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Serum anti-NMDA receptor antibodies are linked to memory impairment 12 months after stroke

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Patients suffering from strokes are at increased risk of developing post-stroke dementia. Serum anti-NMDA receptor autoantibodies (NMDAR1-abs) have been associated with unfavorable post-stroke outcomes. However, their effect on specific cognitive domains remains unclear. We used data from the prospective multicenter DZNE—mechanisms after stroke (DEMDAS) cohort, and measured NMDAR1-abs in serum at baseline. Cognitive function was assessed with a comprehensive neuropsychological test battery at 6- and 12-months follow-up. We employed crude and stepwise confounder adjusted linear and logistic regression models as well as generalized estimating equation models (GEE) to determine the relevance of NMDAR1-abs seropositivity on cognitive function after stroke. 10.2% (58/569) DEMDAS patients were NMDAR1-abs seropositive (IgM:n = 44/IgA:n = 21/IgG:n = 2). Seropositivity was not associated with global cognitive impairment after stroke. However, NMDAR1-abs seropositive patients performed lower in the memory domain ($\beta_{\text{adjusted}} = -0.11$; 95%CI = -0.57 to -0.03) and were at increased risk for memory impairment ($\text{OR}_{\text{adjusted}} = 3.8$; 95%CI = 1.33–10.82) compared to seronegative patients, 12 months after stroke. Further, NMDAR1-abs were linked to memory impairment over time in GEE from 6- to 12-months follow-up ($\text{OR}_{\text{adjusted}} = 2.41$; 95%CI = 1.05–5.49). Our data suggests that NMDAR1-abs contribute to memory dysfunction 1 year after stroke while not affecting other cognitive subdomains. Hence, antineuronal autoimmunity may be involved in distinct mechanisms of post-stroke memory impairment. *Clinical trial name and registration number*: The Determinants of Dementia After Stroke (DEMDAS; study identifier on clinical trials.gov: NCT01334749)

Molecular Psychiatry (2025) 30:1359–1368; <https://doi.org/10.1038/s41380-024-02744-w>

INTRODUCTION

A stroke is a devastating event with far-reaching consequences for further life of the affected person. Despite the development of highly effective treatments in recent decades, the global impact of stroke on individuals, care takers, and economy remains enormous [1]. While modern treatments have significantly reduced death and physical disability after stroke, the burden of cognitive impairment increases with increased survival and aging [2]. A deterioration of cognitive abilities is commonly observed after stroke, including after minor events and even after transient ischemic attacks [3]. However, the precise mechanisms that lead

to cognitive impairment are not known. Hence, effective prevention and treatment strategies are lacking [4]. Serum anti-NMDA-receptor GluN1 (NR1) autoantibodies (NMDAR1-abs), primarily of immunoglobulin (Ig) A and IgM isotype, have been observed in presumably healthy individuals and in patients with various diseases [5–8]. While previously thought to have beneficial effects on ischemic brain lesion evolution in stroke [9, 10], they have been associated with unfavorable post-stroke clinical outcomes, including cognitive outcomes [7, 11, 12]. Recent work suggests impaired neuropsychiatric outcomes after stroke, including cognitive dysfunction, particularly in those with high titers as

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Received: 17 January 2024 Revised: 29 August 2024 Accepted: 2 September 2024
Published online: 30 October 2024

assessed by screening tests [12, 13]. Interestingly, similar findings have been reported in NMDAR1-abs seropositive patients with other diseases (e.g., cancer patients), suggesting a cross-disease pathophysiological relevance of these antibodies [14–16]. It was suggested previously that serum NMDAR1-abs may enter the brain after blood-brain barrier disruption and exert pathological effects [17, 18], which may explain cognitive dysfunction after stroke. However, detailed and differentiated neuropsychiatric data elucidating a potential link between NMDAR1-abs seropositivity and cognitive impairment for stroke outcome are not available. In the present study, we estimated the impact of NMDAR1-abs seropositivity on global cognitive function and various subdomains of cognitive function using high-quality prospective data from the Determinants of Dementia After Stroke (DEMDAS) study.

SUBJECTS AND METHODS

Study design, registrations, and patient population

DEMDAS (study identifier on clinicaltrials.gov: NCT01334749) is an ongoing national multicenter, prospective observational study with the aim to investigate mechanisms leading to cognitive dysfunction and dementia after stroke. Participating centers are listed in Supplementary (Suppl.) Table 1. Within the study, 600 patients with ischemic or hemorrhagic stroke are followed over time in person at seven different sites in Germany. A detailed protocol of the study design has been published previously [19]. In brief, patients aged at least 18 years with stroke defined by a new neurological deficit within the previous 5 days and a new lesion on magnet resonance imaging (MRI) or computer tomography are included. Patients with prior dementia, defined as >64 sum points in the short version of the 'Informant Questionnaire on Cognitive Decline in the Elderly' (IQCODE) [20], and patients with a life expectancy of less than 3 years due to malignancy were excluded. The total follow-up duration for study completion is planned for 5 years. For this investigation, data from the 6- and 12-months follow-up (FU) visits were used. A detailed characterization of study participants was performed at baseline.

Antibody measurements

Blood serum samples were taken from each participant at study inclusion and stored at -80°C before first-time ever thawing for antibody measurement. NMDAR1-abs were measured at the Clinical Immunological Laboratory Prof. Stöcker using reagents of the EUROIMMUN AG with fixed cell-based assays using *GluN1* transfected Human Embryonic Kidney 239 cells, and indirect immunofluorescence as previously described in detail [21]. We measured IgM, IgA, and IgG isotype NMDAR1-abs, and any titer above or equal 1:10 was considered seropositive. Titer dilution steps were 1:10, 1:32, 1:100, 1:320, and 1:1000.

Assessment of cognitive function and definition of outcome parameters

Cognitive abilities in five different domains (language, memory, visuospatial function, executive function, and attention) were assessed by a comprehensive neuropsychological testing. Mainly, the Consortium to Establish a Registry for Alzheimer's Disease Plus (CERAD-Plus) battery in addition to other tests were used, in line with a previous work [22, 23]. CERAD-Plus includes a test for language-specific function, "Semantic and Phonemic Fluency" and "Boston Naming" [22], and additionally we used the language items from Mini Mental State Examination (MMSE) [24]. Furthermore, to examine memory function, CERAD-Plus includes Word List Learning/Recall, Recognition, and Figure Recall. Immediate and delayed recall was tested by the Rey-Osterrieth Complex Figure (ROCF) [25]. To examine visuospatial function, CERAD-Plus includes the Figure Drawing Test and we applied additionally the copy test of ROCF [22, 25]. For executive function, CERAD-Plus uses the Trail Making Test Part B and the Stroop-Colour-Word-Interference Test [26]. We tested attention with the Trail Making Test Part A from CERAD-Plus and additionally with the Digit-Symbol-Substitution Test of the Wechsler Intelligence Scale [27]. Age, sex, and education standardized z-scores were calculated from a reference normative population, as previously described [23]. Next, a domain-specific z-score was averaged for each domain and subsequently, these were calculated into a global cognitive score as previously described, defining the global cognitive function in our study [23]. Performance of less than -1.5 z-score points was used to define impairment in any cognitive

domain or in global cognitive function, respectively [28]. At baseline, cognitive impairment was assessed using Montreal Cognitive Assessment (MoCA) and (if MoCA was unavailable) MMSE scores [29]. Impairment was defined as <26 points in MoCA, or <24 in MMSE [30, 31].

Neuroimaging

Cranial MRI scans (3 Tesla, Siemens Healthineers, Erlangen, Germany) were conducted at baseline. The MRI protocol included 3D T1-weighted magnetization prepared rapid gradient echo (MPRAGE), 3D fluid-attenuated inversion recovery (FLAIR), diffusion-weighted imaging (DWI) with various diffusion directions, T2-weighted turbo spin echo, and T2*-weighted fast low angle shot (FLASH) gradient echo. Intracranial volumes, total brain volumes, and stroke lesion size were derived as previously described [23]. Hippocampal volumes were assessed using the FreeSurfer software (version 5.3; <http://surfer.nmr.mgh.harvard.edu>). Normalized brain volumes were adjusted to total intracranial volumes to account for differences in head size (normalized brain volume = [total brain volume + infract volume]/total intracranial volume). Hippocampal volumes were likewise adjusted to intracranial volumes (normalized hippocampal volume = [right hippocampal volume + left hippocampal volume]/total intracranial volume). Hippocampal volumes were calculated for patients without acute stroke lesions in the hippocampal area.

Statistics

Continuous data is presented as mean with standard deviation (SD) and median along with interquartile range (25th and 75th percentile), while categorical variables are displayed as absolute counts (N) and corresponding percentages (%). We calculated absolute standardized mean differences to evaluate how well the groups of NMDAR1-abs seropositive and seronegative patients are balanced regarding baseline characteristics [32]. To estimate effects of NMDAR1-abs serostatus on global and domain-specific test performance at 6- and 12-months FU, we used linear regression models. Regarding the binary cognitive outcomes at both timepoints, we used logistic regression models. We calculated beta effects and odds ratios (OR) with corresponding 95% confidence intervals (95%CI), respectively. To estimate the effects of NMDAR1-abs seropositivity on cognitive outcome over two timepoints, i.e., 6 to 12 months after stroke, we calculated ORs from generalized estimating equation (GEE) models, comparing seropositive vs. seronegative participants, while encountering the dependency of observations (within subject measurements). To adjust for confounding factors, we conducted a stepwise adjustment: the first model was to explore the unadjusted association (crude, model 1), a partially adjusted model was built with age (continuous), sex (dichotomous), and education (in school educational years, continuous) (model 2), and a fully adjusted model incorporating a propensity score (model 3). The propensity score was calculated from logistic regression models including age (continuous), sex (dichotomous), education (continuous), ever smoking (dichotomous), habitual alcohol consumption (dichotomous), severe chronic disease leading to retirement, obligate support in daily life, or manifest reduction of life quality (dichotomous), previous stroke or transitory ischemic attack (dichotomous), cardiovascular diseases (dichotomous), and other organic brain diseases including other cerebrovascular events excluding stroke or transitory ischemic attack (dichotomous), with NMDAR1-abs serostatus as dependent variable. All confounders were considered to have a potential impact on NMDAR1-abs serostatus at time of assessment and on the respective cognitive outcome.

We additionally investigated post-hoc whether our observed results may be influenced by other neuropsychiatric sequelae: To measure the severity of depressive symptoms, we used the 20-item center for epidemiological studies depression (CES-D) scale, which has been validated for use in German and in stroke patients [33, 34]. To measure fatigue, we used the first 20 items (excluding the visual analog scales, i.e., item 21–24) of the Fatigue Assessment Questionnaire (FAQ), which was also validated for the use in stroke patients [35, 36]. We visually inspected the linear relationship of these measures with z-scores from the memory domain at 6- and 12-month post-stroke and calculated correlation coefficients. Additionally, we investigated whether the observed effects on the memory domain may be explained by hippocampal volume at baseline. Therefore, we correlated baseline hippocampal stroke volumes with the z-scores of the memory domain at 6- and 12-month post-stroke.

Data preparation and statistical analyses were conducted using SPSS Statistics 28.0.1.0 (IBM, Armonk, NY, USA). Data visualization was conducted using R version 4.2.3 with the ggplot2 package and Prism Version 9.4.1 (GraphPad Software, San Diego, CA, USA).

Ethics and standard protocol approvals

The DEMDAS study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the local ethics committees of the participating centers. All participants gave written informed consent to participate in the study and to the analysis of serum biomarkers. Our reporting follows the Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) guidelines [37].

RESULTS

Patient population

Between January 2014 and January 2019, DEMDAS recruited a total of 600 patients, of whom 569 patients were tested for any serum NMDAR1-abs and were thus eligible for our analysis. The median day of blood sampling after index stroke was 1 (IQR: 1–2). The flowchart depicted in Fig. 1 describes the inclusion and

exclusion of patients and provides an overview of the number of neuropsychological examinations after 6 and 12 months. The most common reason for omission of serological antibody testing was patient refusal to have blood drawn, and missing complete autoantibody testing in four patients was due to limited serum amounts. The lack of a complete neuropsychological examination was mainly due to lost follow-ups, patients' inability to complete the examination for health reasons, or because the patient preferred to be followed by mail or telephone, which did not include comprehensive neuropsychological testing. Overall, 450 patients at 6 months (80.7% of seronegatives vs. 63.8% of seropositives) and 423 patients at 12 months (75.5% of seronegatives vs. 62.1% of seropositives) received a complete neuropsychological assessment that allowed calculation of a global cognitive score. An incomplete cognitive assessment testing at least one cognitive domain was obtained in 471 patients at 6-months FU and 438 patients at 12-months FU, respectively. A total of 13 patients died (2 seropositive and 11 seronegative), corresponding to 2.3% of the missing data.

Baseline characteristics

Baseline characteristics of the original study group ($n = 569$) are shown in Table 1, stratified by NMDAR1-abs serostatus, and with corresponding absolute standardized mean differences. Overall, the cohort included 66.4% men and had predominantly ischemic strokes (97.4%). The majority of patients had mild to moderate strokes with a median NIHSS score of 3 (IQR: 1–5). At baseline autoantibody testing, 58 patients (10.2%) were seropositive for any form of NMDAR1-abs, with IgM ($n = 44$) and IgA ($n = 21$) predominating and only 2 patients having NMDAR1-abs of the IgG isotype. Seropositive patients were generally older with a median age of 74 (IQR: 67–80) vs. 68 years (IQR: 60–75). In addition, seropositive patients had more cardiovascular risk factors, including a history of previous ischemic cerebrovascular events (19.0% vs. 12.3%). Imaging data showed similar stroke lesions size in both groups (mean of 0.20% in seropositive vs. 0.15% in seronegative patients). However, hippocampal volumes and total brain volumes (normalized to intracranial volumes) were slightly smaller in seropositive patients (4.3% vs. 4.7%; mean difference: 0.4; 95% CI = 0.2 to 0.6 and 66% vs. 68%; mean difference: 2.4; 95% CI = 0.76 to 3.98). Overall, 24.4% of patients received intravenous thrombolysis therapy, with more seronegative than more seropositive patients (25.0% vs. 17.2%). Cognitive impairment at baseline assessed with bedside screening tests (i.e., <26 points in MoCA, and <24 in MMSE) was noted in 53.4% of the total cohort, without major differences between seropositive and seronegative patients (52.9% of seronegative vs. 58.2% of seropositive patients). Apolipoprotein E genotyping and history of depression at baseline also revealed no differences between seronegative and seropositive patients.

NMDAR1-abs and cognitive outcome—descriptive data

Overall, cognitive test performance (global and domain specific) improved from 6 to 12 months after stroke as shown in Fig. 2. Z-scores for global cognitive function improved in both groups with similar total score point differences ($\Delta = 0.13$ score points in seronegative and $\Delta = 0.14$ in seropositive patients, Fig. 2A). However, seropositive patients started with lower global z-scores at 6 months (mean of -0.31 [SD = 0.64] vs. -0.18 [SD = 0.65], Fig. 2A) thereby also reaching a lower mean global z-score at 12-months FU compared to seronegative patients (mean of -0.17 [SD = 0.72] vs. -0.05 [SD = 0.67]). Likewise, relative amounts of patients with global cognitive impairment were different at 6-months FU with 18/409 (4.4%) seronegative compared to 3/37 (8.1%) seropositive patients. Moreover, at 12-months FU, global cognitive impairment decreased in seronegative to only 11/371 (2.9%) while 3/37 (8.1%) seropositive patients remained impaired (Suppl. Table 2). In cognitive test performance of the memory domain, NMDAR1-abs seropositive patients showed also lower

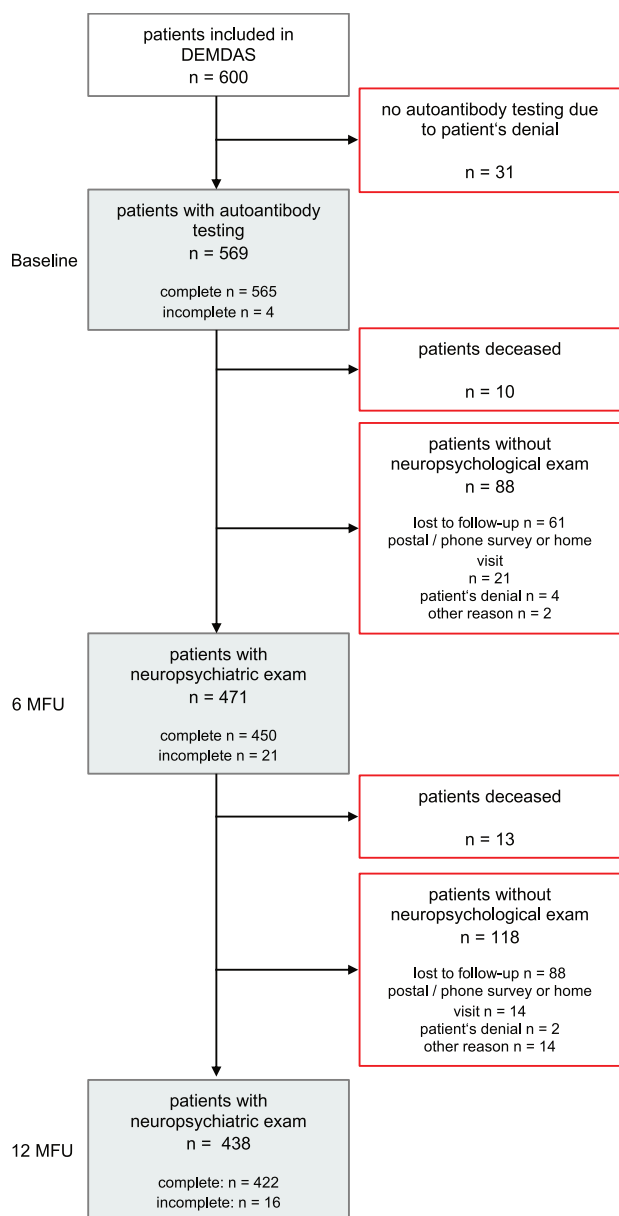


Fig. 1 Flowchart of patient inclusion and exclusion. Gray boxes indicate that participants were included in the analysis, while red boxes represent participants that were excluded from the analysis. Autoantibody indicates anti-NMDAR1 (GluN1) autoantibodies. MFU months follow-up.

Table 1. Baseline characteristics of DEMDAS patients stratified upon anti-NMDAR1 autoantibody serostatus.

Baseline characteristics	Total	Seronegative	Seropositive	ASMD
DEMDAS participants, anti-neuronal antibody testing, n (%)	569 (100)	507 (89.1)	58 (10.2)	
Blood sampling days after index stroke, median (IQR)	1 (1–2)	1 (1–2)	2 (1–2)	0.110
Demographic variables				
Age in years, median (IQR)	69 (60–76)	68 (60–75)	74 (67–80)	0.528
Male sex, n (%)	378 (66.4)	335 (66.1)	42 (72.4)	0.138
Years of education, median (IQR)	13 (12–16)	13 (12–16)	13 (12–17)	0.056
Cardiovascular risk factors and previously existing diseases				
Systolic blood pressure in mmHg, median (IQR)	139 (128–151)	139 (129–151)	139 (122–149)	0.141
Diastolic blood pressure in mmHg, median (IQR)	80 (70–89)	80 (70–89)	79 (70–85)	0.287
Body mass index in kg/m ² , median (IQR)	26.6 (24.3–29.2)	26.5 (24.2–29.2)	27.5 (24.6–29.4)	0.018
Habitual alcohol consumption, n (%)	419 (73.9)	375 (74.3)	41 (70.7)	0.116
Ever smoker, n (%)	342 (60.1)	307 (60.6)	31 (53.4)	0.144
History of hypertension, n (%)	318 (55.9)	282 (57.0)	35 (62.5)	0.113
History of diabetes, n (%)	84 (14.8)	72 (14.2)	12 (20.7)	0.171
History of any hyper-/dyslipidemia, n (%)	165 (29.7)	141 (28.5)	23 (39.7)	0.236
History of peripheral artery disease, n (%)	16 (2.8)	13 (2.6)	3 (5.3)	0.139
History of coronary artery disease, n (%)	32 (5.6)	25 (5.0)	7 (12.1)	0.257
History of myocardial ischemia, n (%)	31 (5.4)	26 (5.1)	5 (8.6)	0.138
History of angina pectoris, n (%)	14 (2.5)	13 (2.6)	1 (1.8)	0.057
History of atrial fibrillation, n (%)	63 (11.2)	55 (11.0)	8 (14.3)	0.100
History of any cardiovascular disease, n (%)	392 (70.0)	347 (69.4)	44 (77.2)	0.177
Previous stroke or TIA, n (%)	74 (13.1)	62 (12.3)	11 (19.0)	0.185
History of other organic brain disease ^a , n (%)	18 (3.2)	15 (3.0)	3 (5.2)	0.070
History of severe disease ^b , n (%)	68 (12.0)	58 (11.5)	9 (15.5)	0.119
History of depression, n (%)	35 (6.1)	33 (6.5)	2 (3.4)	0.209
APOE genotype				0.195
0 ε4 allele, n (%)	383 (78.6)	346 (78.6)	35 (81.4)	
1 ε4 allele, n (%)	96 (19.7)	86 (19.5)	8 (18.6)	
2 ε4 alleles, n (%)	8 (1.6)	8 (1.8)	0 (0.0)	
Index stroke classification				0.070
Ischemic stroke, n (%)	554 (97.4)	493 (97.2)	57 (98.3)	
Hemorrhagic stroke, n (%)	15 (2.6)	14 (2.8)	1 (1.7)	
TOAST, n (%)				0.276
Large artery atherosclerosis	155 (28.0)	134 (27.2)	19 (33.4)	
Cardio embolism	126 (22.7)	113 (22.8)	13 (22.8)	
Small vessel disease	66 (11.9)	59 (12)	7 (12.3)	
Dissection	20 (3.6)	19 (3.9)	1 (1.8)	
Other etiology ^c	42 (7.6)	56 (11.4)	5 (8.8)	
Undetermined etiology	105 (18.8)	96 (19.5)	7 (12.3)	
Diagnostic workup incomplete	41 (7.4)	35 (7.1)	6 (10.5)	
Intravenous thrombolysis, n (%)	139 (24.4)	127 (25.0)	10 (17.2)	0.224

Table 1. continued

Baseline characteristics	Total	Seronegative	Seropositive	ASMD
MRI variables				
Stroke lesion volume in mm ³ , median (IQR)	2248 (528–12,652)	2144 (528–13,136)	2804 (402–9936)	0.073
Normalized stroke lesion volume ^d in %, median (IQR)	0.15 (0.03–0.78)	0.15 (0.03–0.78)	0.20 (0.03–0.61)	0.078
Total brain volume ^e in mm ³ , median IQR	1,042,817 (953,040–1,138,041)	1,048,148 (957,922–1,143,259)	1,015,360 (936,025–1,094,772)	0.324
Normalized brain volume ^d in %, mean SD	68 (5.4)	68 (5.4)	66 (5.2)	0.464
Total hippocampal volume (left + right) in mm ³ , median IQR	7203 (6566–7955)	7252 (6684–7981)	6617 (5742–7430)	0.510
Normalized hippocampal volume ^d in %, mean SD	4.7 (0.6)	4.7 (0.6)	4.3 (0.7)	0.505
Hippocampal stroke, n (%)	41 (7.7)	38 (8.0)	3 (5.7)	0.093
White matter lesions ^f , n (%)	349 (61.4)	307 (60.7)	38 (65.5)	0.190
Chronic stroke lesions, n (%)	161 (29)	144 (29.1)	15 (26.8)	0.051
Clinical/cognitive assessment				
NIHSS, median (IQR)	3 (1–5)	2 (1–5)	3 (1–5)	0.139
Baseline MoCA, median (IQR)	25 (22–27)	25 (22–27)	24 (22–27)	0.286
Cognitive impairment at baseline ^g , n (%)	291 (53.4)	257 (52.9)	32 (58.2)	0.107
IQCODE score, median (IQR)	48 (48–50)	48 (48–49)	48 (48–50)	0.052
Baseline mRS, median (IQR)	2 (1–3)	2 (1–3)	2 (1–2)	0.005

Due to rounding, values might not add to 100%. Missing values were <10% except for *APOE* Genotyping, ($n = 487$), Baseline MoCA ($n = 505$).

ASMD absolute standardized mean difference, TIA transitory ischemic attack, *APOE* Apolipoprotein E, NIHSS National Institutes of Health Scale, MMSE Mini Mental State Examination, MoCA Montreal Cognitive Assessment, IQCODE Informant Questionnaire on Cognitive Decline in the Elderly, mRS modified ranking scale.

^aincluding other cerebrovascular events (excluding ischemic stroke and transitory ischemic attack).

^bsevere disease that led to retirement or obligate support in daily life or manifest reduction of quality of life.

^cother defined causes, and several potential causes.

^ddivided by total intracranial volume.

^ebrain volume + infarct volume.

^fany white matter lesions: punctual, early confluent, wide confluent.

^gMoCA < 26 or MMSE < 24 if MoCA was not available ($n = 64$).

z-scores at both timepoints with similar total score point differences (Fig. 2C). In line, relative amounts of memory impairment were higher in seropositive compared to seronegative patients at 6-months FU (6/40 (15%) vs. 32/425 (7.5%), Suppl. Table 2). Similar to global cognitive impairment, seronegative patients showed less memory impairment with only 15/396 (3.8%), while 6/38 (15.8%) seropositive patients remained impaired after 12 months (Suppl. Table 2). The observed differences in memory performance at the 12-month FU between seropositive and seronegative patients appeared to be mainly driven by those with IgA antibodies. In this subgroup of seropositive patients, z-scores showed only minimal improvement from the 6- to the 12-month FU, in contrast to patients with IgM antibodies (Suppl. Fig. 1C). Z-scores and counts of cognitively impaired individuals in other cognitive domains are shown in Fig. 2B, D, E, F, Suppl. Fig. 1, and Suppl. Table 2.

NMDAR1-abs and cognitive outcome—inferential analyses

With these striking descriptive differences in cognitive test performance, we next analyzed the effect of NMDAR1-abs serostatus on cognitive outcome in stepwise adjusted multiple regression models. Here, neither an effect of NMDAR1-abs serostatus on global cognitive performance, as estimated by linear regression, nor on global cognitive impairment, as estimated by logistic regression models was evident at either timepoint (6-months FU performance: $\beta_{\text{Model3}} = -0.05$; 95%CI =

-0.03 to 0.12 , $p = 0.36$ and 6-months FU impairment: $\text{OR}_{\text{Model3}} = 1.7$; 95%CI = 0.46 to 6.19 , $p = 0.39$; 12-months FU performance: $\beta_{\text{Model3}} = -0.04$; 95%CI = -0.34 to 0.14 , $p = 0.40$ and 12-months FU impairment: $\text{OR}_{\text{Model3}} = 2.8$; 95%CI = 0.74 to 11.06 , $p = 0.13$). For detailed data, please refer to Fig. 3A, B and Suppl. Tables 3–6.

In domain-specific analyses, at 6-months FU, seropositive patients performed worse in the memory domain compared to seronegative patients ($\beta_{\text{Model1}} = -0.10$; 95%CI = -0.55 to -0.03 , $p = 0.03$; $\beta_{\text{Model2}} = -0.09$; 95%CI = -0.53 to -0.01 , $p = 0.04$; $\beta_{\text{Model3}} = -0.09$; 95%CI = -0.49 to 0.04 , $p = 0.09$) (Suppl. Table 3). However, we observed no distinct effect on memory impairment ($\text{OR}_{\text{Model3}} = 1.7$; 95%CI = 0.64 to 4.63 , $p = 0.28$) (Fig. 3A and Suppl. Table 5) as determined by our statistical models. NMDAR1-abs serostatus was not associated with any other domain-specific cognitive outcome at 6-months FU, such as language ($\beta_{\text{Model3}} = 0.03$; 95%CI = -0.18 to 0.36 , $p = 0.52$; Fig. 3A and Suppl. Table 3–4). 12 months after stroke, seropositive patients performed lower in the memory domain ($\beta_{\text{Model1}} = -0.10$; 95%CI = -0.55 to -0.02 , $p = 0.03$, $\beta_{\text{Model2}} = -0.10$; 95%CI = -0.54 to -0.01 , $p = 0.04$, $\beta_{\text{Model3}} = -0.11$; 95%CI = -0.57 to -0.3 , $p = 0.03$), and were at increased risk for memory impairment across all models ($\text{OR}_{\text{Model1}} = 4.8$; 95%CI = 1.73 to 13.12 , $p = 0.01$, $\text{OR}_{\text{Model2}} = 3.7$; 95%CI = 1.28 to 10.41 , $p = 0.02$, $\text{OR}_{\text{Model3}} = 3.8$; 95%CI = 1.33 to 10.82 , $p = 0.01$; Fig. 3B and Suppl. Tables 4 + 6). This association was exclusively evident for the memory domain (Fig. 3B and Suppl. Table 4 + 6). In addition, over 6- to 12-months FU together, the effect of NMDAR1-abs on memory impairment remained consistent

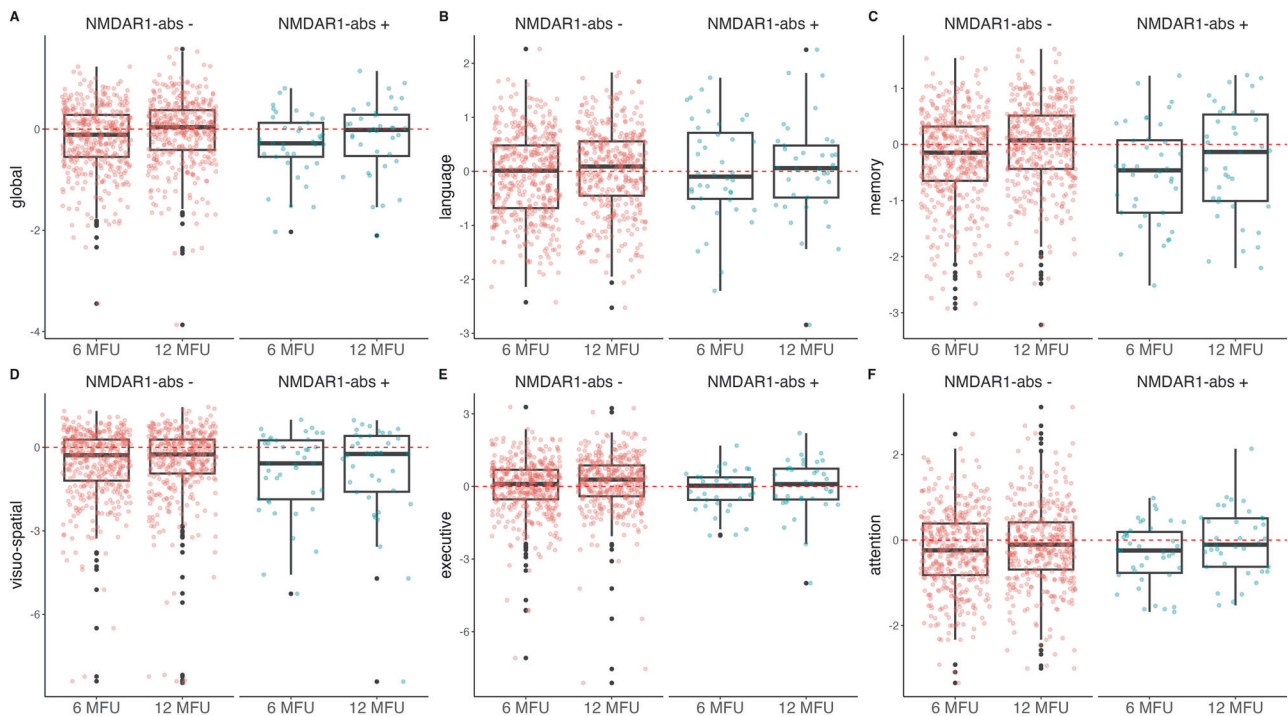


Fig. 2 Global and subdomain scoring from the Consortium to Establish a Registry for Alzheimer's Disease Plus+ battery (CERAD-Plus) from anti-NMDAR1 autoantibody seronegative and seropositive patients. Red dots represent single participants' z-scores of anti-NMDAR1 autoantibody seronegative patients while blue dots display single participants' z-scores of anti-NMDAR1 autoantibody seropositive patients, with a boxplot overlay and emphasized zero-line (red dashed line). **A** Global cognitive test performance. **B** Test performance in the language domain. **C** Test performance in the memory domain. **D** Test performance in the visuo-spatial domain. **E** Test performance in the executive domain. **F** Test performance in the attention domain. Age, sex, and education standardized z-scores were calculated from a reference normative population. MFU months follow-up.

($OR_{Model3} = 2.4$; 95%CI = 1.05 to 5.49, $p = 0.04$; Fig. 3C and Suppl. Table 7). Again, this link was not observed in any other cognitive domain (e.g., language $OR_{Model3} = 0.98$; 95%CI = 0.21 to 4.52, $p = 0.98$; Fig. 3C and Suppl. Table 7). Although the point estimate of the GEE analysis suggested that NMDAR1-abs seropositive patients have an increased risk for global cognitive impairment, this direction was not conclusively confirmed by the confidence interval, and the data were too imprecise to draw conclusions ($OR_{Model3} = 2.11$; 95%CI = 0.65 to 6.88, $p = 0.22$) (Fig. 3C). The complete data are presented in Suppl. Table 7.

Post-hoc exploration of the relationship between other neuropsychiatric outcomes and baseline hippocampal volume with memory performance during follow-up

Mean CES-D and mean FAQ appeared to be similar in seropositive and seronegative patients during follow-up (Suppl. Table 8). We visually explored the linear relationship of depressive symptoms (CES-D) and fatigue symptoms (FAQ) with z-scores of the memory tests and calculated correlation coefficients, which rendered low correlation of these two other neuropsychiatric outcomes at both timepoints (Fig. 4). Additionally, hippocampal volumes at baseline did not show a strong correlation with memory z-scores at both follow-up timepoints (Fig. 5).

DISCUSSION

In this analysis of the DEMDAS stroke cohort, serum prevalence of NMDAR1-abs, mainly of the IgM and IgA isotype, was 10.2%. Seropositivity for NMDAR1-abs was associated with poorer memory performance compared to seronegative patients and with memory impairment at 12 months post-stroke, as well as from 6 to 12 months combined. In contrast, seropositivity was not

associated with global cognitive function or impairment, nor with any of the other cognitive subdomains.

While the proportion of NMDAR1-abs serum prevalence varied between 13 and 23% in other stroke cohorts [12, 38], the prevalence of NMDAR1-abs in our study (10%) was somewhat lower. Similar to another study, serum prevalence was higher in older and in male patients [39]. The high frequency of NMDAR1-abs in healthy individuals and patients with different diseases, calls into question a pathological significance of seropositivity per se. Following ischemic stroke the blood brain barrier is at least focally disrupted, potentially allowing antibodies to access the brain [40]. In agreement with others, we therefore hypothesize that damage to the blood brain barrier may be an additional necessary factor for NMDAR1-abs to exert pathological effects [41–43].

Our data extend recent findings linking serum prevalence of NMDAR1-abs and neuropsychiatric outcome following stroke [11, 12]. In contrast to a recent work [12], we found no clear association of seropositivity with global cognitive function after stroke in this study. This could be explained by a different duration of follow-up (3 years vs. 1 year), as in our study, effects became only clearly apparent after 12 months (not yet after 6 months). Our current and previous data therefore suggest that effects of serum NMDAR1-IgAs and -IgMs may manifest only over time after stroke. A possible explanation could be an antibody-mediated down-regulation of NMDA-receptors with subsequent effects on synaptic plasticity and thereby on long-term regenerative processes after stroke [44–46].

An association between NMDAR1-abs and decreased cognitive abilities has been established in several other cohorts and various disorders [8, 14–16]. It is plausible though that previously observed associations of NMDAR1-abs with global cognitive

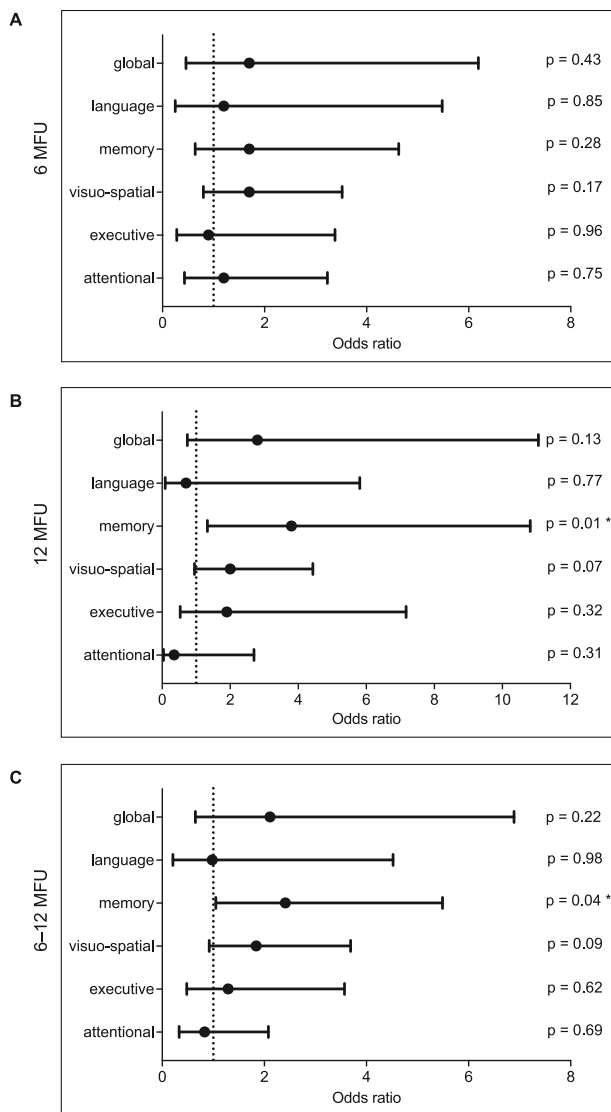


Fig. 3 Global and domain-specific cognitive impairment in association to anti-NMDAR1 autoantibody serostatus. Forest plots representing odds ratios (dots) and corresponding 95% confidential intervals (lines) assessing the association of anti-NMDAR1 autoantibody seropositivity and global and domain-specific binary outcomes in propensity score-adjusted logistic regression models at 6-months follow-up (A), at 12-months follow-up (B), and in logistic GEE analysis from 6- to 12-months follow-up (C). Propensity scores were calculated from logistic regression models including age (continuous), sex (dichotomous), education (continuous), ever smoking (dichotomous), habitual alcohol consumption (dichotomous), severe disease (dichotomous), previous stroke or transitory ischemic attack (dichotomous), cardiovascular diseases (dichotomous), and other organic brain diseases (dichotomous), with NMDAR1-abs serostatus as dependent variable. MFU months follow-up. * Indicates statistical significance with the p-value threshold set at <0.05.

performance are mainly attributable to dysfunction in the memory domain, as supported by findings regarding immediate memory, from one respective study [12].

The link between NMDAR1-abs seropositivity and reduced memory function is particularly intriguing and biologically plausible given the high density of NMDA-receptors in the hippocampus. In fact, we found slightly smaller hippocampal volumes in seropositive patients already at baseline. However, the effect of seropositivity on the memory impairment was only evident after 12 months, and correlations of memory z-scores at follow-up timepoints and

hippocampal volumes at baseline were low. Baseline hippocampal volumes therefore do not appear as the main factor for memory function in the long term after stroke. However, whether seropositivity impacts hippocampal volumes over time upon blood brain barrier impairment due to stroke remains to be determined. While some studies question whether NMDA-receptors are internalized after binding of IgA/M NMDAR1-abs [47, 48], evidence shows at least IgA binding to hippocampal NMDA receptors, however, with lower affinity than IgG, possibly leading to local 'latent' autoimmunity [14, 41, 49]. Effects on memory performance may be primarily attributed to NMDAR1-abs IgA antibodies, as observed differences in our cohort were most pronounced in patients with IgA isotype antibodies. However, the sample size was too small to further investigate the effects by isotype stratification.

NMDAR1-abs seropositive patients in our study were more likely to have ischemic cerebrovascular events before the index stroke compared with seronegative patients (19% vs. 12%). This may suggest that seropositive patients have a higher baseline risk of cerebrovascular events [7], or that NMDAR1-abs formation is induced by cerebrovascular events, although other studies have challenged this notion [7, 11, 43].

Cardiovascular risk factors as potential confounders did not lead to major changes in point estimates in our memory domain analyses. In line with previous work [50], this suggests that vascular risk factors do not particularly influence memory function. In contrast, intravenous thrombolytic therapy could modify the effect of NMDAR1-abs on cognitive outcomes, as tissue-type plasminogen activator (tPA) has been shown to affect blood brain barrier integrity and neuroinflammation as well as neuronal survival by altering NMDAR signaling in endothelial and neuronal cells [51]. Since our analysis revealed too large CIs after excluding patients treated with rtPA (data not shown), future studies with larger cohorts should investigate the role of rtPA in this context.

Interestingly, neuropsychiatric outcomes (i.e., depression, fatigue) did not appear to be substantially different in NMDAR1-abs seropositive compared to seronegative patients at either follow-up timepoint, which is in contrast to previous study results [12, 52]. Since these outcomes were generally only weakly correlated with memory function in our study, we infer that memory function is not majorly impacted by these conditions, highlighting a specific and independent mechanisms despite additional neuropsychiatric findings.

In a previous analysis of the DEMDAS cohort, we found a robust connection between small vessel disease burden in baseline MRI and cognitive impairment at follow-up timepoints at global and subdomain levels, with exception of the memory domain [23]. This again suggests distinct underlying mechanisms leading to memory impairment vs. impairment in other cognitive domains after stroke. Taken together, our data might reveal a novel subtype of post-stroke cognitive impairment characterized by memory dysfunction, however exact mechanisms of post-stroke inflammation and potential autoimmunity remain unclear [53].

Strengths and limitations

A clear strength of the study is the detailed neuropsychological testing with the CERAD battery and the size of the prospectively analyzed cohort. However, the relatively small number of seropositive patients ($n = 58$) limits the precision of our estimates as indicated by large CIs. Another major limitation is the short follow-up time and the limited access to imaging data as no MRI was acquired after 12 months. Future investigation of domain-specific cognitive outcomes and concurrent data on hippocampal volumes over time might elucidate imaging correlates of memory impairment in NMDAR1-abs seropositivity after stroke. The frequency of missing data from patients at 6 and 12 months was 16.8% and 22.8%, respectively, which is not unusual in observational studies. Although baseline characteristics were not

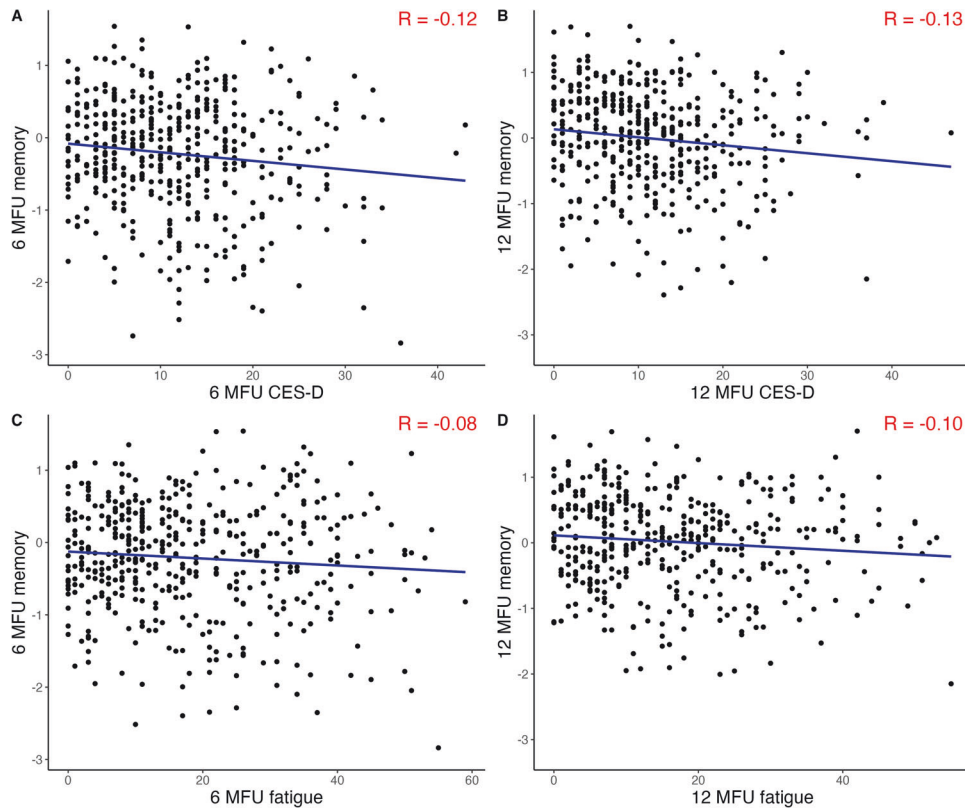


Fig. 4 Scatter plots and correlation coefficients for depression and fatigue with memory function at both follow-up timepoints. **A + B** x-axis: Center for Epidemiological Studies Depression (CES-D), y-axis: z-scores for memory function at 6- and 12-months, respectively. R: correlation coefficient. **C + D** x-axis: Fatigue Assessment Questionnaire (FAQ), y-axis: z-scores for memory function at 6- and 12-months respectively. R correlation coefficient, MFU months follow-up.

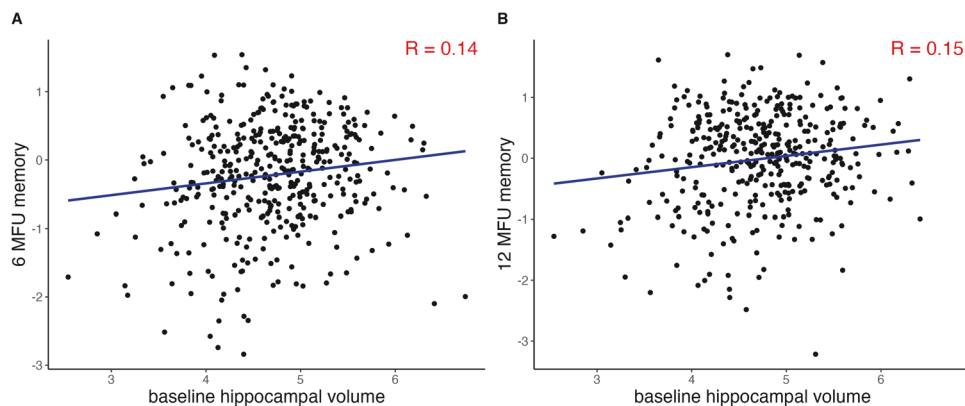


Fig. 5 Scatter plots and correlation coefficients for baseline hippocampal volumes with memory function at both follow-up timepoints: **X-axis**: Baseline hippocampal volume, y-axis: z-scores for memory function at (A) 6- and (B) 12-months, R correlation coefficient, MFU months follow-up.

different between patients who dropped out of the study and those who remained in the study, lost-to-follow-up may be differential. In this line, follow-up rates were higher in seronegative participants than in seropositive patients.

Our data add to a growing body of evidence derived from clinical observations pointing towards a functional relevance of NMDAR1-abs for cognitive outcomes after stroke, particularly memory function. The underlying distinct pathophysiological mechanisms need to be studied in the future using experimental stroke models and large patient cohorts.

DATA AVAILABILITY

All primary data and analyses scripts are available from the responsible principal investigator (matthias.endres@charite.de) upon reasonable request.

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ACKNOWLEDGEMENTS

ME received funding from DFG under Germany's Excellence Strategy—EXC-2049 – 390688087, the DFG via KFO 5023 BeCAUSE-Y, project 2 EN343/16-1, Collaborative Research Center ReTune TRR 295- 424778381, BMBF, DZNE, DZHK, EU, Corona Foundation, and Fondation Leducq. The DEMDAS study was funded by the German Center for Neurodegenerative Diseases (DZNE), reference number MC002.

AUTHOR CONTRIBUTIONS

FAA: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing—original draft, Writing—review & editing. PSS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing—original draft, Writing—review & editing. RR: Formal analysis, Investigation, Methodology, Writing—review & editing. PG: Formal analysis, Methodology, Writing—review & editing. BT: Investigation, Writing—review & editing. MKG: Data curation, Formal analysis, Methodology, Writing—review & editing. RF: Data curation, Formal analysis, Methodology, Writing—review & editing. AD: Data curation, Formal analysis, Investigation, Methodology, Writing—review & editing. MG: Investigation, Resources, Supervision, Writing—review & editing. GCP: Investigation, Resources, Supervision, Writing—review & editing. IZ: Investigation, Resources, Supervision, Writing—review & editing. MD: Investigation, Methodology, Resources, Supervision, Writing—review & editing. HP: Investigation, Resources, Supervision, Writing—review & editing. ME: Conceptualization, Investigation, Methodology, Resources, Supervision, Writing—original draft, Writing—original draft, Writing—review & editing.

FUNDING

Open Access funding enabled and organized by Projekt DEAL.

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COMPETING INTERESTS

ME reports grants from Bayer and fees paid to the Charité from Abbot, Amgen, AstraZeneca, Bayer Healthcare, Boehringer Ingelheim, BMS, Daiichi Sankyo, Sanofi, Novartis, Pfizer, all outside the submitted work.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41380-024-02744-w>.

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