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ORIGINAL PAPER

Dysfunction of the blood-cerebrospinal fluid-barrier and *N*-methyl-D-aspartate glutamate receptor antibodies in dementias

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Abstract *N*-Methyl-D-aspartate glutamate receptor (NMDA-R) antibodies (Abs) could play a role in neurodegenerative disorders. Since, in these diseases, NMDA-R Abs were detected in serum, but only sporadic in cerebrospinal fluid (CSF), the origin and impact of the Abs are still unresolved. We examined the presence of NMDA-R Abs in serum and CSF using a cell-based immunofluorescence assay as well as the function of the blood-CSF-barrier (B-CSF-B) by determination of Q albumin (ratio of albumin in CSF and serum) in patients with mild cognitive impairment (MCI; $N=59$) and different types of dementia, Alzheimer's disease (AD; $N=156$), subcortical ischemic vascular dementia (SIVD; $N=61$), and frontotemporal dementia (FTD; $N=34$). Serum IgA/IgM NMDA-R Abs and/or a disturbed B-CSF-B were sporadically present in all investigated patients' groups. In AD, these Abs often developed during the disease course. Patients with either no hippocampal atrophy and/or no AD-related characteristic changes in CSF, referred to "non-classical" AD, were characterized by seropositivity at diagnosis and loss of function of the B-CSF-B showed a progressive decline in cognitive functions and a poor prognosis. Our data indicate that NMDA-R Abs are present in different types of dementia and elderly healthy individuals. In combination with disturbed B-CSF-B integrity, they seem to promote their pathological potential on cognitive decline, and thus, a subgroup of dementia patients with these unique characteristics might inform clinicians.

Keywords NMDA-R antibodies · Blood-CSF-barrier (B-CSF-B) · NMDA-R dementia · Alzheimer's disease (AD) · Subcortical ischemic vascular dementia (SIVD) · Frontotemporal dementia (FTD) · Mild cognitive impairment (MCI)

Introduction

L-Glutamate is the main excitatory neurotransmitter in the brain. Since this amino acid is involved in cognitive functions, a dysfunction of the *N*-methyl-D-aspartate glutamate-receptor (NMDA-R) contributes to neuropsychiatric disorders [27]. These effects may be linked with a dysregulated immune system: Initially, Dalmau described IgG antibodies (Abs) in anti-NMDAR encephalitis [7], followed by several studies investigating Abs against the NR1 or GluN1/GluN2 subunits of the NMDA-R in other neuropsychiatric diseases [25]. Surprisingly, NMDA-R Abs were also detected in serum of healthy volunteers and are probably a hallmark of normal aging [3, 21], since the overall frequency of autoantibodies increases with age [22].

Although the origin and impact of NMDA-R Abs are still not completely resolved, their presence in serum of schizophrenic patients with a history of a disturbed blood–brain barrier (BBB) [22, 38] suggests that Abs develop their pathogenic potential when the BBB is compromised [3, 22]. Typical neurodegenerative disorders like

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dementias are very frequent during aging and affect the BBB [14, 33, 37]. The BBB separates the brain tissue from the blood circulation and the B-CSF-B separates the blood from the cerebrospinal fluid (CSF). Both barriers protect the central nervous system (CNS), since they prevent the passage of microorganisms and other potential toxic substances from blood into the brain.

To prove our hypothesis, that, in dementia, the presence of NMDA-R autoantibodies in the periphery might be detrimental if, in parallel, the integrity of B-CSF-B is disturbed, we investigated the function of B-CSF-B and the distribution of NMDA-R Abs between blood and CSF in patients with mild cognitive impairment (MCI), which is considered as the intermediate state between normal aging and dementia, and in patients with Alzheimer's disease (AD), subcortical ischemic vascular dementia (SIVD), and frontotemporal dementia (FTD), and compared them with neuropsychiatrically healthy elderly subjects. Moreover, we addressed the question if seropositivity could develop during the course of disease, and whether once detected, NMDA-R Abs are associated with the clinical course of dementia.

Materials and methods

Study population

The study was performed in accordance with German laws, the Declaration of Helsinki, and the guidelines of the local institutional review board. We screened all available serum and CSF samples of patients from an established scientific biobank at Magdeburg University's Department of Psychiatry for the presence of NMDA-R antibodies. We included 59 MCI patients (63–92 years, mean age 77.14; 40 female, 19 male), 34 FTD patients (61–86 years; mean age 76.68; 20 female, 14 male), 61 SIVD patients (55–92 years; mean age 78.25; 41 female, 20 male), and 156 AD patients (59–93 years; mean age 80.61; 106 female, 50 male; demographic data are shown in Table 1). "Classical" AD was defined as hippocampal and/or mesiotemporal atrophy (MRI, CT) and characteristic changes in CSF values (p tau > 50 pg/ml, total tau > 350 pg/ml, Aβ1-42 < 485 pg/ml, Aβ ratio < 0.8). In total, 145 patients (100 female, 45 male) met these criteria and were grouped, therefore, into "classical" cases (clinical data are shown in Suppl. Table 1). In the residual 11 patients, we could not confirm the initial diagnosis "Alzheimer's dementia", since the above-mentioned characteristics of "classical" AD were absent or minimally present. These 11 patients (6 female, 5 male) were defined as "non-classical" cases (clinical data are shown in Suppl. Table 2).

32 healthy age-matched control persons without neurodegenerative disorders (50–89 years; mean age 72.41; 24 female, 8 male) participated at the study, 8 of them with CSF samples (diagnosis of these patients are shown Suppl. Table 3).

The patients were diagnosed at the university hospital for psychiatry and psychotherapy of Magdeburg. Clinicians had access to detailed clinical files, including the medical histories by proxy and referral letters from the general practitioners. Magnetic resonance imaging (MRI) of the brain or cerebral computer tomography (CT), cerebrospinal fluid analysis, mini-mental-state examination (MMSE), electroencephalography (EEG), and routine blood analysis (including differential blood cell count, levels of C-reactive protein, glucose, lipids, liver enzymes, and thyroid hormones) were performed. Patients with a history of immune disease, immunomodulating treatment, cancer, chronic terminal disease, severe cardiovascular disorder, substance abuse, and severe trauma or clinical/paraclinical findings indicating these disorders were excluded.

Serum samples were centrifuged at 1000x*g* for 10 min; CSF samples were centrifuged at 30x*g* for 5 min. All samples were stored at −80 °C until analysis.

Detection of NMDA-R antibodies

Antibody testing of all samples was performed at the Institute for Experimental Immunology in Lübeck, using a standardized laboratory technology as previously described [34, 39, 42]. Briefly, plasmids that contain glutamate receptor-type NMDA (subunits NR1a/NR1a) were transfected into HEK293 cells [8]. Recombinant cells were grown on cover glasses and fixed with acetone. Coated cover glasses were cut into millimeter-sized fragments (biochips) and

Table 1 Demographic data of study cohort at diagnosis

Characteristics	Patients group						Controls
	MCI	SIVD	FTD	Total	AD "classical"	"Non-classical"	
Total	59	61	34	156	145	11	32
Age (years; mean)	77.14	78.25	76.68	80.61	80.58	81.00	72.41
Gender (female/male)	40/19	41/20	20/14	106/50	100/45	6/5	24/8

Table 1summarizes number, age, and gender of neuropsychiatric healthy controls and patients suffering from MCI, SIVD, FTD, and AD, total and divided in "classical" and "non-classical" cases

used side-by-side with fragments containing untransfected cells as substrates in the indirect immunofluorescence test. The slides were incubated with patient samples at a starting dilution of 1:10 (serum) or undiluted (CSF). After incubation for 30 min at room temperature, the slides were washed in phosphate-buffered saline (PBS)-Tween for 5 min. Bound antibodies were labeled using Fluorescein-conjugated goat anti-human IgG, IgA, or IgM antibodies for 30 min and washed in PBS-Tween for 5 min. PBS-buffered glycerol (containing triethylenediamine to reduce bleaching) was added and a cover glass was applied. Samples were classified as positive or negative on the basis of the intensity of the surface immunofluorescence of transfected cells in direct comparison with nontransfected cells and control samples. The titer of an antibody was defined as the maximum dilution at which immunoreactivity was visible. All test results were confirmed in a blinded separate analysis.

Determination of B-CSF-B integrity

The B-CSF-B function was evaluated by Q albumin, since albumin is produced exclusively in the liver and its presence in CSF is totally dependent on its transudation from the systemic circulation [36]. Therefore, the concentration of albumin was analyzed in CSF and, in parallel, also in serum. Q albumin was calculated by dividing the detected concentration of albumin in CSF by that detected in serum (result: Q albumin). Since normal aging is associated with a decline in B-CSF-B functions, we calculated an age-dependent reference value of B-CSF-B to differentiate modifications of B-CSF-B induced by aging from disorder-related alterations.

To quantify the immunoglobulin content in CSF, we calculated the quotient by dividing the detected concentrations of IgM, IgA, or IgG in CSF by the detected concentrations of IgM, IgA, and IgG serum. Q IgM, IgG, and IgG were calculated by dividing the detected concentration of the antibody isotypes in CSF by that detected in serum (result: Q IgM; Q IgG, and Q IgA).

Follow-up study and treatment of AD patients after diagnosis

In a preliminary follow-up study, we determined the presence of NMDA-R Abs in serum of 27 NMDA-R-AD patients, 11 NMDA-R+ AD patients, 8 "non-classical" AD patients, and 21 elderly healthy volunteers without any neurodegenerative diseases for up to 3 years after the first blood draw (demographic data of study cohort registered in Suppl. Table 4).

AD is a progressive disorder that could only hold up for a certain time by treatment of patients with acetylcholinesterase inhibitors (e.g., Rivastigmine) or Memantine, a non-competitive antagonist at glutamatergic NMDA-Rs. 33 patients received Rivastigmine (21 NMDA-R- AD patients, 10 NMDA-R+ AD patients, and 3 "non-classical" AD NMDA-R- AD patients) and 12 patients were treated with memantine (6 NMDA-R- AD patients, 1 NMDA-R+ AD patient, and 5 "non-classical" AD).

Mini-mental state examination (MMSE)

The mini-mental state examination (MMSE) was used to measure the cognitive function of patients and, therefore, the effect of treatment. Since the patients had different MMSE values at diagnosis, we calculated the percentages (day 0 = 100%) to detect positive or negative developments in the course of disorder.

Statistical analysis

To determine differences between the indicated groups, ANOVA, student's *t* test, and Fisher's exact tests were performed. The *p* values were corrected by the Bonferroni method. Significance was defined as $p < 0.05$ (*), $p < 0.005$ (**) or $p < 0.001$ (***). To detect alterations in the integrity of the blood-CSF-barrier (B-CSF-B) due to a dementia, reference value of an intact B-CSF-B in aged individuals (Q albumin) was calculated: age/15 + 4.

Results

Detection of NMDA-R antibodies

IgG was not detected in any of the 350 samples. In some individuals, both IgM and IgA Abs were detectable; therefore, we included the column "IgA and/or IgM" meaning the total number of seropositive people (Table 2). In none of the CSF samples, NMDA-R Abs were detectable.

In our present study cohort, 3.1% of neuropsychiatric healthy persons had IgA and/or IgM NMDA-R Abs in serum, 6.25% IgA and/or IgM. Of note, none of the healthy subjects with available CSF data was seropositive.

In patients suffering from frontotemporal dementia (FTD), we detected only the isotype IgA (8.8%) in serum. 16.4% of patients with subcortical ischemic vascular dementia (SIVD) had NMDA-R Abs IgA and/or IgM in serum, 8.2% IgA and 11.5% IgM.

In mild cognitive impairment (MCI), a possible preliminary stage of AD, 5.1% of patients had positive IgA and/or IgM NMDA-R Abs, 3.4% IgA and 3.4% IgM. 10.9% of AD patients had NMDA-R autoantibodies subtypes IgA and/or IgM, more patients with IgM (8.3%) than IgA (3.9%). Although we detected no significant differences in the Ab

Table 2 Seroprevalence of IgA and IgM NMDA-R antibodies in MCI, several dementia forms and persons without neurodegenerative disorders, and correlation with a disturbed blood-CSF-barrier

Group	Total	NMDA-R Abs in serum					
		IgA		IgM		IgA and/or IgM	
		N	%	*N*	%	*N*	%
MCI	59	2	3.4	2	3.4	3	5.1
SIVD	61	5	8.2	7	11.5	10	16.4
FTD	34	3	8.8	0	0	3	8.8
AD							
Total	156	6	3.9	13	8.3	17	10.9
"Classical"	145	3	2.1	4	2.8	6	4.1
"Non-classical"	11	3	27.3	9	81.8	11	100
Controls	32	1	3.1	1	3.1	2	6.25

Table 2 specifies the seroprevalence of NMDA-R Abs in patients suffereing from MCI, SIVD, FTD, and AD (total, "classical", "non-classical") and neuropsychiatric healthy controls, once as total number (*N*) and again as percentage (%)

Since some individuals have both IgA and IgG NMDA-R Abs in serum, the column "IgA and/or IgM" lists the total number of seropositive persons, the column "IgA" and "IgM" register the persons with the indicated Ab subtype

titers (Suppl. Figure 1), we identified a group of 11 patients who were admitted to our hospital with cognitive failures and a first diagnosis AD that presented clinically as "non-classical" form of AD. In these patients, at least one of the characteristics that define the disorder AD, such as typical changes in Aβ and tau proteins and a hippocampal atrophy, were only detected to a minor degree or were absent (Suppl. Table 2). Surprisingly, these patients substantially contributed to the seropositivity of AD patients, since all of this "non-classical" AD patients had IgA and/or IgM NMDA-R Abs, 81.8% IgM and 27.3% IgA. In the remaining "classical" AD cases, 2.8% of patients had IgM and 2.1% had IgA NMDA-R Abs in serum. We found no association between the concentration of total antibodies in serum and the presence of NMDA-R autoantibodies as well as the level of C-reactive protein (CRP) as a marker for inflammation and the level of IgM or IgA in serum (Suppl. Figure 2).

Association between NMDA-R Abs in serum and B-CSF-B dysfunction

An impairment of the B-CSF-B function was detected in 22% of our elderly volunteers without a neurodegenerative disorder. However, the frequency of a B-CSF-B dysfunction was higher in each dementia group, between 32.1% (AD) and 39.3% (SIVD). A simultaneous presence of NMDA-R Abs in serum was low in MCI and FTD (1.7% in MCI, 2.9% in FTD) and higher in SIVD (8.2%) and AD patients (7.1%; $p=0.036$). When we looked closely at the large group of AD patients, again the group of 11 patients with "non-classical" AD contributed significantly to the frequency of patients with a disrupted B-CSF-B and a positive Ab titer in serum, since all of these individuals had a dysfunction of B-CSF-B and NMDA-R Abs (Table 2).

Changes in CSF associated with "non-classical" AD

Although we could not detect NMDA-R autoantibodies in CSF of our study cohort, we were interested whether we could detect any differences in CSF between patients with "classical" AD symptoms that were seropositive (NMDA-R+ AD) or seronegative (NMDA-R- AD) and patients with a "non-classical" form of AD who were characterized by presence of NMDA-R Abs in serum and a dysfunction of B-CSF-B ("non-classical" AD). Therefore, we determined changes in CSF immunoglobulins and cell count. We found that the presence of NMDA-R Abs in serum does not influence any of Ab isotypes (Fig. 1a): NMDA-R- AD has a mean Q IgM of 0.6926, Q IgA of 1.912 and a Q IgG of 3.859; the mean Q IgM in NMDA-R+ AD is 0.3800, Q IgA 1.618 and Q IgG is 3.045.

The level of all tested isotypes is significantly higher in CSF of "non-classical" AD patients compared to NMDA-R- AD and NMDA-R+ AD. This is also reflected by the calculated CSF/serum ratios: the mean Q IgM was 1.500 in "non-classical" AD compared to 0.6926 in NMDA-R- AD ($p=0.0025$) and 0.3800 in NMDA-R+ AD ($p=0.0003$). The mean Q IgA in "non-classical" AD was 3.738, in NMDA-R- AD 1.912 (p NMDA-R- AD: <0.0001) and in NMDA-R+ AD 1.618 ($p<0.0001$). The Q IgG in "non-classical" AD patients was 6.450, in NMDA-R- AD 3.485 ($p=0.0058$) and in NMDA-R+ AD 3.045 ($p<0.0001$).

However, neither the cell count in CSF nor the distribution of cell types (mainly lymphocytes and monocytes) was significantly different between the three investigated

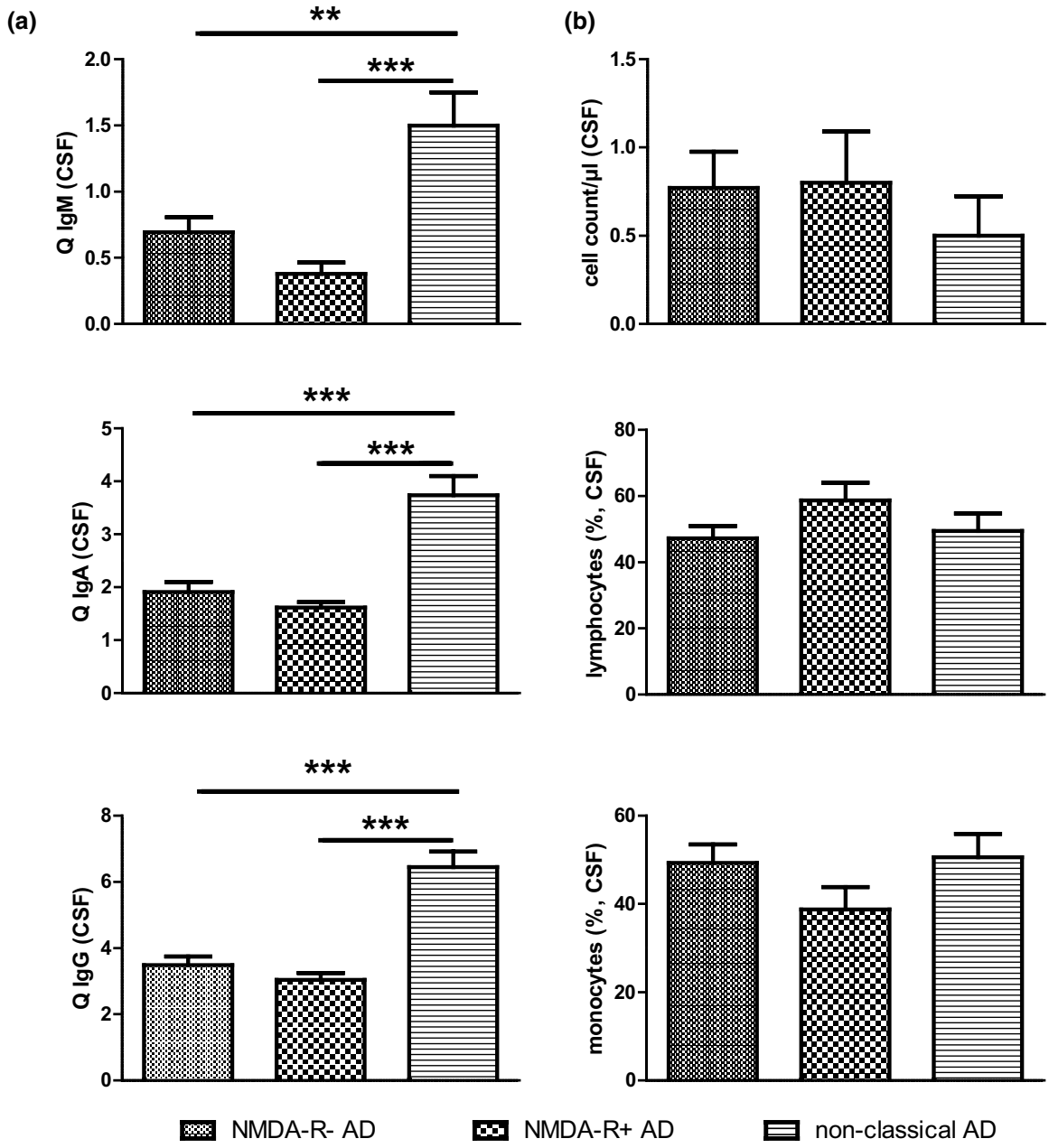

Fig. 1 Changes in CSF immunoglobulins and cell count in classical AD with or without NMDA-R Abs and "non-classical" AD. To identify changes that are associated with "non-classical" AD, classical AD either without NMDA-R Abs (NMDA-R- AD) or with NMDA-R Abs (NMDA-R+ AD) in CSF, we calculated the quotient (Q) between the content of IgM, IgA and IgG in CSF and serum (**a**) and the total number of cells and the main cell types, lymphocytes and monocytes, in CSF using ANOVA test (**b**). $^{**}p < 0.005$; $^{***}p < 0.001$

patients groups. The mean total cell count was in "non-classical" AD patients 0.500, in NMDA-R- AD 0.7703 and in NMDA-R+ AD 0.800. The percentage of lymphocytes ranged between 47.23% in NMDA-R- AD, 49.43% in "non-classical" AD, and 58.70% in NMDA-R+ AD. In "non-classical" AD, mean 50.57% of cells in CSF were monocytes, 49.35% in NMDA-R- AD and 38.80% in NMDA-R+ AD (Fig. 1b).

Course of NMDA-R Abs seropositivity in a follow-up study

At diagnosis, the frequency of patients with classical symptoms associated with AD and positive NMDA-R Ab titers were rather low (4.1%). Therefore, we were interested whether during the progressive course of this disorder seropositivity would develop. On the other hand, we also

wanted to know whether the isotype could switch or the autoantibodies were undetectable, potentially induced by medication. To gain first insight into this theme, we performed a preliminary follow-up study with 27 NMDA-R-AD patients, 11 NMDA-R+ AD patients, 8 "non-classical" AD patients and 21 elderly healthy volunteers.

IgM or IgA NMDA-R Abs were detected in serum of 11 NMDA-R+ AD and 8 "non-classical" AD patients. At the same time, IgA and IgM NMDA-R Abs were detected in only one patient (WK). While in NMDA-R+ AD the Abs were mostly detected in serum in the course of the disorder (72.7%), in "non-classical" AD the Abs were very often (75%) present from the day of diagnosis. In this AD form, 7 patients had IgM NMDA-R Abs in serum and only one (12.5%) IgA (Table 3).

IgA NMDA-R Abs were detected in four patients with NMDA-R+ AD, two at the day of diagnosis and the other two during the course of disorder. On the contrary, 87.5% of NMDA-R+ AD patients developed IgM NMDA-R Abs in the weeks and months after diagnosis and only one patient was IgM positive at diagnosis (Table 3).

Once detected, the NMDA-R Abs kept present in serum in 85% of patients. In three cases, the Abs that were found at diagnosis could not be detected in the further course. These patients received rivastigmine for treatment.

Table 3 shows the results of correlation analysis between the seropositivity of NMDA-R Ab and the integrity of the blood-CSF-barrier, which was calculated by Q albumin (corrected: age/15 + 4), received by Fisher's exact tests

	NMDA-R Abs	B-CSF-B disturbation			B-CSF-B disturbation			Fisher's exact test/Bonferroni correction
			No	Yes		No	Yes	
			N	*N*		%	%	
MCI	No	*N*	38	18	%	64.4	30.5	1.000/1.000
	Yes	*N*	1	1	%	3.4	1.7	
SIVD	No	*N*	32	19	%	52.5	31.1	0.495/1.000
	Yes	*N*	5	5	%	8.2	8.2	
FTD	No	*N*	19	12	%	55.9	35.3	1.000/1.000
	Yes	*N*	2	1	%	5.9	2.9	
AD "total"	No	*N*	100	39	%	64.1	25.0	0.004/0.036**
	Yes	*N*	6	11	%	3.8	7.1	
AD "classical"	No	*N*	100	39	%	69.0	26.9	0.192/1.000
	Yes	*N*	6	0	%	4.1	0	
AD "non-classical"	No	*N*	0	0	%	0	0	–
	Yes	*N*	0	11	%	0	100	
Controls	No	*N*	7	2	%	77.8	22.2	–
	Yes	*N*	0	0	%	0	0	

The p values were corrected by Bonferroni method; $^{**}p < 0.005$

Table 4 IgA and/ or IgM NMDA-R autoantibodies at diagnosis or in the clinical course of disorder in patients with "classical" AD and NMDA-R Abs and in "non-classical" AD

Group	Total	NMDA-R Abs in serum					
		IgA		IgM		IgA and/or IgM	
		N	%	*N*	%	*N*	%
At diagnosis							
NMDA-R + AD	11	2	50	1	12.5	3	27.3
"Non-classical" AD	8	1	100	5	71.4	6	75
During course							
NMDA-R + AD	11	2	50	7	87.5	8	72.7
"Non-classical" AD	8	0	0	2	28.6	2	25

In Table 4, the chronological presence of NMDA-R Abs is illustrated in seropositive patients either with the "classical" or "non-classical" form of AD at diagnosis or during course of disease. Shown is the total number of cases (*N*) as well as the percentages (%)

NMDA-R Abs influence the MMSE

The presence of NMDA-R Abs in the periphery *per se* does not influence the MMSE. Three months after diagnosis and start of medication, patients with NMDA-R- AD (mean 105.2%) and NMDA-R+ AD (mean 110.3%) showed improved cognitive functions that were still present after one year (mean 116.8 and 116.4%). However, in "non-classical" AD, the cognitive decline continues (mean after three months: 84.5%; $p=0.0189$; mean after one year: 71.4%). Two years after onset of medication, the MMSE value decreased in all three groups, but compared to NMDA-R- AD (mean 76.00%) and NMDA-R+ AD (83.74%), the cognitive decline was more pronounced in "non-classical" AD (mean: 43.89%; $p=0.0247$; Fig. 2a).

"Non-classical" AD patients have a poorer prognosis

AD is a progressive disorder that finally leads to death. Within the 3 years after diagnosis, 8 of 38 (21.1%) "classical" AD patients died. Although not significant, 3 of the 8 patients (37.5%) in the group of "non-classical" AD died within the 3 years after diagnosis.

Taken together, the observed patients which were first identified by "non-classical" AD symptoms showed a unique pattern of characteristics (summarized in Fig. 2b). According to the high frequency of NMDA-R Abs in serum we suggest to designate this AD form "NMDA-receptor dementia".

Discussion

In our present study, we investigated the association between the presence of NMDA-R-specific serum Abs (IgA and/or IgM) in MCI and several forms of dementia (AD, SIVD and FTD). Though, we identified a group of patients initially diagnosed with AD that is characterized by the seroprevalence of these Abs, combined with dysfunction of B-CSF-B.

Our findings support other publications indicating that MCI and dementias are associated with IgM and IgA NMDA-R Abs in serum, but not in CSF [2, 10, 35]. Although the origin and biological impact of these autoantibodies is still unresolved, these findings indicate that the origin of the autoimmunity is located in the periphery. NMDA-Rs are expressed in peripheral tissues, such as kidney, lungs, spleen, testis and ovaries [16, 17] and by several cell types like osteoblasts and osteoclasts [19] and T

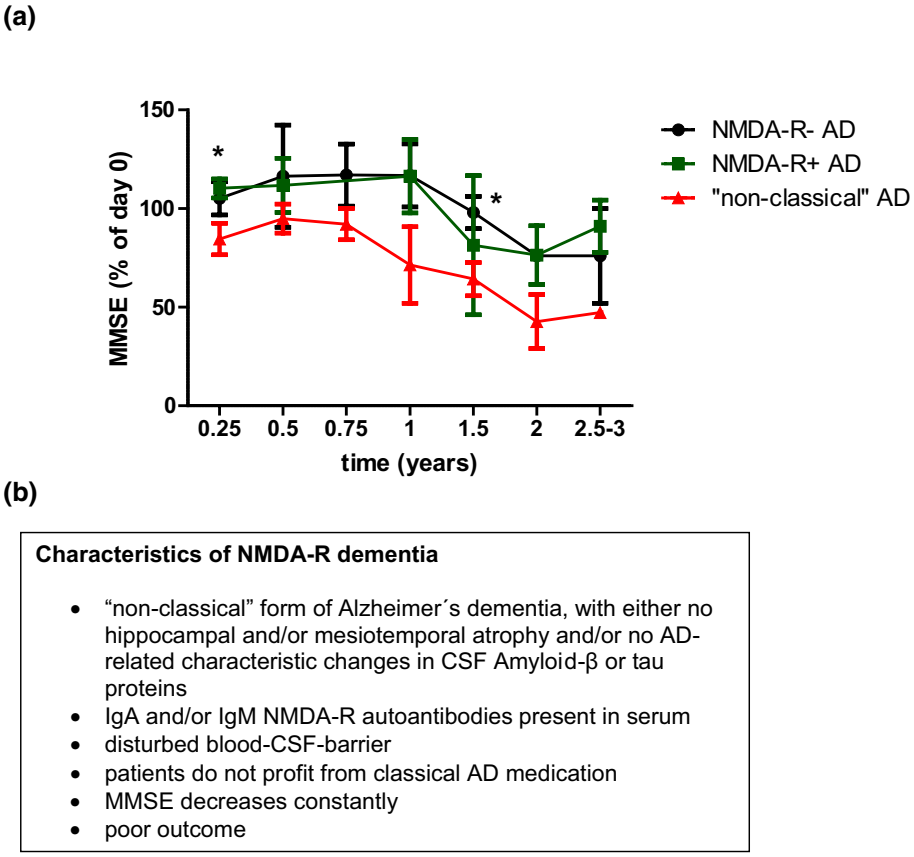

Fig. 2 MMSE of patients with "classical" AD with or without NMDA-R antibodies and "non-classical" AD and characteristics of NMDA-R dementia. In Fig. 2a, the mini-mental state examination (MMSE) is used to determine the cognitive function of dementia patients. Within the examined patient's groups "classical" AD with (NMDA- + AD) or without NMDA-R antibodies (NMDA-R- AD) and "non-classical" AD, the individual MMSE values were different. To determine the trend of changes in MMSE values, MMSE values at time point diagnosis (day 0) were defined 100%. Changes in MMSE values (improvements, impairments) were calculated as % from day 0. Statistics were performed using ANOVA test, $*p<0.05$. The group of "non-classical" AD cases shows unique characteristics. These are summarized in Fig. 2b. To distinguish this patient cohort from other types of dementia, we suggest terming this disorder "NMDA-R dementia"

lymphocytes [30, 32] and autoantibodies could there origin from there.

Furthermore, our data cover the results published by Doss et al. who found the highest frequency of NMDA-R Abs in "unclassified" dementia [10]. Among all dementia groups, "non-classical" AD patients had the highest frequency of NMDA-R seropositivity and B-CSF-B dysfunction.

In general, AD patients benefit from drugs like rivastigmine or mementine, indicated by an improved or even stable MMSE value within at least the first year of treatment, independent of the presence of NMDA-R Abs in serum. Treatment of "non-classical" AD patients with these drugs resulted in a rapid decline in cognitive function, in the course of disorder they never ever reached 100% MMSE level at diagnosis, implicating no beneficial effect of medication. Therefore, we hypothesize that the presence of NMDA-R Abs, which are detected in serum of dementia patients but also in neurodegenerative healthy individuals, which was described by our and other groups [2, 4–6, 21], has no pathological impact by itself [3]. However, upon breakdown of B-CSF-B integrity, these Abs can pass the B-CSF-B, enter the brain and bind to their main target cells, resulting in a diminished cognitive function.

Studies aiming at the integrity of blood–brain-barrier/B-CSF-B in AD showed controversial results: some authors detected an elevated CSF/serum albumin ratio in AD patients while others found no differences compared to controls [12, 24, 31, 41]. Therefore, it was speculated that only a subgroup of AD patients develops measurable B-CSF-B disruption. We identified the subgroup "non-classical" AD/NMDA-R dementia characterized by B-CSF-B disruption.

Although it remains to be clarified why we could not detect Abs in CSF of our seropositive patients independent from B-CSF-B integrity, we might speculate that the NMDA-R Abs are bound on the surface of their target cells and thereby the side of the NMDA-R Abs, which is detected in our test system, is no more available. A first hint for this theory came from ApoE-/- mice with an open BBB: Five days after intravenous application of NMDA-R1 Ab, these Ab were not detectable in CSF, but were instead recovered in different parts of the brain indicating that the brain serves as "immunoprecipitator" for serum NMDA-R1 Abs (Ehrenreich et al., personal communication). It is still unclear to which extent these data can be transferred to human situation. However, in schizophrenia, it was shown that patients with a last or present history of BBB disturbance, e.g., by brain trauma or birth complication, are more likely to develop more pronounced neurological symptoms upon NMDA-R Abs seropositivity [22, 40]. But there are several other reasons resulting in a diminished B-CSF-B integrity: In neurodegenerative disorders, changes in the expression of key vascular genes and receptors in brain capillaries and arteries can compromise (directly or indirectly) several BBB functions [1, 43]. Severe regional cerebral blood flow (CBF) reductions led to accumulation of glutamate and lactate [11], a diminished transport of energy substrates and nutrients across the BBB and a reduced clearance of potential neurotoxins. In AD, the attenuated CBF results in Aβ accumulation and neuroinflammation. Finally, the B-CSF-B/ BBB breaks down and also huge molecules like Abs have access to the brain. In a mouse model it was demonstrated that expression of anti-NR2 does not induce neuronal damage until BBB breakdown, indicating the importance of an intact BBB for the prevention of anti-NR2 transport from the systemic circulation into CNS [26].

Second, virus-induced destruction of neurons are discussed to disturb B-CSF-B integrity. Acute infections or reactivation of Mycoplasma, Herpes-simplex virus (HSV), and Epstein-Barr virus (EBV) have been described in patients with anti-NMDA-R Abs [15, 20, 44]. Such viral infections could be important for the pathophysiology of dementias, since, recently, an association between the risk of developing AD and the presence of HSV Abs has been described [28, 29].

Normal aging is associated with reductions in CBF and, therefore, diminished cerebral protein synthesis [23] and another factor that contributes to a compromised BBB [13]. To differentiate changes of B-CSF-B induced by aging from disorder-induced ones, we calculated an age-dependent reference value of B-CSF-B.

Since a significant proportion of SIVD patients had a compromised B-CSF-B and NMDA-R Abs, another possible mechanism could account for the dysfunction of B-CSF-B: neurotoxicity, induced by embolic or thrombic vascular occlusion. After the splitting of NMDA-Rs by thrombin-activated serine proteases [18] brain-specific NMDA-R fragments could enter the bloodstream and initiate a peripheral immune response producing NMDA-R Abs [9].

Our present study has certain limitations that need to be considered. First, we cannot exclude the influence of vascular risk factors to B-CSF-B, because AD is partially overlapping with SIVD. Most patients with AD suffered from vascular risk factors, such as hypertension, hyperlipidemia, diabetes mellitus and metabolic syndrome. Second, the influence of medication (rivastigmine, memantine) remains unclear. Third, our study cohort consisted of only some persons without a neurodegenerative disorder. Besides, the NMDA-R Abs seropositive cases, especially in the groups of MCI and FTD were few so that it was not possible to prove a connection with the function of B-CSF-B. More patients have to be recruited to compare these groups with the two described cohorts of AD and SIVD patients.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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