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Depressive symptoms and anti-N-methyl-D-aspartate-receptor GluN1 antibody seropositivity in the PROSpective cohort with incident stroke

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ABSTRACT

Background: Anti-NMDA-receptor GluN1 antibodies (NMDAR1-abs) are present in an autoimmune encephalitis with severe neuropsychiatric symptoms. We aimed to estimate the impact of serum NMDAR1-abs on depressive symptoms years after first-ever ischemic stroke (IS).

Methods: Data were used from the PROSpective Cohort with Incident Stroke-Berlin (PROSCIS-B; NCT01363856). Serum NMDAR1-abs (IgM/IgA/IgG) were measured within 7 days after IS using cell-based assays. We defined seropositivity as titers $\geq 1:10$, thereof low titers as $\leq 1:100$ and high titers as $> 1:100$. We used the Center for Epidemiological Studies–Depression (CES-D) scale to measure depressive symptoms at year one, two and three following IS. We calculated crude and confounder adjusted weighted generalized linear models to quantify the impact of NMDAR1-abs on CES-D assessed at three annual time-points.

Results: NMDAR1-abs were measured in 583 PROSCIS-B IS patients (mean age = 67 [SD = 13]; 42%female; median NIHSS = 2 [IQR = 1–4]) of whom 76 (13%; IgM: n = 49/IgA: n = 43/IgG: n = 2) were seropositive, 55 (9%) with low and 21 (4%) with high titers. CES-D regarded over all follow-up time-points was higher in seropositive patients ($\beta_{\text{crude}} = 2.56$ [95%CI = -0.34 to 5.45]; $\beta_{\text{adjusted}} = 2.26$ [95%CI = -0.68 to 5.20]) and effects were highest in patients with high titer (low titers: $\beta_{\text{crude}} = 1.42$ [95%CI = -1.79 to 4.62], $\beta_{\text{adjusted}} = 0.53$ [95%CI = -2.47 to 3.54]; high titers: $\beta_{\text{crude}} = 5.85$ [95%CI = 0.20 to 11.50]; $\beta_{\text{adjusted}} = 7.20$ [95%CI = 0.98 to 13.43]).

Conclusion: Patients with serum NMDAR1-abs (predominantly IgM&IgA) suffer more severe depressive symptoms after mild-to-moderate IS compared to NMDAR1-abs seronegative patients.

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1. Introduction and background

Stroke is a devastating event, however, modern therapeutic options greatly reduced stroke related physical disability and mortality (Collaborators, 2019). Neuropsychiatric sequelae are frequent – even after minor strokes – with depression occurring in at least one third of stroke survivors (Ferro et al., 2016; Dong et al., 2020). Depression after stroke associates with impaired rehabilitation, functional and vascular outcome, and also greatly increases mortality (Dar et al., 2017). Not much is known on the occurrence of depressive symptoms in the long-term after stroke and a sound pathophysiological concept is lacking. Neuroinflammation, however, is considered to play an important role (Fang et al., 2019; Arwert et al., 2018).

In 2007, Dalmau et al. described the pathobiology of a severe multi-stage neuropsychiatric disease associated with immunoglobulin (Ig)G antibodies targeting the GluN1 (also NR1) subunit of N-methyl-D-aspartate receptors (NMDAR1-abs) (Dalmau et al., 2007). Circulating NMDAR1-abs of the IgA and IgM isotype were found in healthy individuals and patients with various diseases, including stroke patients (Doss et al., 2014; Steiner et al., 2014).

NMDAR1-abs are considered to contribute to neuropsychiatric outcome as suggested by previous studies (Pruss et al., 2012; Dahm et al., 2014; Sperber et al., 2022; Daguano Gastaldi et al., 2023). One study found an association of serum NMDAR1-abs with depression after stroke (Deutsch et al., 2021). However, these data are in contrast to a previous study, which linked serum NMDAR1-abs with anti-depressive effects (Pan et al., 2021). Overall, the impact of NMDAR1-abs on depressive symptoms after stroke seems unclear. Therefore, we aimed to estimate effects of serum NMDAR1-abs on depressive symptoms over three years after first ischemic stroke (IS).

2. Methods

Data and scripts supporting the findings of this investigation are available from the corresponding author upon reasonable request.

2.1. Study design, patients and ethics

The prospective cohort with incident stroke – Berlin (PROSCIS-B, ClinicalTrials.gov identifier: NCT01363856) is a university hospital based prospective cohort study of stroke patients, with the primary aim to study stroke secondary risks. Details on the study design, in- and exclusion criteria were published previously (Liman et al., 2013). Briefly, patients with first ever stroke according to world health organization (WHO) criteria, (Hatano, 1976) within the past seven days were included. Excluded were patients with brain malignoma, brain metastasis of a malignoma of other origin, and those participating in an intervention study. Patients with National Institutes of Health Stroke Scale (NIHSS) score >15 and were excluded from analyses, to increase homogeneity, as only few patients presented with a severe stroke event (i.e. NIHSS >15) in this study. PROSCIS-B was approved by the local ethics committee and conducted in concordance with the declaration of Helsinki. The study was completed in 2016.

2.2. Antibody measurement

Blood drawings were conducted at time of baseline visit. NMDAR1-abs were measured from patients sera with fixed cell-based assays (CBA) in a specialized laboratory, as previously described (Dalmau et al., 2007; Ramberger et al., 2015). Any titer $\geq 1:10$ of NMDAR1-abs of the immunoglobulin (Ig-) A, IgM and IgG isotype were considered seropositive. We a priori defined subgroups with titers $\leq 1:100$ defined as a low titer group and titers $>1:100$ as a high titer group, in line with previous analyses (Sperber et al., 2019, 2022). Other measurements included a tissue reactivity assay using monkey cerebellum, GAD65 IgG antibody measurement, GABA-b IgG antibody measurement, AQP4 IgG

antibody measurement, LGI1 IgG antibody measurement, and CASPR2 IgG antibody, all with fixed CBAs in line with NMDAR1-abs measurement.

2.3. Outcome

Symptoms of depression were assessed annually up to three years (3 times per individual) after IS with the Center for Epidemiologic Studies Depression Scale (CES-D, 20 items, 4 domains, range 0–60 points, with 0 points minimal indicating no depressive symptoms and 60 points indicating the maximum level of depressive symptoms) by telephone interview and postal letter, starting at one year after stroke. All letters received within the consequent three months after dispatch were included in this study. Depression was considered present at a cut-off value of ≥ 16 (Parikh et al., 1988). Vital status was obtained by the local registry office in Berlin.

2.4. Statistics

The impact of NMDAR1-abs seropositivity and subgroups compared to seronegativity in PROSCIS-B stroke patients was estimated using time-specific weighted generalized linear models, an approach which has been described previously in detail. (Daza et al., 2017). We visualized the relationships between variables (underlying assumptions) using a directed acyclic graph, and choose the following set of variables for our adjusted analysis to minimize confounder bias: (Shrier and Platt, 2008) age in years, sex (binary: female and male), depression before stroke (assessed as anti-depressive treatment before stroke, please see Supplemental Methods I for which ATC codes were used), education (assessed in three levels according to the German schooling system), smoking (binary: yes and no), alcohol consumption (binary: yes and no), stroke etiology defined by the Trial of Org 10,172 in Acute Stroke Treatment (TOAST) classification (large artery atherosclerosis, cardioembolic, small-vessel occlusion, stroke of other determined etiology and stroke of undetermined etiology) and body mass index (BMI). The graph can be viewed in the Supplemental Methods II, Supplemental Material. A propensity score was calculated from these variables. We estimated correlation coefficients (β s) with 95% confidence intervals (95%CI) from crude and propensity score adjusted models using Stata version 14.2 (Stata Corp., College Station, TX, USA). Figures were designed in R i386 3.5.1 with the ggplot2 package.

3. Results

After excluding six patients with severe strokes, a total of 621 participants were initially included. However, five participants declined to provide consent for blood biomarker analyses, and antibody measurements were not possible in 33 participants due to reasons, such as the absence of collected samples, technical issues, or insufficient sample volume. The median day of blood sampling after stroke was 4 (IQR = 3 to 5). We included 583 mild-to-moderate IS patients with a mean age of 67 years (standard deviation [SD] = 13) with a median NIHSS of 2 (boundaries of the interquartile range [IQR] = 1 to 4) of whom 242 participants were female (42%), into the analysis. Thirty-eight patients (6%) were taking an anti-depressive medication before stroke. Seventy-six patients (13%) were seropositive for NMDAR1-abs, with 49 patients with IgM antibodies, 43 with IgA antibodies and only two patients with IgG NMDAR1-abs. Among seropositive patients, 55 (9%) had low titers (1:10–1:100) and 21 (4%) high titers ($>1:100$) of NMDAR1-abs. Patient characteristics stratified by NMDAR1-abs serostatus are presented in Table 1. From those other antibodies measured, only one patient had serum LGI1 antibodies, with a titer of 1:10 (See Supplemental Table 1). No substantial differences were found between NMDAR1-abs seropositive and seronegative patients, despite that more seronegative patients were female compared to seropositive patients, had a slightly higher median NIHSS (3 [IQR = 1–5] vs. 2 [IQR = 1–4]) on the day of the

Table 1
Baseline characteristics of PROSCIS-B participants, stratified according to anti-NMDA-receptor GluN1 antibody serostatus.

	Anti-NMDA-receptor GluN1 antibody serostatus	
	seronegative	seropositive
patients ^a n (%)	507 (82)	76 (13)
anti-NMDAR GluN1 antibodies n (%)		
IgM	–	49 (8)
IgA	–	43 (7)
IgG	–	2 (>0)
age (years)		
mean (SD)	67 (13)	66 (14)
median (IQR)	69 (59–76)	67 (56–77)
female sex n (%)	204 (40)	22 (29)
blood pressure (mmHg) mean (SD)		
systolic	139 (22)	139 (24)
diastolic	77 (15)	78 (13)
body mass index (kg/m ²) median (IQR)	27 (24–29)	28 (24–31)
habitual alcohol consumption n (%)	179 (36)	23 (31)
current smoker n (%)	139 (28)	22 (30)
total cholesterol (mg/dl) mean (SD) ^b	199 (48)	198 (50)
high density lipoprotein (mg/dl) mean (SD) ^c	52 (16)	49 (17)
low density lipoprotein (mg/dl) mean (SD) ^c	122 (41)	124 (43)
triglyceride (mg/dl) mean (SD) ^d	136 (80)	152 (80)
history of: n (%)		
hypertension	336 (66)	46 (61)
diabetes mellitus	107 (21)	21 (28)
peripheral artery disease	34 (7)	6 (8)
coronary heart disease	80 (16)	16 (21)
atrial fibrillation	106 (21)	18 (24)
estimated GFR (ml/min) mean (SD)	77 (21)	79 (22)
NIHSS median (IQR)	2 (1–4)	3 (1–5)
NIHSS 0–4 n (%)	386 (76)	54 (71)
NIHSS 5–15 n (%)	121 (24)	22 (29)
TOAST n (%) arterial atherosclerosis	128 (25)	25 (33)
cardioembolic	121 (24)	18 (24)
small vessel disease	87 (17)	6 (8)
other	15 (3)	2 (3)
undetermined etiology	156 (31)	25 (33)
Presence of chronic infarct lesions in MRI ^{e,f} n (%)	94 (27)	10 (23)
MR-DWI lesion volume in ml ^{e,g} median (IQR)	0.94 (0.30–3.71)	1.67 (0.41–6.07)
years of school n (%)		
≤10	345 (72)	51 (68)
>10	136 (28)	24 (32)
MMSE median (IQR)	28 (26–30)	29 (27–30)
Cognitive impairment (MMSE≤26) n (%)	144 (29)	16 (22)
Anti-depressive medication before stroke n (%)	30 (6)	5 (7)

SD, Standard deviation; IQR, boundaries of the inter quartile range between the 25th and 75th percentile; MI, myocardial infarction; PAD, peripheral artery disease; CHD, coronary heart disease; BMI, Body Mass Index; GFR, glomerular filtration rate calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula; HDL, high density lipoprotein; LDL, low density lipoprotein; NIHSS, National Institutes of Health Stroke Scale; TOAST, stroke etiology according to Trial of Org 10,172 in Acute Stroke Treatment; mRS, modified Rankin Scale; MMSE, Mini Mental State Examination.

Due to rounding values might not add to 100%.

^a 38 participants were missing antibody measurements; Missing values were <10% in all characteristics except for.

^b 'total cholesterol' missing: n = 57.

^c 'HDL' and 'LDL' missing: n = 38.

^d 'Triglycerides' missing: n = 49.

^e MRIs obtained retrospectively with different MRIs and protocols.

^f 'presence of chronic infarct lesions in MRI' missing: n = 203.

^g MR-DWI, magnet resonance defusion weighted imaging.

baseline assessment and a greater median infarct lesion volume in diffusion weighted MRI (1.67 ml [IQR = 0.41–6.07] vs. 0.94 ml [IQR = 0.30–3.71]), although lesions were rather small in the total cohort (median 1.04 ml [IQR = 0.35–4.49]).

One year after stroke, 411 CES-D scores were available; 357 CES-D scores two years after and 324 CES-D scores three years after stroke. We provide baseline characteristics of patients with a CES-D assessment at year three (study completion) contrasted to those patients without CES-D at year three (drop-outs), which showed no major differences between the strata. Except, more patients with a CES-D at year three had a higher school education and also baseline MMSE was higher in these patients (see [Supplemental Table II](#)). The mean CES-D value in the total cohort one years after stroke was 11 (SD = 10) points, 11 (SD = 9) points two years after stroke, and 11 (SD = 9) points three years after stroke. One-hundred-three (25%) patients could be categorized as depressed (CES-D≥16) one year after IS, of whom 33% of NMDAR1-abs seropositive patients were depressed (n = 17) and 24% (n = 86) of NMDAR1-abs seronegative patients were depressed. Corresponding difference in percentage points was 9 (95% CI = –5 to 22). Two years after stroke, 31% of seropositive patients compared to 26% of seronegative patients were depressed and at year three after stroke 25% of seropositive patients compared to 23% of seronegative patients were depressed. At year one after stroke, 8% (n = 6) of NMDAR1-abs seropositive compared to 3% (n = 16) of NMDAR1-abs seronegative patients died (13% vs. 6% at year two, and 21% vs. 8% at year three after stroke, respectively). [Fig. 1](#) shows CES-D scores of PROSCIS-B subjects over three years after stroke, stratified by NMDAR1-abs serostatus. NMDAR1-abs seropositive patients had a higher level of depression regarded over three years after IS ($\beta_{\text{crude}} = 2.56$ [95%CI = –0.34 to 5.45]; $\beta_{\text{adjusted}} = 2.26$ [95%CI = –0.68 to 5.20]). The observed effect was mainly driven by patients with high titers, as subgroup analysis revealed (low titers: $\beta_{\text{crude}} = 1.42$ [95%CI = –1.79 to 4.62], $\beta_{\text{adjusted}} = 0.53$ [95%CI = –2.47 to 3.54]; high titers: $\beta_{\text{crude}} = 5.85$ [95%CI = 0.20 to 11.50]; $\beta_{\text{adjusted}} = 7.20$ [95%CI = 0.98 to 13.43]). Outcome data from our statistical models are summarized in [Table 2](#).

4. Discussion

In our analyses, NMDAR1-abs seropositive patients had a higher level of depression over three years after mild-to-moderate IS, with the greatest effect seen in patients with high NMDAR1-abs titers. The findings are in line with previous observations, supporting unfavorable neuropsychiatric outcome of patients with serum NMDAR1-abs following an ischemic stroke event and suggesting an important role of these antibodies for outcomes after stroke.

Post-stroke depression is highly prevalent, but its underlying causes remain poorly understood. In our study, we only included patients who had experienced a mild to moderate ischemic stroke event, yet, we observed that many of them exhibited symptoms of depression to a varying extend. Remarkably, even one year after stroke, a period during which a significant recovery would be expected, 25% (n = 103) of the patients could be classified as depressed. For comparison, the point-prevalence assessed with the CES-D in a non-stroke comparable western population was estimated at ~7% ([Jahn et al., 2018](#); [Roth et al., 2020](#)). Despite the alarming numbers, there is currently no well-defined pathobiological concept for depression and depressive symptoms after stroke. While the overall disease burden certainly plays a role, there may be biological factors that contribute to the occurrence of this frequent sequelae. Depression, in- and outside stroke pathology has been linked to neuroinflammation ([Wijeratne and Sales, 2021](#); [Endres et al., 2022](#)). Upon blood brain barrier disruption, circulating agents – including intrinsic factors– can potentially enter the brain and contribute to functional changes, that may lead to depression.

NMDAR1-abs have been detected in serum of individuals with different diseases and in the healthy population ([Doss et al., 2014](#); [Dahm et al., 2014](#); [Daguano Gastaldi et al., 2023](#); [Zerche et al., 2015](#); [Finke et al., 2017](#)). In line with these studies, we observed a similar serum prevalence of NMDAR1-abs in the present stroke cohort, suggesting that these antibodies were present before the stroke event. This is further supported by the swift blood sampling after the stroke event, which

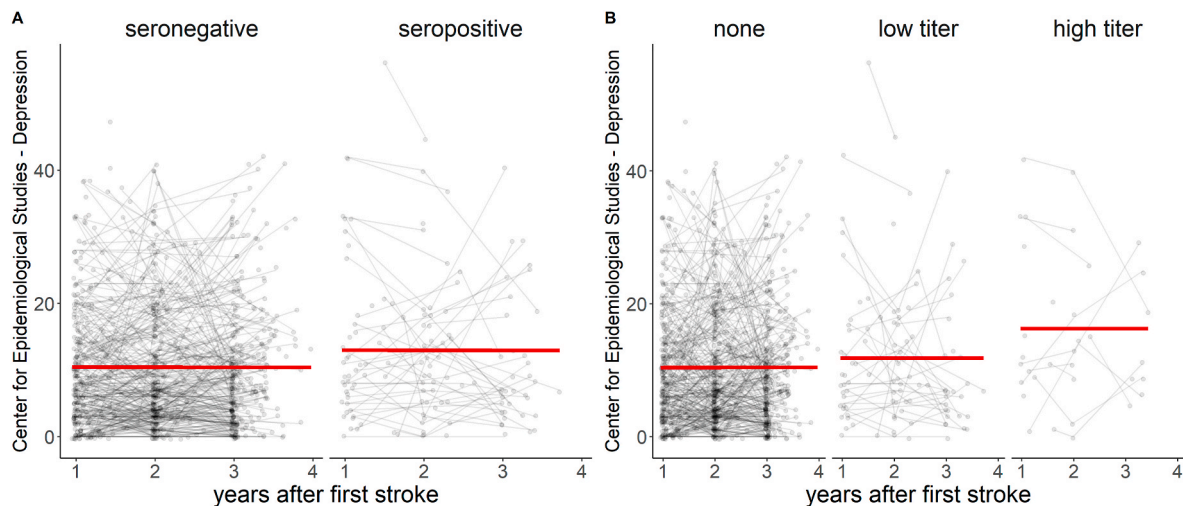


Fig. 1. Anti-N-methyl-D-aspartate receptor antibody serostatus and depressive symptoms over time.

Center for Epidemiological Studies – Depression values assessed at annual time-points for **A**, Anti-NMDA receptor GluN1 antibody (NMDAR1-abs) seropositive and NMDAR1-abs seronegative patients and **B**, for NMDAR1-abs seropositive patients with low titer (serum titer of 1:10–1:100) and high titer (serum titer of 1:320 and 1:1000). Grey dots represent assessed raw data, combined by respective subject. Red lines represent fitted lines over time from weighted linear mixed models. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2

Effects of anti-NMDA-receptor antibody seropositivity on depressive symptoms over three years in patients with ischemic stroke.

Antibody serostatus	Unadjusted		Adjusted ^a	
	β	95% CI	β	95% CI
NMDAR1-abs seronegative	(ref.)	–	(ref.)	–
NMDAR1-abs seropositive	2.56	–0.34 to 5.45	2.26	–0.68 to 5.20
titers \leq 1:100	1.42	–1.79 to 4.62	0.53	–2.47 to 3.54
titers $>$ 1:100	5.85	0.20 to 11.50	7.20	0.98 to 13.43

Antibody serostatus, anti-NMDAR antibody (NMDAR1-abs) seroprevalence. β , effect size (points on the Center for Epidemiological Studies – Depression [CES-D] min value = 0 points, max value = 60 points) in relation to reference group. 95% CI, 95% confidence interval. ref., reference group. ^aAdjusted, analysis adjusted for a propensity score built from age, sex, anti-depressive treatment before stroke, years of school education, smoking, alcohol consumption and the Trial of Org 10,172 in Acute Stroke Treatment (TOAST) classification for stroke etiology using logistic regression (binary outcome: seropositive and seronegative) and an ordinal logistic regression (titer level subgroups titers $>$ 1:10 \leq 1:100 and titers $>$ 1:100).

would render an antibody formation due to the stroke unlikely. However, it is possible that the overall serum prevalence or titer levels of NMDAR1-abs were higher before the stroke, as NMDAR1-abs may have bound to their respective receptors immediately after blood-brain barrier disruption. In a separate stroke cohort, we assessed NMDAR1-abs serostatus in a small group of NMDAR1-abs seropositive patients for up to seven consecutive days after a severe stroke, with the initial assessment within 36 h of the stroke event. We observed that i. Seropositive patients remained seropositive ii. Seronegative patients remained seronegative and iii. Titer levels remained relatively constant over time in most individuals (data not published). MRI was not part of the primary protocol of this present study, therefore their informative value is limited (different MRI protocols and missing data, see Table 1). However, despite this limitation, available images do not suggest that seropositive patients have a higher number of chronic lesions compared to seronegative patients (see Table 1). These findings challenge a notion that seropositivity is a consequence of a cerebrovascular event. However, we detected a lower percentage of seropositive patients with SVD stroke compared to seronegative patients, and likewise a higher proportion of seropositive patients with LAA stroke. Although we cannot explain this finding, NMDAR1-abs may relate to inflammatory

processes, which are more likely to occur in larger arteries.

In autoimmune encephalitis, severe neuropsychiatric symptoms have been linked to intrathecal IgG isotype NMDAR1-abs (Dalmau et al., 2007). However, the role of NMDAR1 antibodies of IgA and IgM isotypes, outside the encephalitis syndrome, is less clear. In stroke, these antibodies may enter the brain after blood brain barrier disruption and affect NMDAR functioning, resulting in observable clinical effects. Cognitive decline, including memory deficits, has been linked to circulating NMDAR1 antibodies in cancer patients, parkinsons disease, patients with slow progressive cognitive impairment (Doss et al., 2014; Pruss et al., 2012; Deutsch et al., 2021; Finke et al., 2017). We observed a dose-dependent relationship in our data (beta for any seropositivity 2.56, beta for low titer: 1.42 and beta for high titers 5.58). This biological gradient suggests a causative link of NMDAR1-abs in the pathobiology of depression after stroke (Hill, 1965). Nevertheless, there is still uncertainty whether any seropositivity (i.e. with any titer) and seropositivity with low titers truly impact depressive symptoms, as our confidence intervals do not allow a definite conclusion and effect estimates may be different in the total stroke population. The observed effect was quite large, rendering confounding to fully explain the observed effects, unlikely. Larger studies are needed to address remaining uncertainty. The integrity of the blood-brain barrier seems to play a role in the pathological effects of NMDAR1 antibodies, as high serum prevalence was found in healthy individuals. (Dahm et al., 2014; Daguano Gastaldi et al., 2023).

A body of evidence supports a central role of glutamate homeostasis in depressive disorders, (Hashimoto, 2009) with a central role of NMDA-receptor antagonists for anti-depressive treatment (Iadarola et al., 2015; Amidfar et al., 2019). While we do not currently have a proof of concept to present, we consider two potential mechanisms underlying our findings: firstly, our results may be explained by impaired recovery after the stroke event. NMDA-receptor mediated neuronal communication and plasticity is important for neuronal regeneration and recovery (Dhawan et al., 2011). Receptor down-regulation, as we expect after NMDAR1-abs binding, may interfere with these processes, and subsequently leading to impaired structural recovery, which is a known risk factor for depression (Hardingham and Bading, 2010; Levite, 2014; Loubinoux et al., 2012). Secondly, the diverging antagonistic properties of NMDAR1-abs and other NMDA-receptor antagonists may explain contrary effects. Ketamine, as well as other anti-depressive drugs (e.g. memantine) are

non-competitive NMDA-receptor antagonists (Hashimoto, 2009). In contrast, we suspect NMDAR1-abs to internalize NMDA-receptors leading to NMDA downregulation (Kreye et al., 2016; Wenke et al., 2019). The observed depressive symptoms in NMDAR1-abs could be attributed to a potential accumulation of glutamate due to NMDA-receptor internalization following antibody binding (Hashimoto, 2009). Elevated glutamate levels have been observed in the blood, CSF, and postmortem brains of individuals with major depressive disorders in both mice and humans (Amidfar et al., 2019). In the end, the intricate interactions of NMDA-receptors with other receptors and signaling proteins, as well as the so called “NMDA-receptor paradox” (cell-survival signaling vs. pro-death signaling) render many possible explanations for a link between NMDAR1-abs and depression after stroke (Hardingham et al., 2002). Importantly, observed effects may also be linked to an overrepresentation of autoantibodies (e.g. antibody flooding) and not functionally linked to NMDA-receptor antagonization. However, this study design cannot illuminate on pathomechanistic causality. While clinical observational studies provide accumulating evidence of a connection between NMDAR1-abs seropositivity and depressive symptoms, a comprehensive pathway concept based on experimental evidence is needed.

Our findings also indicate that depressive symptoms tend to persist beyond the short-term in stroke patients. Additionally, there is no substantial decline in the prevalence of depression over time. Although the effect size suggests a potentially large impact of NMDAR1-abs seropositivity with high titers on depression, the low sample size limits our certainty, and the actual impact may be less severe. Interestingly, and in contrast to the general prevalence of depression, the percentage of seropositive patients that could be categorized as depressed after stroke decreased. This finding may be explained by the high number of death cases in the NMDAR1-abs seropositive group (21% of NMDAR1-abs seropositive patients vs. 8% of seronegative patients at year three after stroke). Larger studies are needed to confirm the significance of our observed effect of NMDAR1-abs seropositivity on depressive symptoms in stroke patients.

4.1. Strength and limitations

In this large prospective IS cohort, we provide evidence for an important role of serum NMDAR1-abs for depressive symptoms in the long-term course after stroke. Sequential measurements of depressive symptoms over time address the fluctuating course of depressive symptoms, and the effect may have been missed with a one-time only assessment of depressive symptoms. However, some fluctuations of NMDAR1-abs titer levels were reported in a previous study, therefore, it renders unclear whether our titer-level grouping is robust (Pan et al., 2021). Lack of follow-up data due to depressive symptoms is a known issue in clinical studies, however we tried to reduce the bias resulting from missing data by inverse probability weighting. Unfortunately, we were not able to discriminate the causes of death in this study; therefore, it is unclear if death may have been related to depression or other concomitant diseases. We included a range of potential confounding variables which we regarded as important, including a variable indicating whether the patient received anti-depressive medication before the stroke. However, this variable only approximates which patient was depressed before stroke and we lack a more representative measurement of depressive symptoms before the stroke event. Hence, it is possible that the influence of this significant confounding variable has been underestimated, although it is unlikely to fully account for the observed effect. Additionally, bias due to other unmeasured confounders may still be present.

To conclude, mild-to moderate IS patients with NMDAR1-abs seropositivity have more severe depressive symptoms during long-term follow-up compared to NMDAR1-abs seronegative patients. The results provide evidence from a clinical observational study regarding the novel concept, that serum autoimmunity, specifically the presence of anti-

neuronal autoantibodies, play a role in causing depression after an ischemic stroke.

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Declaration of competing interest

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Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbih.2023.100705>.

References

- Amidfar, M., Woelfer, M., Reus, G.Z., Quevedo, J., Walter, M., Kim, Y.K., 2019. The role of NMDA receptor in neurobiology and treatment of major depressive disorder: evidence from translational research. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 94, 109668.
- Arwert, H.J., Meesters, J.J.L., Boiten, J., Balk, F., Wolterbeek, R., Vliet Vlieland, T.P.M., 2018. Poststroke depression: a long-term problem for stroke survivors. *Am. J. Phys. Med. Rehabil.* 97 (8), 565–571.
- Collaborators, G.B.D.N., 2019. Global, regional, and national burden of neurological disorders, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 18 (5), 459–480.
- Daguano Gastaldi, V., Bh Wilke, J., Weidinger, C.A., Walter, C., Barnkothe, N., Teegen, B., et al., 2023. Factors predisposing to humoral autoimmunity against brain-antigens in health and disease: analysis of 49 autoantibodies in over 7000 subjects. *Brain Behav. Immun.* 108, 135–147.
- Dahm, L., Ott, C., Steiner, J., Stepniak, B., Teegen, B., Saschenbrecker, S., et al., 2014. Seroprevalence of autoantibodies against brain antigens in health and disease. *Ann. Neurol.* 76 (1), 82–94.
- Dalmau, J., Tuzun, E., Wu, H.Y., Masjuan, J., Rossi, J.E., Voloschin, A., et al., 2007. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann. Neurol.* 61 (1), 25–36.
- Dar, S.K., Venigalla, H., Khan, A.M., Ahmed, R., Mekala, H.M., Zain, H., et al., 2017. Post stroke depression frequently overlooked, undiagnosed, untreated. *Neuropsychiatry* 7 (6), 906–919.
- Daza, E.J., Hudgens, M.G., Herring, A.H., 2017. Estimating inverse-probability weights for longitudinal data with dropout or truncation: the xtrcpiw command. *STATA J.* 17 (2), 253–278.
- Deutsch, N.R., Worthmann, H., Steixner-Kumar, A.A., Schuppner, R., Grosse, G.M., Pan, H., et al., 2021. Autoantibodies against the NMDAR subunit NR1 are associated with neuropsychiatric outcome after ischemic stroke. *Brain Behav. Immun.* 96, 73–79.
- Dhawan, J., Benveniste, H., Luo, Z., Nawrocky, M., Smith, S.D., Biegon, A., 2011. A new look at glutamate and ischemia: NMDA agonist improves long-term functional outcome in a rat model of stroke. *Future Neurol.* 6 (6), 823–834.
- Dong, L., Sanchez, B.N., Skolarus, L.E., Stulberg, E., Morgenstern, L.B., Lisabeth, L.D., 2020. Sex difference in prevalence of depression after stroke. *Neurology* 94 (19), e1973–e1983.
- Doss, S., Wandinger, K.P., Hyman, B.T., Panzer, J.A., Synofzik, M., Dickerson, B., et al., 2014. High prevalence of NMDA receptor IgA/IgM antibodies in different dementia types. *Ann Clin Transl Neurol* 1 (10), 822–832.
- Endres, M., Moro, M.A., Nolte, C.H., Dames, C., Buckwalter, M.S., Meisel, A., 2022. Immune pathways in etiology, Acute phase, and chronic sequelae of ischemic stroke. *Circ. Res.* 130 (8), 1167–1186.
- Fang, M., Zhong, L., Jin, X., Cui, R., Yang, W., Gao, S., et al., 2019. Effect of inflammation on the process of stroke rehabilitation and poststroke depression. *Front. Psychiatry.* 10, 184.
- Ferro, J.M., Caeiro, L., Figueira, M.L., 2016. Neuropsychiatric sequelae of stroke. *Nat. Rev. Neurol.* 12 (5), 269–280.
- Finke, C., Bartels, F., Lutt, A., Pruss, H., Harms, L., 2017. High prevalence of neuronal surface autoantibodies associated with cognitive deficits in cancer patients. *J. Neurol.* 264 (9), 1968–1977.
- Hardingham, G.E., Bading, H., 2010. Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nat. Rev. Neurosci.* 11 (10), 682–696.
- Hardingham, G.E., Fukunaga, Y., Bading, H., 2002. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nat. Neurosci.* 5 (5), 405–414.
- Hashimoto, K., 2009. Emerging role of glutamate in the pathophysiology of major depressive disorder. *Brain Res. Rev.* 61 (2), 105–123.
- Hatano, S., 1976. Experience from a multicentre stroke register: a preliminary report. *Bull. World Health Organ.* 54 (5), 541–553.
- Hill, S.B., 1965. The Environment and Disease: Association or Causation?.
- Iadarola, N.D., Niciu, M.J., Richards, E.M., Vande Voort, J.L., Ballard, E.D., Lundin, N.B., et al., 2015. Ketamine and other N-methyl-D-aspartate receptor antagonists in the treatment of depression: a perspective review. *Ther. Adv. Chronic Dis* 6 (3), 97–114.
- Jahn, R., Baumgartner, J.S., van den Nest, M., Friedrich, F., Alexandrowicz, R.W., Wancata, J., 2018. [Criterion validity of the German version of the CES-D in the general population]. *Psychiatr. Prax.* 45 (8), 434–442.
- Kreye, J., Wenke, N.K., Chayka, M., Leubner, J., Murugan, R., Maier, N., et al., 2016. Human cerebrospinal fluid monoclonal N-methyl-D-aspartate receptor autoantibodies are sufficient for encephalitis pathogenesis. *Brain* 139 (Pt 10), 2641–2652.
- Levite, M., 2014. Glutamate receptor antibodies in neurological diseases: anti-AMPA-GluR3 antibodies, anti-NMDA-NR1 antibodies, anti-NMDA-NR2A/B antibodies, anti-mGluR1 antibodies or anti-mGluR5 antibodies are present in subpopulations of patients with either: epilepsy, encephalitis, cerebellar ataxia, systemic lupus erythematosus (SLE) and neuropsychiatric SLE, Sjogren's syndrome, schizophrenia, mania or stroke. These autoimmune anti-glutamate receptor antibodies can bind neurons in few brain regions, activate glutamate receptors, decrease glutamate receptor's expression, impair glutamate-induced signaling and function, activate blood brain barrier endothelial cells, kill neurons, damage the brain, induce behavioral/psychiatric/cognitive abnormalities and ataxia in animal models, and can be removed or silenced in some patients by immunotherapy. *J. Neural. Transm.* 121 (8), 1029–1075.
- Liman, T.G., Zietemann, V., Wiedmann, S., Jungehuelsing, G.J., Endres, M., Wollenweber, F.A., et al., 2013. Prediction of vascular risk after stroke - protocol and pilot data of the Prospective Cohort with Incident Stroke (PROSCIS). *Int. J. Stroke* 8 (6), 484–490.
- Loubinoux, I., Kronenberg, G., Endres, M., Schumann-Bard, P., Freret, T., Filipkowski, R. K., et al., 2012. Post-stroke depression: mechanisms, translation and therapy. *J. Cell Mol. Med.* 16 (9), 1961–1969.
- Pan, H., Steixner-Kumar, A.A., Seelbach, A., Deutsch, N., Ronnenberg, A., Tapken, D., et al., 2021. Multiple inducers and novel roles of autoantibodies against the obligatory NMDAR subunit NR1: a translational study from chronic life stress to brain injury. *Mol. Psychiatry.* 26 (6), 2471–2482.
- Parikh, R.M., Eden, D.T., Price, T.R., Robinson, R.G., 1988. The sensitivity and specificity of the Center for Epidemiologic Studies Depression Scale in screening for post-stroke depression. *Int. J. Psychiatr. Med.* 18 (2), 169–181.
- Pruss, H., Holtje, M., Maier, N., Gomez, A., Buchert, R., Harms, L., et al., 2012. IgA NMDA receptor antibodies are markers of synaptic immunity in slow cognitive impairment. *Neurology* 78 (22), 1743–1753.
- Ramberger, M., Peschl, P., Schanda, K., Irshchick, R., Hoftberger, R., Deisenhammer, F., et al., 2015. Comparison of diagnostic accuracy of microscopy and flow cytometry in evaluating N-methyl-D-aspartate receptor antibodies in serum using a live cell-based assay. *PLoS One* 10 (3), e0122037.
- Roth, D.L., Haley, W.E., Sheehan, O.C., Liu, C., Clay, O.J., Rhodes, J.D., et al., 2020. Depressive symptoms after ischemic stroke: population-based comparisons of patients and caregivers with matched controls. *Stroke* 51 (1), 54–60.
- Shrier, I., Platt, R.W., 2008. Reducing bias through directed acyclic graphs. *BMC Med. Res. Methodol.* 8, 70.
- Sperber, P.S., Siegerink, B., Huo, S., Rohmann, J.L., Piper, S.K., Pruss, H., et al., 2019. Serum anti-NMDA (N-Methyl-D-Aspartate)-Receptor antibodies and long-term clinical outcome after stroke (PROSCIS-B). *Stroke* 50 (11), 3213–3219.
- Sperber, P.S., Gebert, P., Broersen, L.H.A., Huo, S., Piper, S.K., Teegen, B., et al., 2022. Serum anti-NMDA-receptor antibodies and cognitive function after ischemic stroke (PROSCIS-B). *J. Neurol.* 269 (10), 5521–5530.
- Steiner, J., Teegen, B., Schiltz, K., Bernstein, H.G., Stoecker, W., Bogerts, B., 2014. Prevalence of N-methyl-D-aspartate receptor autoantibodies in the peripheral blood: healthy control samples revisited. *JAMA Psychiatr.* 71 (7), 838–839.
- Wenke, N.K., Kreye, J., Andrzejak, E., van Casteren, A., Leubner, J., Murgueitio, M.S., et al., 2019. N-methyl-D-aspartate receptor dysfunction by unmutated human antibodies against the NR1 subunit. *Ann. Neurol.* 85 (5), 771–776.
- Wijeratne, T., Sales, C., 2021. Understanding why post-stroke depression may be the norm rather than the exception: the anatomical and neuroinflammatory correlates of post-stroke depression. *J. Clin. Med.* 10 (8).
- Zerche, M., Weissenborn, K., Ott, C., Dere, E., Asif, A.R., Worthmann, H., et al., 2015. Preexisting serum autoantibodies against the NMDAR subunit NR1 modulate evolution of lesion size in Acute ischemic stroke. *Stroke* 46 (5), 1180–1186.