1	Leucine-rich, glioma inactivated 1
CASPR2	Contactin associated protein 2
AMPAR1	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor 1
AMPAR2	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor 2
GABAb	Gamma-aminobutyric acid b

antibodies is warranted.

Anti-NMDAR-antibodies (Abs) are currently the most studied antineuronal antibodies in psychiatric disorders, as well as the most prevalent of autoantibodies known to directly cause autoimmune encephalitis (Dalmau and Graus, 2018). The presence of anti-NMDAR-Abs among individuals with psychotic disorders has mostly been investigated in serum, with results on all isotypes (i.e. IgG, IgA, and IgM) varying from 0 to 11 %, and a meta-analysis finding anti-NMDAR-Abs to be present in 8 % of patients with psychotic disorders (Pollak et al., 2014). When including only IgG anti-NMDAR-Abs in the meta-analysis—IgG Abs being the only isotype known to be clinically relevant in autoimmune encephalitis—the seroprevalence in patients with psychotic disorders was 1.46 %, which was significantly higher than in healthy controls (Pollak et al., 2014). The clinical importance is, however, still unknown, with a more recent meta-analysis finding no clear significant difference when comparing the prevalence of anti-NMDAR-Abs to healthy controls (Cullen et al., 2021). Presence of other antineuronal antibodies, as for example Leucine-rich, glioma inactivated 1-Abs (LGI1-Abs), can also result in autoimmune encephalitis presenting with psychotic symptoms (Herken and Prüss, 2017); however, the prevalence of these other antineuronal antibodies is even less investigated. A recent review reports varying findings in the few studies investigating IgG, IgM, and IgA anti-Contactin associated protein 2-Abs (CASPR2-Abs) in serum, with up to 2.5 % of patients—but also 2.9 % of healthy controls—showing seropositivity (Colijn and Ismail, 2019). Seropositivity of LGI1-Abs was found in up to 2 % of patients (in three studies), with no significant differences compared to controls (Colijn and Ismail, 2019).

Antineuronal antibodies need to reach the central nervous system in order to be pathogenic; however, knowledge on the prevalence of antineuronal antibodies in the CSF of patients with psychotic disorders is still limited. A handful of studies have investigated IgG NMDAR-Abs in the CSF of patients with psychotic disorders (Colijn and Ismail, 2019), with prevalence ranging from 0 to 0.6 % (Endres et al., 2020; Guasp et al., 2021; Oviedo-Salcedo et al., 2018a), whereas other IgG cell-surface antibodies (e.g. CASPR2-Abs and LGI1-Abs) were not present in the CSF from patients with psychotic disorders in the largest study to date (Endres et al., 2020). To the best of our knowledge, only two studies have examined the prevalence of antineuronal antibodies in the CSF of patients with psychosis with comparison to healthy controls (Bien et al., 2021; Theorell et al., 2021); one including 103 patients and 40 controls (Bien et al., 2021), the other 71 patients and 49 healthy controls (Theorell et al., 2021). Neither found any of their participants to be positive for any IgG isotype antineuronal autoantibodies in CSF.

To gain further knowledge on the prevalence of antineuronal antibodies and assess their clinical relevance, large studies on CSF of patients with psychotic disorders and healthy controls are needed. We screened CSF and serum of a broad group of patients with isolated psychotic disorders, not selected due to an indication of probable autoimmune encephalitis, and individually matched healthy controls, for the presence of the seven most common surface and synaptic antineuronal IgG antibodies in autoimmune encephalitis; NMDAR NR1 subunit Abs, glutamic acid decarboxylase 65 (GAD65) Abs, LGI1-Abs, CASPR2-Abs, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor 1 and 2 Abs (AMPAR1 and AMPAR2), and gamma-aminobutyric acid b (GABAb) receptor B1/B2 Abs. We thereby present the largest study to date on antineuronal antibodies in the CSF of patients with recent onset, non-affective psychotic disorders compared to individually matched healthy controls.

2. Methods

The study is approved by The Regional Committee on Health Research Ethics in the Capital Region of Denmark (j.no: H-16030985) and The Danish Data Protection Agency (j.no: RHP-2016-020, I-Suite no.: 04945). The study is part of the larger PSYCH-FLAME biobank.

The methods have previously been described in detail in our pre-published study protocol (Jeppesen et al., 2021).

2.1. Participants

Patients were recruited via psychiatric departments and outpatient clinics in the Capital Region of Denmark, whereas the sex- and age-matched healthy controls were recruited via a Danish website, forsøgsperson.dk. Patients and controls came from the same region. All participants had to be aged 18–50 years at time of inclusion. To be included, cases were to have been diagnosed with a non-affective psychotic disorder (ICD10 F20/F22-29) within a year prior to inclusion. See the pre-published protocol for a detailed overview of exclusion criteria, including rationale for the individual criteria (Jeppesen et al., 2021). In brief, exclusion criteria were a prior diagnosis of a psychotic disorder, prior or recent severe neurological or general medical condition, contraindications to lumbar puncture, regular use of anti-inflammatory medication, and treatment with electroshock therapy within three months prior to inclusion. In general, patients did therefore not fulfill current criteria for autoimmune encephalitis (Graus et al., 2016; Pollak et al., 2020). Controls were additionally excluded when any prior or current psychiatric disorder could not be ruled out. Patients were screened via electronic health records, and controls were screened via phone using a series of screening questions (Jeppesen et al., 2021). On the day of inclusion, all participants answered screening questions regarding prior and current mental and physical health, as well as current intake of medication, alcohol and drugs, and contraindications against lumbar puncture. Informed consent was obtained for all participants prior to inclusion.

2.2. Clinical assessment

Both patients and controls underwent an interview using WHO Schedules for Clinical Assessment in Neuropsychiatry (SCAN, version 2.1). All interviews were performed by certified interviewers. For the patients, the interview was used to validate diagnoses given by clinical doctors prior to inclusion, whereas for healthy controls it was used to rule out psychiatric disorders. Positive and Negative Symptom Scale (PANSS) and the Scale for Assessment of Positive/Negative Symptoms (SAPS/SANS) were used to rate psychopathology severity. Cognition was assessed by Mini-Mental State Examination (MMSE), Trail Making Test (TMT) A, and the Brief Assessment of Cognition in Schizophrenia

(BACS). Neurological status was assessed using the Neurological Evaluation Scale (NES) for detection of neurological soft signs.

Data on duration of illness and medication status for patients were collected from electronic health records and validated by self-report. Height, weight, smoking status, and alcohol intake was self-reported. Patients and controls were further evaluated using a broader range of psychopathology scales, cognitive tests, and rating scales assessing level of functioning. For details hereon, see our pre-published protocol (Jeppesen et al., 2021).

2.3. Biological samples

Venous blood samples were collected for all cases and controls prior to the lumbar puncture.

Lumbar puncture was carried out according to current consensus guidelines (Engelborghs et al., 2017). Immediately following the lumbar puncture, samples were transported via taxi to Rigshospitalet, where they were centrifuged, aliquoted and frozen.

Samples were collected from June 29, 2016 to September 30, 2021.

2.4. Laboratory analyses

Laboratory personnel were blinded to all clinical information, including case/control status. Analyses were performed in the nationally accredited laboratory for testing of anti-neuronal antibodies at the Dept. of Clinical Immunology, Odense University Hospital. Antineuronal antibodies were detected by indirect immunofluorescence using commercial fixed cell-based assays (CBAs) transfected with the following antigens: NMDAR NR1 subunit, GAD65, LGI1, CASPR2, AMPAR1, AMPAR2 and GABAb receptor B1/B2 (Euroimmun, Lübeck, Germany). Serum samples were analyzed in dilution 1:10, and serum samples positive for anti-CASPR2 were additionally analyzed in dilution 1:100. CSF was analyzed undiluted. Analyses were performed according to the manufacturer's recommendations. Both kit controls and inhouse serum controls were analyzed in each sample run, and both positive and negative controls were used. Kit controls are ready to use, and inhouse serum controls are analyzed as patient samples. Reactive samples were re-analyzed twice with CBA and with a tissue-based assay (TBA), using monkey cerebellum as substrate (Euroimmun, Lübeck, Germany). Results of CBAs were categorized as negative, borderline positive, weakly positive, moderately positive, or strongly positive. Samples were considered autoantibody positive and included in the analyses if they were at least weakly positive in two or more CBAs.

2.5. Outcomes

2.5.1. Primary outcomes

Our primary outcomes were the presence of any one of the seven antineuronal autoantibodies (NMDAR-Abs, GAD65-Abs, LGI1-Abs, CASPR2-Abs, AMPAR1-Abs, AMPAR2-Abs and GABAb receptor B1/B2 Abs) in 1) CSF and 2) either serum or CSF.

2.5.2. Secondary outcomes

As secondary outcomes, we analyzed contrasts of presence of each antineuronal antibodies in serum and CSF between patients and controls.

2.6. Statistical analyses

Demographic data were compared using two-sample *t*-tests for continuous outcomes, and Pearson χ^2 test for categorical ones (e.g., sex). BACS z-scores with healthy controls as references were calculated as described by Keefe et al. (2004).

Differences between patients and healthy controls of presence of antineuronal antibodies (dichotomous variables; yes/no) were analyzed with Pearson χ^2 tests. Two-sided tests with $p < 0.05$ were considered significant. All statistical analyses were performed twice by two individual authors (RJ and RHBC) in R version 4.0.2 (R Core Team, 2018).

3. Results

We included 104 patients and 104 individually matched healthy controls. Population characteristics for the two groups are described in Table 1. In both groups, 64 participants (61.5 %) were male. Mean age for the patient and control group were 26.1 (SD 6.6) and 26.6 (SD 6.7) years, respectively ($p = 0.634$). BMI did not differ significantly between

Table 1
Population characteristics.

	Patients (N = 104)	HC (N = 104)	
Demographics			
Sex, N Males	64 (61.5 %)	64 (61.5 %)	p = 1.000[a]
Age, mean ± SD	26.1 ± 6.6	26.6 ± 6.7	p = 0.634[b]
Range	18.3–50.4	18.3–49.5	
BMI, mean (SD)	23.5 (±4.6)	23.8 (±4.0)	p = 0.603[b]
Smokers (%)	40 (38.5 %)	14 (13.5 %)	**p < 0.001**[a]
Weekly alcohol intake (n)	(80)	(104)	
Mean ± SD	3.3 ± 5.0	5.3 ± 7.4	**p = 0.035**[b]
Range	0–24	0–50	
Time since first F2 diagnosis, months (n)	(97)		
Mean ± SD	3.1 ± 4.1	NA	NA
Range	0–20.0	NA	
Antipsychotic treatment (n)	(104)	(104)	
N yes	81 (77.9 %)	0 %	NA
Inpatients (n)	(104)	(104)	
N yes	52 (50.0 %)	0 %	NA
Clinical factors			
Psychopathology			
Total PANSS (n)	(79)	(104)	
Mean ± SD	62.5 ± 13.0	31.7 ± 2.0	**p < 0.001**[b]
Range	33–97	30–38	
SAPS global summary score (n)	(102)	(104)	
Mean ± SD	6.3 ± 3.1	0.1 ± 0.3	**p <**
Range	0–13	0–2	**0.001**[b]
SANS global summary score (n)	(95)	(104)	**p<**
Mean ± SD	8.1 ± 4.5	0.5 ± 1.1	**0.001**[b]
Range	0–17	0–6	
Cognition			
BACS composite (n)	(66)	(101)	
Mean ± SD	−0.78 ± 0.90	0.00[c] ± 0.61	**p <**
Range	−3.35-1.12	−1.70-1.32	**0.001**[b]
MMSE (n)	(74)	(103)	**p <**
Mean ± SD	28.5 ± 1.7	29.4 ± 0.9	**0.001**[b]
Range	21–30	26–30	
TMT A (n)	(100)	(103)	**p <**
Mean ± SD	24.1 ± 8.3	19.4 ± 5.5	**0.001**[b]
Range	13–58	10–40	
Neurology			
Neurological deficits of any kind (%)	9 (8.8 %)	6 (5.8 %)	p = 0.399[a]
Neurological soft signs (n)	(77)	(103)	**p <**
Mean ± SD	9.5 ± 6.9	4.3 ± 3.2	**0.001**[b]
Range	0–34	0–17	

HC: Healthy controls, n: number of subjects with data, BMI: Body-mass Index, PANSS: Positive and Negative Symptom Scale, SAPS/SANS: Scale for Assessment of Positive/Negative Symptoms, BACS: Battery for Assessment of Cognition in Schizophrenia, TMT: Trail-Making Test, MMSE: Mini-Mental State Examination. Bold p-values highlight statistically significant differences between patients and controls (ie, $p<0.05$).

[a] Pearson's Chi-squared test.

[b] Welch two-sample *t*-test.

[c] HC used as reference for z-score.

patients and controls (p = 0.603). The patients smoked more (38.5 % vs 13.5 %, p < 0.001), whereas the cases had a higher weekly alcohol intake (3.3 vs 5.3 units per week, p = 0.035). At time of lumbar puncture, 36 patients (34.6 %) had been diagnosed with a schizophrenia spectrum disorder, and the remaining 68 patients (65.4 %) with other non-organic, non-affective psychotic disorders.

Mean time since first psychotic disorder diagnosis was 3.1 months (SD 4.1), 52 patients (50 %) were inpatients, and 81 patients (77.9 %) were treated with antipsychotic drugs at time of inclusion. Patients were moderately ill with a mean PANSS total score of 62.5 (SD 13.0), neurological soft sign score of 9.5 (SD 6.9), and a mean total MMSE score of 28.5 (SD 1.7).

3.1. Primary outcomes

No patients or healthy controls had positive reactions for any antineuronal antibody in CSF in two or more CBAs. All CSF samples were negative using TBA.

In serum, one patient (0.96 %) and two controls (1.92 %) tested positive at least twice for any antineuronal antibody using CBA (p = 0.561). A borderline positive result for any antibody was found in the serum of one patient (0.96 %) and two controls (1.92 %) (p = 0.561). All serum samples were negative using TBA. An overview of results can be found in Table 2, and images of the positive samples can be found in Supplementary Fig. 1.

Discrepancies in results between consecutive CBAs were seen in multiple samples, both from patients and controls. An overview of results from retest of initially positive samples, including results from analyses of follow-up samples, can be found in Table 3. No statistically significant contrasts between patients and controls were found in any round of CBAs. One-year follow-up samples were available for four of the controls that tested positive. None of the follow-up samples were found positive for any antibody.

Table 2
Overview of results.

	Patients positive[a] (N = 104)	HC positive[a] (N = 104)	p-value[b]
Primary analyses			
Any antineuronal autoantibody in the CSF	0 (0.00 %)	0 (0.00 %)	1.000
Any antineuronal autoantibody in CSF or serum	1 (0.96 %)	2 (1.92 %)	0.561
Secondary analyses, CSF			
NMDAR1-Ab	0 (0.00 %)	0 (0.00 %)	1.000
CASPR2-Ab	0 (0.00 %)	0 (0.00 %)	1.000
LGI1-Ab	0 (0.00 %)	0 (0.00 %)	1.000
GABAb receptor 1-Ab	0 (0.00 %)	0 (0.00 %)	1.000
AMPAR1-Ab	0 (0.00 %)	0 (0.00 %)	1.000
AMPAR2-Ab	0 (0.00 %)	0 (0.00 %)	1.000
GAD65-Ab	0 (0.00 %)	0 (0.00 %)	1.000
Secondary analyses, serum			
NMDAR1-Ab	0 (0.00 %)	0 (0.00 %)	1.000
CASPR2-Ab	1 (0.96 %)	1 (0.96 %)	1.000
LGI1-Ab	0 (0.00 %)	0 (0.00 %)	1.000
GABAb receptor 1-Ab	0 (0.00 %)	0 (0.00 %)	1.000
AMPAR1-Ab	0 (0.00 %)	0 (0.00 %)	1.000
AMPAR2-Ab	0 (0.00 %)	0 (0.00 %)	1.000
GAD65-Ab	0 (0.00 %)	1 (0.96 %)	0.316

HC: Healthy controls, CSF: Cerebrospinal fluid.
[a] number of participants whose samples are weakly, moderately, or strongly positive for antibodies (in two or more CBA tests).
[b] Pearson's Chi-squared test.

3.2. Secondary outcomes: specific antibodies

No statistically significant differences were found regarding the prevalence of any single antineuronal antibody in CSF or serum, neither when including all borderline positive and positive results, nor when including only those positive in at least two CBAs (all p-values >0.3).

None of the seven antineuronal antibodies were detected in any CSF sample in two or more CBAs.

The serum of one patient (0.96 %) and one healthy control (0.96 %) was found weakly positive for CASPR2-Abs, while serum samples from one patient (0.96 %) and two controls (1.92 %) were borderline positive hereof. In analyses of serum diluted 1:100, all samples were CASPR2-Ab negative.

The serum of one healthy control (0.96 %) was moderately positive for GAD65-Abs. The control did not have type 1 diabetes, and the serum from the 1-year follow-up visit was tested negative for GAD65-Abs.

No apparent clinical or paraclinical similarities were found between individuals with samples positive of antibodies (see Table 4).

4. Discussion

This study is the largest to date on the presence of antineuronal antibodies in the CSF of patients with psychotic disorders matched with healthy controls. We screened a broad group of patients with recent diagnoses of psychotic disorders, not fulfilling current criteria for autoimmune encephalitis (Graus et al., 2016; Pollak et al., 2020). We found no CSF samples to be consistently positive for any of the seven antibodies included in this study. The serum of three patients and four controls tested either positive or borderline positive for any of the antineuronal antibodies in at least two CBAs, and we found no significant differences between patients and healthy controls in any of our analyses. None of the participants had positive TBA, and none of the healthy controls that initially tested seropositive were positive at the 1-year follow-up visit.

The absence of antibodies in the CSF of our broad group of patients with psychotic disorders corroborates previous findings, with four studies including 71, 103, 105 and 124 patients, respectively, not finding antineuronal IgG antibodies in CSF (Bien et al., 2021; Guasp et al., 2021; Oviedo-Salcedo et al., 2018a; Theorell et al., 2021). Only two of these studies (Bien et al., 2021; Theorell et al., 2021) included healthy controls, and as in our study, none of the CSF samples from controls were positive for antineuronal antibodies.

The prevalence of antibodies found in the serum of our patients falls within the range observed in previous studies of IgG antineuronal antibodies (Bien et al., 2021; Colijn and Ismail, 2019; Pollak et al., 2014), and the lack of differences between patients and controls also complies with what has previously been observed (Bien et al., 2021; Colijn and Ismail, 2019; Warren et al., 2019). Our findings of discrepancies between results from consecutive CBA tests highlight the need for cautious interpretation of CBA findings that are not strongly positive, due partially to the margin of error of +/− 1 when evaluating the pattern and intensity of the specific fluorescence used for autoantibody testing. We found no antineuronal antibodies in any of the available follow-up samples of the healthy controls that were initially tested seropositive, indicating transient seropositivity. Furthermore, we found no CASPR2-Ab seropositivity when diluted 1:100, indicating low antibody titers, which has been argued not to be of clinical significance (Bien et al., 2017).

The recent, larger study by Endres et al. (2020), without controls, examined the serum and CSF of >450 patients with schizophreniform disorders and found IgG anti-NMDAR-Abs in only 2 % of serum and 0.3 % of CSF samples. Additionally, a recent study including 1661 patients suspected of autoimmune encephalitis found seropositivity of IgG anti-NMDAR-Abs in only 3.8 %, despite these patients having been selected based on symptoms indicating possible autoimmune encephalitis (Ariño et al., 2021). The number of participants needed to identify a

Table 3
Comparison of individual test runs.

	CBA test 1	CBA test 2	CBA test 3	Analyzed as autoantibody positive[a]	TBA test (monkey cerebellum)	CBA test of follow-up sample[b]
CSF						
Patient 1	GAD65 (+) LGI1 (+)	NMDAR (+)	Negative	No	Negative	Not performed
Serum						
Patient 2	CASPR2 (+)	CASPR2 +	CASPR2 +	CASPR2 +	Negative	Not performed
Patient 3	CASPR2 (+)	CASPR2 (+)	CASPR2 (+)	CASPR2 (+)	Negative	Not performed
Patient 4	CASPR2 (+)	Negative	Negative	No	Negative	Not performed
Patient 5	CASPR2 (+)	Negative	Negative	No	Negative	Not performed
Patient 6	CASPR2 (+)	Negative	Negative	No	Negative	Not performed
Patient 7	CASPR2 (+)	Negative	Negative	No	Negative	Not performed
Patient 8	GABAb-R (+)	Negative	Negative	No	Negative	Not performed
Healthy control 1	CASPR2 (+)	CASPR2 +	CASPR2 +	CASPR2 +	Negative	Negative
Healthy control 2	CASPR2 (+)	CASPR2 (+)	CASPR2 (+)	CASPR2 (+)	Negative	Negative
Healthy control 3	CASPR2 (+)	CASPR2 (+)	CASPR2 (+)	CASPR2 (+)	Negative	Negative
Healthy control 4	CASPR2 (+)	Negative	Negative	No	Negative	Not performed
Healthy control 5	CASPR2 (+)	Negative	Negative	No	Negative	Not performed
Healthy control 6	GAD65 +	GAD65 ++	GAD65 ++	GAD65 ++	Negative	Negative

(+): Borderline positive, +: Weakly positive, ++: Moderately positive, CSF: cerebrospinal fluid, CBA: Cell-based assay, TBA: Tissue-based assay.
[a] Interpreted as negative if results were not consistent in at least two CBAs.
[b] Sample collected at least one year after first inclusion.

Table 4
Clinical information on individuals with positive findings.

	Patient positive in >2 CBA tests		Healthy controls positive in >2 CBA tests		
	Pt 2	*Pt avg*	HC 1	HC 6	*HC avg*
CBA serum result	CASPR2 +		CASPR2 +	GAD65 ++	
Age (years)	30.9	*26.1*	25.3	27.5	*26.6*
BMI	27.3	*23.5*	20.1	26.0	*23.8*
Illness duration (months)	3.8	*3.1*	NA	NA	*NA*
PANSS total	60	*62.5*	30	34	*31.7*
SAPS global score	2	*6.3*	0	0	*0.1*
SANS global score	17	*8.1*	0	0	*0.5*
BACS composite[a]	−1.69	*−0.78*	−0.34	−0.35	*0.00*
TMT A	00:45	*00:24*	00:27	00:22	*00:19*
MMSE total	28	*28.5*	30	30	*29.4*
Neurological deficits	No	*NA*	No	No	*NA*
Neurological soft sign score	23	*9.5*	0	0	*4.3*
Hs-CRP	3.76	*1.59*	1.31	0.58	*1.18*
Leukocytes (10^9/L)	7.6	*5.93*	5.6	5.0	*5.60*
HbA1c	36	*31.9*	29	33	*31.8*
CSF WBC (cells/μL)	2	*2.18*	2	2	*1.98*

CBA: Cell-based assay, Pt: Patient, HC: Healthy control, avg.: average, BMI: Body-mass Index, PANSS: Positive and Negative Symptom Scale, SAPS/SANS: Scale for Assessment of Positive/Negative Symptoms, BACS: Battery for Assessment of Cognition in Schizophrenia, TMT: Trail-Making Test, MMSE: Mini-Mental State Examination.
[a] HC used as reference for z-score.

possible difference between healthy controls and patients with a broad spectrum of psychotic disorders not fulfilling current criteria for autoimmune encephalitis (Graus et al., 2016; Pollak et al., 2020), would thus need to be much higher than the 208 included in our study; expecting a seropositive prevalence in patients of 2.3 % as reported by Endres et al. (2020) and in healthy controls of 0.23 % as reported in a recent review (Lang and Prüss, 2017), achieving a power of 80 % with Pearson's χ^2 tests would require as many as 390 participants in each group.

The patients included in our study represent a broad spectrum of psychotic disorders with regards to diagnoses, symptoms, and severity. They were not selected based on indication of probable autoimmune encephalitis, and many patients that would have otherwise been suspected thereof were excluded from our study due to previous general medical conditions such as seizures or diseases with impact on the immune system such as autoimmune disorders (Herken and Prüss, 2017; Pollak et al., 2020). Additionally, as recently reported in a separate paper, only 2 of the 104 patients had increased white blood cell count in the CSF (i.e., >5 cells/μL currently defined as the clinical cut-off for neuroinflammation) (Jeppesen et al., 2022). The fact that we—and other studies including similar patient groups (Bien et al., 2021; Guasp et al., 2021; Oviedo-Salcedo et al., 2018a; Theorell et al., 2021)—find no antineuronal antibodies in CSF samples, suggests that autoimmune encephalitis with isolated psychiatric symptoms is a very rare diagnosis. Of note, one of the previous studies included a markedly older population, including patients with recurrent schizophrenia spectrum disorders, patients with substance abuse disorder, and patients with affective psychotic syndromes (Oviedo-Salcedo et al., 2018b), and another included patients with warning signs of autoimmune encephalitis such as previous seizures (Guasp et al., 2021). A more widespread CSF testing strategy in patients with recent-onset psychotic disorders was recently proposed by Guasp and colleagues, suggesting CSF antibody testing in the CSF in patients with MRI or EEG abnormalities, serum antibodies or concurrent neurological symptoms or other relevant comorbidities (Guasp et al., 2021). Investigation of antineuronal antibodies is however expensive, rendering selection of relevant patients important. Around 80 % of patients with autoimmune encephalitis have been found to have abnormal routine CSF tests when examining measures such as white blood cell count and oligoclonal bands (Broadley et al., 2021; Hébert et al., 2020). Thus, in the absence of other red flags raising suspicion for autoimmune encephalitis such as focal neurological disorders or seizures (Pollak et al., 2020), a more feasible and cost-effective way of approaching selection of patients is initial screening of CSF with inexpensive routine analyses to decide if investigation of antineuronal antibodies is indicated.

4.1. Strengths and limitations

Our study complies with all criteria for a high-quality study as listed in the Newcastle-Ottawa Scale (Wells et al., 2000), increasing the validity of our findings; we comprehensively evaluated the diagnoses of our patients, as well as the psychopathological status of our controls. This allowed for detailed validation of both case and control status. Patients and controls were included from the same region and were individually matched regarding both sex and age. Patients were included from both in- and outpatient clinics from a broad geographical area, ensuring

representativeness of patients with recent onset psychotic disorders in Denmark. Additionally, sampling and handling of biological material from patients and controls were identical, and all laboratory analyses were performed blinded to case/control status.

Our study is possibly limited by the requirements to partake in our study, where patients had to be able to consent to participate in a research study and cooperate with the procedure of the lumbar puncture. Moreover, though our intervention was kept as brief as possible, we included a very thorough assessment of multiple aspects of psychopathology, cognition, and clinical variables. Hence, the most severely ill patients might not have been able to be included in our study, as indicated by the mean PANNS score of 62.5 and the relatively high cognitive scores; however, another recent study on CSF antineuronal autoantibodies in first-episode psychosis patients averaging a PANSS score of 93 found results similar to ours (Bien et al., 2021). Another limitation to our study is the use of fixed CBA, inclusion of only IgG subtype antibodies, and use of TBA only in cases positive of well-known antibodies on CBA. IgA and IgM subtypes, as well as currently undiscovered antineuronal antibodies, might possibly play a yet unknown role in non-encephalopathic autoimmune psychosis. Furthermore, the generalizability of our data is possibly limited by the age of our participants (18–50 years), as some types of autoimmune encephalitis are more prevalent in those older than 50 (e.g. anti-LGI1-Ab mediated encephalitis), and prevalence of antibodies in the CSF generally increases with increasing age. Additionally, two thirds of the included patients were male, while some autoimmune encephalitis syndromes (e.g., NMDA-R-Ab mediated) is markedly more prevalent among women. Lastly, our exclusion of affective psychosis may have served as a limitation, as a majority of patients with isolated psychiatric syndromes of autoimmune encephalitis have been observed with mood disorders (Kayser et al., 2013).

4.2. Conclusions and perspectives

This largest study to date on antineuronal antibodies in the serum and CSF of 104 patients with recent onset psychotic disorders and 104 individually matched healthy controls revealed no significant differences between the two groups. No CSF samples were positive for antibodies, whereas antineuronal antibody seropositivity was found in one patient and two healthy controls. The true prevalence of CSF and serum antineuronal antibodies in patients with a broad spectrum of psychotic disorders and healthy controls remains largely unknown, but it seems apparent that CSF antibodies are very rare in both groups. With the low prevalence found by us and others, studies including a much higher number of participants would be needed to detect a possible difference, and selection of patients to be screened for antibodies with routine CSF measures such as white blood cell count seems relevant.

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