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ORIGINAL RESEARCH

Vasoregulatory Autoantibodies and Clinical Outcome After Ischemic Stroke—PROSCIS-B

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BACKGROUND: Vasoregulatory autoantibodies including autoantibodies targeting G-protein–coupled receptors might play a functional role in vascular diseases. We investigated the impact of vasoregulatory autoantibodies on clinical outcome after ischemic stroke.

METHODS AND RESULTS: Data were used from the PROSCIS-B (Prospective Cohort With Incident Stroke–Berlin). Autoantibody-targeting receptors such as angiotensin II type 1 receptor (AT1R), endothelin-1 type A receptor, complement factor-3 and -5 receptors, vascular endothelial growth factor receptor-1 and -2, vascular endothelial growth factor A and factor B were measured. We explored associations of high antibody levels with (1) poor functional outcome defined as modified Rankin Scale >2 or Barthel Index <60 at 1 year after stroke, (2) Barthel Index scores over time using general estimating equations, and (3) secondary vascular events (recurrent stroke, myocardial infarction) or death up to 3 years using Cox proportional hazard models. We included 491 patients with ischemic stroke with data on autoantibody levels and outcome. In models adjusted for demographics and vascular risk factors, high autoantibody concentrations (quartile 4) targeting complement factor C3a receptor, vascular endothelial growth factor receptor-2, and vascular endothelial growth factor B were associated with poor functional outcome at 1 year: (odds ratio, 2.0 [95% CI, 1.1–3.6]; odds ratio, 1.8 [95% CI, 1.1–3.2]; and odds ratio, 2.1 [95% CI, 1.2–3.6], respectively) and with lower Barthel Index scores over 3 years (complement factor C3a receptor: adjusted β=−3.3 [95% CI, −5.7 to −0.5]; VEGF-B: adjusted β=−2.4 [95% CI, −4.8 to −0.06]). Patients with high autoantibody levels were not at higher risk for secondary vascular events or death.

CONCLUSIONS: High levels of autoantibodies against vascular endothelial growth factor receptor-2, vascular endothelial growth factor B, and complement factor C3a receptor measured are associated with poor functional outcome after stroke but not with recurrent vascular events or death.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT01363856.

Key Words: autoantibodies ■ ischemic stroke ■ prospective studies ■ vascular endothelial growth factor A ■ vascular endothelial growth factor B ■ vascular endothelial growth factor receptor-1

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Functional autoantibodies can target endothelial antigens as well as circulating proteins (eg, cytokines, growth factors) via variable antigen region–specific interactions and impact vasoregulation.[1,2] Agonistic autoantibodies directed against G-protein–coupled receptors (GPCRs), for example, antibodies against the angiotensin II receptor 1 (AT1R) or the endothelin-1 type A receptor (ETAR), are typically encountered in

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CLINICAL PERSPECTIVE

What Is New?

- Vasoregulatory autoantibodies including autoantibodies targeting G-protein–coupled receptors might play a functional role in vascular diseases and are linked to worse outcomes in experimental brain ischemia. However, data from stroke studies are lacking.
- In a large cohort of patients with ischemic stroke, high levels of vasoregulatory antibodies against, for example, vascular endothelial growth factor receptor 2 and complement factor C3a receptor are associated with poor functional outcome (modified Rankin Scale score >2, functional dependency) but not with recurrent vascular events or death.

What Are the Clinical Implications?

- Our findings provide first evidence that high levels of vasoregulatory autoantibodies might impact outcome after stroke—further research is needed to determine the underlying pathophysiological mechanisms and the potential as future treatment targets.

Nonstandard Abbreviations and Acronyms

AT1R	angiotensin II type-1 receptor
BBB	blood–brain barrier
BI	Barthel Index
C3aR	complement factor C3a receptor
ETAR	endothelin-1 type A receptor
GPCR	G-protein–coupled receptor
mRS	modified Rankin Scale
NIHSS	National Institute of Health Stroke Scale
PROSCIS-B	Prospective Cohort With Incident Stroke–Berlin
VEGFR	vascular endothelial growth factor receptor

vascular diseases and well known to affect cardiovascular function (eg, dysregulation of blood pressure).[2,3]

Our knowledge of autoantibodies as mediators of autoimmune processes and pathogenesis in acquired and systemic vasculopathies has been expanding rapidly lately.[4,5]

Autoantibodies against AT1R were first identified in women with preeclampsia with a prevalence of >90%.[6] Autoantibodies against AT1R from these patients with preeclampsia directly induced endothelial dysfunction in experimental models.[7] Elevated autoantibody levels against AT1R and ETAR are independently associated with death in systemic sclerosis-associated pulmonary arterial hypertension.[8,9] High levels of autoantibodies against GPCR have been identified in renal allograft rejection,[10] malignant hypertension,[11] idiopathic cardiomyopathy,[12] and chronic heart failure[13] and as markers for disease severity in patients with heart failure[14] and COVID-19.[15] Furthermore, agonistic GPCR antibodies were associated with adverse health outcomes and inflammation in a large cohort of older individuals.[16]

The impact of these "vasoregulatory" autoantibodies in patients with cerebrovascular diseases remains largely unclear to date and was therefore the main focus of this study. In experimental models of stroke and traumatic brain injury, autoantibody activation of specific endothelial receptors in the acute phase is associated with poor outcome, for example, via disruption of the blood–brain barrier and edema.[17,18]

In this study, our primary hypothesis is that high levels of autoantibodies against AT1R are associated with poor functional outcome in patients with first-ever ischemic stroke. Second, we aimed to investigate the impact of high levels of other vasoregulatory autoantibodies on functional and vascular outcomes up to 3 years after ischemic stroke.

METHODS

Data Availability

The data and software script that support the findings of this study are available from the qualified principal investigator of PROSCIS-B (Prospective Cohort With Incident Stroke–Berlin) (T.G. Liman, thomas.liman@charite.de) upon reasonable request.

Ethics Approval

Patients or their legal representative gave informed consent for study participation. The study was approved by the ethics committee of the Charité-Universitätsmedizin Berlin (EA1/218/09).

The PROSCIS-B Study

The PROSCIS-B (ClinicalTrials.gov identifier: NCT01363856) is a prospective observational hospital-based cohort study of patients after a first-ever stroke. The protocol and study design have been published previously.[19] Briefly, patients with first-ever ischemic or hemorrhagic stroke or venous sinus thrombosis were recruited at the 3 tertiary stroke units of the Charité-Universitätsmedizin Berlin and interviewed within 7 days after onset of stroke symptoms. An extensive clinical and technical examination as well as biomarker sampling were performed at baseline as described in detail elsewhere.[19]

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Stroke survivors were followed-up annually after recruitment by telephone interviews assessing vital status, cognitive function, functional outcome, and other outcomes. Information on vital status was complemented by contacting the registry office for death certificates if patients were lost to follow-up.

Study Population

Patients aged ≥18 years who suffered from first-ever stroke according to the World Health Organization criteria were included.[20] Exclusion criteria were prior stroke, brain tumor or metastasis, or participation in an interventional study (eg, experimental studies such as clinical trials, where the researcher intercedes as part of the study design). We restricted this analysis to patients with ischemic stroke and known status on functional outcome at 1 year after stroke. Information on functional outcome was obtained by telephone interview at 1 year follow-up (365±30 days).

Assessment of Vasoregulatory Autoantibodies

Blood samples were collected within 7 days after onset of symptoms with median day 4 (interquartile range, 3–5). After 30 to 60 minutes at room temperature, serum was obtained by centrifugation at room temperature at 1500*g* for 15 minutes and then aliquoted into 0.5-mL tubes. Samples were stored at −80 °C until assays were run.

A sandwich ELISA (CellTrend GmbH, Luckenwalde, Germany) was developed to measure levels of autoantibodies of immunoglobulin G (IgG)-targeting receptors angiotensin II type-1 (AT1R), endothelin-1-type-A (ETAR), complement factor C3a receptor (C3aR) and complement factor C5a receptor, vascular endothelial growth factor receptor (VEGFR)-1 and -2, as well as vascular endothelial growth factor (VEGF)-A and -B. All these assays are described in detail elsewhere.[2,8,21,22] Assay precision and confirmatory studies are shown in Table S1. Results are presented as units/mL. Each serum sample was measured in triplicate. The laboratory was blinded to patients' baseline characteristics and follow-up data.

Patient Characteristics

Factors possibly associated with poor outcome 1 year after incident stroke included sociodemographic parameters (age, sex, institutionalization before stroke [care home, retirement home, or assisted living]; and stroke-related risk factors [body mass index, active smoking, hypertension, diabetes type I or II, atrial fibrillation, myocardial infarction (MI), coronary artery disease, dyslipidemia, peripheral arterial disease, pathogenetic subtype of ischemic stroke according to the Trial of ORG 10172 in Acute Stroke Treatment classification, stroke severity according to National Institute of Health Stroke Scale (NIHSS)]), elevated CRP (C-reactive protein) levels, leukocyte counts, and estimated glomerular filtration rate (eGFR).[23]

Outcomes

The primary outcome was poor functional outcome 1 year after stroke defined as disability, dependency, or death using the modified Rankin Scale (mRS score >2) or Barthel Index (BI) score <60 as previously defined.[24] Secondary outcomes were changes in BI scores over time, unfavorable functional outcome defined as mRS score >1 1 year after stroke, and secondary vascular events or death up to 3 years after stroke.

The BI measures performance in 10 activities of daily living and ranges from 0 to 100 in 5-point increments, with 100 indicating normal physical functioning.[25] BI scores were assessed annually up to 3 years after index stroke. The reliability of telephone BI assessments has been demonstrated before.[26] Although it is an ordinal scale, stroke researchers have advocated analyzing the BI as a continuous variable because of increased power to detect associations, the ability to describe the course of change over time in linear form, and avoidance of potential misclassification due to crude categorization.[27–29] mRS and BI have been validated for German language and for use in telephone interviews.[23,30]

For secondary vascular events or death, we used a combined end point of recurrent stroke, MI, and all-cause death within 3 years. Recurrent strokes and MIs were self-reported and obtained by telephone interview or postal mail contact. Additionally, we screened the Charité University Hospital medical records for any unreported end points. To validate reported recurrent stroke events or MIs, we obtained medical records from the responsible hospital or the treating physician. An end point committee consisting of 2 senior vascular neurologists independently rated and validated the clinical end points without having knowledge of the individual antibody status of each patient. Vital status was obtained directly from the Berlin local registration office. Only confirmed end points were used in all analyses.

Our primary exposure of interest was levels of autoantibodies targeting AT1R. Secondary exposures were all other vasoregulatory autoantibodies such as autoantibodies against GPCR (AT1R, ETAR, C3aR, C5aR), tyrosine kinase receptor (VEGFR1, VEGFR2), and growth factors (VEGF-A, VEGF-B) as shown in Table 1.

Statistical Analysis

For descriptive purposes, univariate analyses were performed using appropriate statistical tests; for example, continuous data of 2 groups were compared

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Table 1. Baseline Characteristics

No.	590
Age, y, mean (SD)	67 (13)
Age group, y, n (%)	
<55	101 (17.1)
55–64	135 (22.9)
65–74	183 (31)
≥75	171 (29)
Female sex, n (%)	226 (38.3)
NIHSS at admission, median (IQR)	2 (1–5)
0–4 (n, %)	441 (74.7)
5–15	144 (24.4)
>15	5 (0.8)
Body mass index, median (IQR)*	
<25	26.9 (24.2–26.9)
25 to <30	205 (35)
≥30	139 (25)
Thrombolysis, n (%)	122 (20.7)
Active smoking, n (%)*	165 (28)
Institutionalization before stroke	12 (2)
Regular alcohol consumption, n (%)*	205 (34.7)
Ischemic stroke subtype, n (%)	
Large-artery atherosclerosis	156 (26.4)
Cardioembolic	142 (24.1)
Small-artery occlusion	93 (15.8)
Other causes	18 (3.1)
Undefined	181 (30.7)
Cardiovascular risk factors (n, %)	
History of myocardial infarction	21 (3.6)
Coronary artery disease	91 (15.4)
Diabetes	129 (21.9)
Atrial fibrillation	126 (21.4)
Peripheral arterial disease	40 (6.8)
Arterial hypertension	385 (65.3)
Dyslipidemia	122 (20.7)
Laboratory parameters, mean (SD)	
eGFR (CKD-EPI formula)	77.7 (20.7)
C-reactive protein, mg/dL	1.3 (2.1)
Leukocyte counts (×10^9 per L)	8.2 (3.5)
Vasoregulatory autoantibodies in units, median (IQR)	
AT1R	8.3 (5.1–11.1)
ETAR	4.6 (2.4–8.8)
C3aR	7.3 (4.9–10.5)
C5aR	3.5 (2.0–5.9)
VEGFR1	27.3 (22.9–34.1)
VEGFR2	1.6 (0.9–3.0)
VEGF-A	13.1 (7.8–22.4)
VEGF-B	3.1 (2.0–8.4)

AT1R indicates angiotensin II type 1; C3aR, C5aR, complement factor-3 and -5; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; ETAR, endothelin-1 type A; IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale; VEGF-A, VEGF-B, vascular endothelial growth factor A, B; and VEGFR1, VEGFR2, vascular endothelial growth factor receptor-1, -2.

*Restricted to patients without missing values in the respective category.

using a nonparametric test (Wilcoxon signed-rank test) or Student's *t* test if data were normally distributed. Categorical variables were compared using the Pearson χ^2 test. To identify which autoantibodies were independently associated with dichotomized outcomes (eg, mRS score >1), multivariable logistic regression was used to estimate odds ratios (ORs) and 95% CIs, after adjusting for possible confounders in 3 models: Model 1 adjusted for sex and age; model 2 further adjusted for stroke severity, stroke pathogenesis, prestroke dependency, hypertension, diabetes, peripheral arterial disease, history of MI, coronary artery disease, smoking, and dyslipidemia; and model 3 further adjusted for high-sensitivity CRP levels, leukocyte counts, and eGFR.

CRP and eGFR were included as potential modifiers of endothelial function and vasoregulation as well as factors associated with the primary outcome in univariate analyses. Leukocytes were included to adjust for stroke-induced changes in circulating white blood cells.

Generalized estimating equations were used to assess the association between high levels of vasoregulatory autoantibodies and functional status over time with repeated measurements of BI as performed in a previous analysis.[31] Adjustments for possible confounders were applied in 3 sequential models as described above.

For the combined end point of recurrent stroke, MI, or all-cause death, we conducted event-free survival analyses comparing cumulative hazards of autoantibody levels. Event-free survival time was measured until 1 of the events of the combined end point occurred, the study ended, or the participant was lost to follow-up, whichever happened first. Dropouts were censored at time of last contact. Cox proportional hazard models were used to estimate hazard ratios (HRs) for a combined end point within 3 years after the index event in patients with antibody levels in the fourth quartile versus the first quartile as reference. Cox proportional hazards regression models were adjusted for confounding factors (age, sex, NIHSS score, stroke subtype according to Trial of ORG 10172 in Acute Stroke Treatment classification, cardiovascular risk factors [current smoking, hypertension, peripheral arterial disease, atrial fibrillation, diabetes, coronary artery disease, or history of MI]).

All multivariable analyses were restricted to patients without missing values in the respective category. All tests were 2-tailed, and statistical significance was determined at an alpha level of 0.05.

Statistical analyses were performed using SPSS version 28 (SPSS Statistics; IBM, Armonk, NY). We followed the Strengthening the Reporting of Observational Studies in Epidemiology guidelines for reporting.

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RESULTS

Study Population

We included 491 patients with ischemic stroke who had data on autoantibody levels and outcomes in our primary analysis. In detail, between March 2010 and May 2013, 627 patients with first-ever ischemic stroke from tertiary university stroke units were recruited for PROSCIS-B. Measurements of vasoregulatory autoantibodies at baseline were available for analysis in 590 (94%) patients and obtained at a median of 4 days (interquartile range, 3–5) after stroke onset. Of these patients, information on functional status (mRS) at 1 year was available in 491 (83%). Detailed information on patient inclusion and exclusion is provided in the flowchart of the Figure.

Patient Characteristics

Patient characteristics at baseline and levels of autoantibodies against GPCR (AT1R, ETAR, C3aR, C5aR), tyrosine kinase receptor (VEGFR1, VEGFR2), and growth factors (VEGF-A, VEGF-B) are shown in Table 1. Mean age was 67 years (SD±13), and 226 (38.3%) were women. Median NIHSS score at admission was 2 (interquartile range, 1–5). Of all stroke survivors with data on functional outcome at year 1 (n=526), 79 had an mRS score of 3 to 5 1 year after index stroke or died (n=27), and 18 had a BI score of <60. Of all 491 patients with data on baseline autoantibody levels and functional outcome at year 1, 102 patients had an mRS score of 3 to 6 or BI score of <60 at year 1.

No statistically significant differences were found for baseline data (age, sex, body mass index, NIHSS score, active smoking, prestroke institutionalization, regular alcohol consumption, ischemic stroke subtype, cardiovascular risk factors; levels of eGFR, CRP, and leukocytes) between patients with autoantibody status included in the analyses and those without autoantibody measurements.

Autoantibody levels were highly correlated among each other (Figure S1).

Outcomes

Poor Functional Outcome (Primary Outcome)

In univariate analyses, factors associated with poor functional outcome defined as an mRS score >2 or BI score <60 were age, sex, regular alcohol consumption, atrial fibrillation, diabetes, coronary artery disease, peripheral arterial disease, ischemic stroke subtype, eGFR, levels of CRP, and NIHSS score as shown in Table 2. High levels of autoantibodies against C3aR,

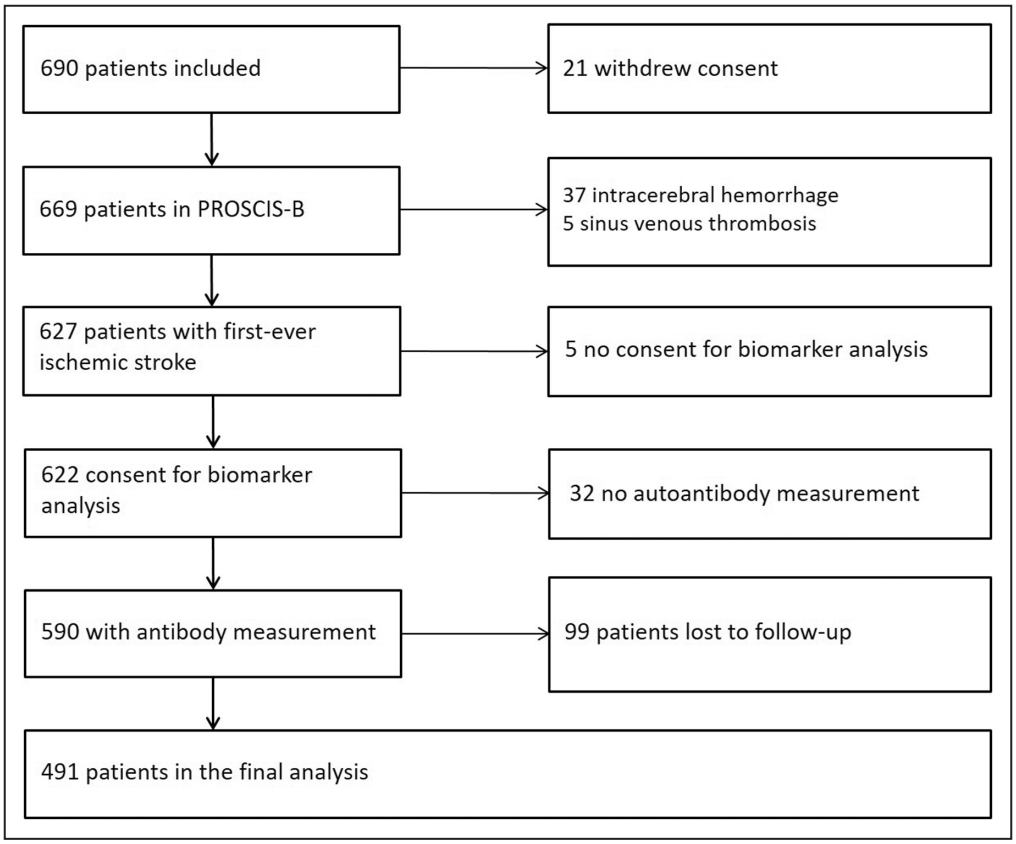

Figure 1. Flowchart of study inclusion and exclusion.
PROSCIS-B indicates Prospective Cohort With Incident Stroke–Berlin.

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Table 2. Factors Associated With Poor Functional Outcome Defined as Modified Rankin Scale Score 3 to 6 or Barthel Index <60 at 1 Year After First Ischemic Stroke in Univariate Analyses

	Good outcome	Poor outcome	*P* value
n	389	102	
Age, mean (SD)	65 (12.9)	73 (10.6)	0.03
Age group, n (%)			0.02
<55	79 (20.3)	6 (5.9)	
55–64	93 (23.9)	17 (16.7)	
65–74	125 (32.1)	31 (30.3)	
≥75	92 (23.7)	48 (47.1)	
Female sex, n (%)	140 (36.0)	50 (49.0)	0.02
NIHSS, median (IQR)	2 (1–4)	3 (2–7)	<0.01
NIHSS group, n (%)			<0.01
0–4	312 (80.2)	63 (62.0)	
5–15	75 (19.3)	36 (36.0)	
>15	2 (0.5)	3 (3.0)	
BMI, median (IQR)*	26.8 (24.2–29.4)	28.0 (23.7–31.4)	0.3
BMI group, n (%)			
<25	140 (36)	34 (35.0)	
25 to <30	164 (42)	32 (33.0)	
≥30	85 (22)	32 (32.0)	
Thrombolyses, n (%)	82 (21)	23 (22)	0.7
Active smoking, n (%)*	107 (27.8)	21 (20.8)	0.16
Institutionalization before stroke, n (%)	7 (1.8)	1 (1.0)	0.9
Regular alcohol consumption, n (%)*	149 (39.5)	24 (24.0)	0.05
Ischemic stroke subtype (n, %)			0.02
Large-artery atherosclerosis	107 (27.5)	25 (24.5)	
Cardioembolic	80 (20.6)	36 (35.3)	
Small-artery occlusion	61 (15.7)	14 (13.7)	
Other causes	12 (3.1)	5 (4.9)	
Undefined	129 (33.2)	22 (21.6)	
Cardiovascular risk factors, n (%)			
History of myocardial infarction	10 (2.6)	5 (5.0)	0.2
Coronary artery disease	50 (12.9)	25 (24.5)	0.005
Diabetes	69 (17.7)	35 (33.3)	0.001
Atrial fibrillation	68 (17.5)	38 (37.3)	0.001
Peripheral arterial disease	17 (4.4)	14 (13.7)	0.002
Arterial hypertension	239 (61.4)	71 (69.6)	0.13
Dyslipidemia	83 (21.3)	22 (21.6)	0.9
Laboratory parameters, mean (SD)			
eGFR (CKD-EPI formula)	79.2 (19.4)	69.3 (21.6)	0.04
C-reactive protein (mg/dL)	1.05 (1.9)	2.2 (2.8)	<0.01
Leukocyte counts ($\times 10^9$ per L)	7.9 (3.9)	8.2 (2.8)	0.1
Vasoregulatory autoantibodies			
Quartile 4, n (%)			
AT1R	96 (24.7)	30 (29.0)	0.3
ETAR	100 (25.7)	23 (22.0)	0.21
C3aR	90 (23.1)	35 (35.0)	0.03
C5aR	93 (24)	30 (30.0)	0.33
VEGFR1	94 (24.2)	31 (31.0)	0.38
VEGFR2	92 (23.7)	35 (34.0)	0.02
VEGF-A	91 (23.4)	30 (29.0)	0.2
VEGF-B	90 (23.1)	34 (34.0)	0.03

AT1R indicates angiotensin II type 1; BMI, body mass index; C3aR, C5aR, complement factor-3 and -5; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; ETAR, endothelin-1 type A; IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale; VEGF-A, VEGF-B, vascular endothelial growth factor A, B; and VEGFR1, VEGFR2, vascular endothelial growth factor receptor-1, -2.

*Restricted to patients without missing values in the respective category.

VEGFR2 and VEGF-B in the fourth quartile were significantly more frequent in patients with stroke with poor functional outcome. Results of multivariable logistic regression analyses are shown in Table 3. Confounder adjustment was performed in 3 different models. After adjustment for age and sex (model 1), levels of autoantibodies against C3aR, VEGFR2, and VEGF-B were associated with increased risk of a poor outcome (for C3aR: OR, 1.9 [95% CI, 1.2–3.1]; for VEGFR2: OR, 1.9 [95% CI 1.2–3.1]; and for VEGF-B: OR, 2.0 [95% CI, 1.2–3.2]). In model 2, the OR for poor outcome for autoantibodies against C3aR, VEGFR2, and VEGF-B in the fourth quartile were 2.0 (95% CI, 1.2–3.6), 1.8 (95% CI, 1.1–3.1), and 2.1 (95% CI, 1.2–3.6), respectively. In model 3 adjusted for confounders of model 2 plus levels of CRP, eGFR, and leukocytes counts, the OR for poor outcome for autoantibodies against C3aR, VEGFR2, and VEGF-B in the fourth quartile were 2.0 (95% CI, 1.1–3.6), 1.8 (95% CI, 1.1–3.2), and 2.1 (95% CI, 1.2–3.6), respectively.

Autoantibody levels were highly correlated among each other (Figure S1). Each autoantibody was run in a separate model.

Changes in BI Score Over Time

In repeated measures analyses of BI over time using generalized estimating equations, VEGF-B and C3aR in the highest quartile were associated with lower BI scores over 3 years of follow-up (C3aR: adjusted $\beta = -3.3$ [95% CI −5.7 to −0.5]; VEGF-B: adjusted $\beta = -2.4$ [95% CI −4.8 to 0.06]) compared with other quartiles as shown in Table 4.

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Table 3. Multivariate Logistic Regression Analyses for Poor Functional Outcome at 1 Year After Stroke Defined as Modified Rankin Scale Score >2 or Barthel Index Score <60

Autoantibodies	AT 1 R-IgG	ETAR-IgG	C3a R-IgG	C5aR-IgG	VEGFR1-IgG	VEGFR2-IgG	VEGFA-IgG	VEGFB-IgG
	Odds ratios with 95% CIs for quartile 4 vs other (≥95th percentile versus other)							
Model 1	1.3 (0.8–2.1)	0.8 (0.5–1.5)	1.9 (1.2–3.1)	1.5 (0.9–2.4)	1.6 (0.97–2.7)	1.9 (1.2–3.1)	1.5 (0.9–2.6)	2.0 (1.2–3.2)
Model 2	1.3 (0.7–2.2)	0.8 (0.5–1.5)	2.0 (1.2–3.6)	1.7 (0.96–3.0)	1.7 (0.97–3.0)	1.8 (1.1–3.1)	1.5 (0.9–2.7)	2.1 (1.2–3.6)
Model 3	1.3 (0.7–2.3)	0.9 (0.5–1.6)	2.0 (1.1–3.6)	1.5 (0.97–3.1)	1.7 (0.97–3.0)	1.8 (1.1–3.2)	1.6 (0.9–2.8)	2.1 (1.2–3.6)

AT1R indicates angiotensin II type 1; C3aR, C5aR, complement factor-3 and -5; ETAR, endothelin-1 type A; VEGF-A, VEGF-B, vascular endothelial growth factor A, B; and VEGFR1, VEGFR2, vascular endothelial growth factor receptor-1, -2.

*Model 1: adjusted for sex and age; **model 2: model 1+stroke severity, stroke pathogenesis (Trial of ORG 10172 in Acute Stroke Treatment classification), prestroke dependency, regular alcohol consumption, cardiovascular risk factor (hypertension, diabetes, peripheral arterial disease, history of myocardial infarction, coronary artery disease, smoking, dyslipidemia); ***model 3: model 2+eGFR, CRP, leukocytes.

Unfavorable Functional Outcome Defined as mRS Score >1

Levels of autoantibodies against C3aR, VEGFR2, and VEGF-B were associated with increased risk of unfavorable functional outcome defined as mRS score >1 at 1 year after stroke for autoantibodies against C3aR, VEGFR2, and VEGF-B in the fourth quartile were 2.1 (95% CI, 1.2–3.7), 1.8 (95% CI, 1.0–3.1), and 2.1 (95% CI, 1.2–3.6) in adjusted models (model 3) as shown in Table S2. In further analyses that included all autoantibodies in 1 model, only high levels of VEGFR2-IgG were independently associated with an mRS score >1 (OR, 1.9 [95% CI, 1.2–3.2]).

Results of dose–response multivariable regression analyses for quartiles 2 to 4 of autoantibody levels and associations with outcomes (poor functional outcome, BI over time) using quartile 1 as reference are shown in Table S3. In our cohort, 122 patients (21%) received thrombolyses. In sensitivity analyses, we also adjusted for thrombolyses in multivariable logic regression analyses without any major changes (C3a R-IgG: OR, 2.0 [95% CI, 1.1–3.6]; VEGFR2-IgG: OR, 1.8 [95% CI, 1.1–3.2]; VEGFB-IgG: OR, 2.1 [95% CI, 1.2–3.7]).

Combined Vascular End Point

Five MIs, 44 recurrent ischemic strokes, and 50 deaths occurred during follow-up. Total patient-years at risk were 1761 years, median follow-up time was 3.0 (interquartile range, 2.9–3.4) years. Results of Cox proportional hazard models are shown in Table S4. In summary, no associations were found for high levels of autoantibodies with the combined vascular end point consisting of recurrent ischemic stroke, MI, or all-cause death.

DISCUSSION

Our study demonstrates that high levels of autoantibodies against VEGFR2, VEGF-B, and the C3a receptor are associated with poor functional outcome after first-ever ischemic stroke but not with recurrent vascular risk or death. To the best of our knowledge, this is the first study to investigate the impact of vasoregulatory autoantibodies including GPCR antibodies on clinical outcomes after stroke.

Overall, only few prospective studies have investigated associations between vasoregulatory autoantibodies and clinical outcomes in cardiovascular medicine.[2] Most available studies examined GPCR antibodies targeting AT1R and ETAR in rheumatic and hypertensive diseases.[5,8] For instance, agonistic autoantibodies against AT1R and ETAR were significantly higher and more frequent in patients with systemic sclerosis–associated pulmonary arterial hypertension (n=81) and in connective tissue disease–associated pulmonary arterial hypertension (n=110) compared with other forms of pulmonary hypertension (n=106).[9] In addition, high levels of these autoantibodies predicted pulmonary arterial hypertension development (HR, 3.5 [95% CI, 1.5–5.6]) and death (HR, 2.7 95% CI, 1.2–6.1) in systemic sclerosis. Furthermore, a recent systematic review with meta-analysis explored the impact of autoantibodies against AT1R in nongravid hypertension and preeclampsia.[32] Ten studies including 757 patients (456 with hypertension only and 301 with preeclampsia) and 344 controls were analyzed. Associations of anti-AT1R antibodies with hypertension were more pronounced in preeclampsia than in nongravid hypertension (pooled OR, 32.84 [95% CI, 17.19–62.74]; pooled OR, 4.18 [95% CI, 2.20–7.98], respectively). Moreover, Abadir and colleagues[16] examined a set of physical function tests and outcomes in community-dwelling adults from Baltimore (n=255) and explored correlations with autoantibodies targeting AT1R. They found that high levels were strongly associated with weaker grip strength, reduced walking speed, and higher rates of falls. They also observed a significant increase in inflammation and blood pressure in patients with high anti-AT1R autoantibody levels, as well as attenuation of the decline in grip strength and a decreased time to death with treatment with ATR1 blockers.

In our mild-to-moderate ischemic stroke cohort, autoantibodies against AT1R seem to have no impact

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Table 4. Adjusted Repeated Measures Analyses of Barthel Index Over Time After Stroke Using Generalized Estimating Equations

Autoantibodies	AT 1 R-IgG	ETAR-IgG	C3a R-IgG	C5aR-IgG	VEGF1R-IgG	VEGF2R-IgG	VEGFA-IgG	VEGFB-IgG
	Change in Barthel Index score over time with 95% CI for quartile 4 versus quartile 1–3 as reference							
Model 1	−0.3 (−2.5–2.4)	−0.2 (−2.6 to 2.3)	−3.3 (−6.1 to −0.5)	−1.2 (−3.8 to 1.3)	−2.2 (−4.8 to 0.4)	−1.5 (−4.0 to 0.9)	−1.8 (−3.6 to 0.7)	−2.5 (−5.1 to −0.08)
Model 2	−0.3 (−2.5–2.1)	−0.3 (−2.5 to 2.0)	−3.1 (to 5.2 to −0.5)	−1.3 (−3.7 to 1.1)	−2.3 (−4.8 to 0.2)	−1.3 (−3.6 to 1.1)	−1.5 (−4.0 to 1.2)	−2.3 (−4.7 to −0.06)
Model 3	−0.3 (−2.6 to 1.0)	−0.2 (−2.5 to 2.0)	−3.1 (−5.7 to −0.5)	−1.4 (−3.8 to 1.1)	−2.3 (−4.9 to 0.2)	−1.2 (−3.6 to 1.1)	−1.5 (−4.1 to 1.2)	−2.4 (−4.8 to −0.06)

AT1R indicates angiotensin II type 1; C3aR, C5aR, complement factor-3 and -5; ETAR, endothelin-1 type A; VEGF-A, VEGF-B, vascular endothelial growth factor A, B; and VEGFR1, VEGFR2, vascular endothelial growth factor receptor-1, -2.

*Model 1: adjusted for sex and age; **model 2: model 1+stroke severity, stroke pathogenesis (Trial of ORG 10172 in Acute Stroke Treatment classification), pre-stroke dependency, regular alcohol consumption, cardiovascular risk factor (hypertension, diabetes, peripheral arterial disease, history of myocardial infarction, coronary artery disease, smoking, dyslipidemia); ***model 3: model 2+eGFR, CRP, leukocytes.

on clinical outcome after stroke. Thus, our primary hypothesis could not be confirmed. However, we found clear associations between high levels of autoantibodies against VEGFR2, VEGF-B, and C3aR and poor functional outcome at 1 year after stroke.

In experimental stroke studies, activation of the VEGFR2—as proposed for the agonistic autoantibodies targeting VEGFR2 in this study—induces angiogenesis in the chronic phase after cerebral ischemia, while in the acute phase, activation of VEGFR2 increases blood–brain barrier (BBB) dysfunction and edema growth.[33–36]

In our study, only IgG-type autoantibodies were measured.[9] Therefore, it is likely that the antibodies we measured in the acute phase of stroke are preexisting and not induced by brain ischemia. Thus, preexisting autoantibodies could enter the brain through the leaky BBB after cerebral ischemia and initiate their potential effects. This might be one explanation of the associations between high levels of autoantibodies targeting VEGFR2 measured in the acute phase of stroke and poor outcome at 1 year as demonstrated by our results.

For VEGF-B, experimental stroke studies found that larger cerebral lesions and more severe neurological deficits are present in VEGF-B–deficient mice after focal brain ischemia.[36] VEGF-B could stimulate neurogenesis and serves as an important proangiogenic factor essential for favorable long-term outcome in mouse models of ischemic stroke.[37] In contrast with the agonistic effects of autoantibodies on vascular receptors such as VEGFR2, autoantibodies targeting VEGF-B are thought to reduce receptor binding, for example, by blockade of or competition for the receptor binding site, and thus reduce signaling effects of VEGF-B.

Furthermore, complement activation plays an important role in brain injury after ischemic stroke and is associated with unfavorable outcome.[37,38] In a model of focal cerebral ischemia, C3a-deficient mice showed a significant reduction of stroke lesion volume and oxidative stress.[39] Moreover, Atkinson and colleagues[40] found that C3a-deficient animals showed a significant improvement in survival, neurological deficit and infarct size 24 hours after experimental stroke. In a prospective study of patients with ischemic stroke, high C3a levels measured within 10 days after stroke onset were associated with a poor outcome (mRS score >2) at 3 months after stroke (OR for upper tertile compared with lowest, 3.8 [95% CI, 1.1–14.0]).[41] In addition, Zhang and colleagues[42] reported in a cohort of 1451 young Chinese patients with stroke that low C3a levels were associated with favorable functional outcome defined as mRS score <3 at 3 months after stroke.

In experimental models, signaling through C3aR in the acute phase of ischemic stroke can contribute to

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tissue damage and increase stroke lesion volume via inflammatory endothelial activation and BBB dysfunction.[43,44] The involvement of C3 in the pathophysiology of ischemic stroke evolution is also supported by human genetic studies.[45] In our study, activation of the C3a receptor as assumed for high levels of autoantibodies against C3aR leads to reduced functional outcome. Pathophysiological mechanisms such as BBB dysfunction and increased infarct lesion in the acute phase might play a critical role. However, functional and brain imaging studies are required to prove this hypothesis.

The lack of association between vasoregulatory autoantibodies and vascular risk remains unclear. One explanation is that activation of the C3aR and VEGFR2 and blocking of VEGF-B effects primarily leads to larger infarct lesions and edema growth via increased BBB permeability (with clinically reduced functional outcome) but did not affect the risk of recurrent vascular events over the long term. Otherwise, C3aR-dependent vascular inflammation and endothelial dysfunction might also lead to an increased vascular risk from a pathophysiological point of view. However, low event rates might also limit our results (44 recurrent strokes, 5 MIs).

Our study has a number of limitations. First, biomarker studies of associations between vasoregulatory autoantibodies and clinical outcome were merely defined as secondary analyses in the PROSCIS-B study.[19] Thus, analysis in this study has to be considered as exploratory. Second, mostly mild to moderate ischemic strokes (median NIHSS score of 2) in patients who were able to give informed consent could be included. This limits the generalizability of our study results beyond this patient group, and extrapolation should be done with caution. Third, although we adjusted for several potential confounders, there is the possibility of residual confounding; for example, only ≈65% of patients had brain imaging with magnetic resonance imaging, so we did not adjust for brain imaging parameters such as white matter hyperintensities or infarct volume. However, because most of the variables included in our models are strong predictors for poor clinical outcome (eg, stroke severity, age, atrial fibrillation), we believe that residual confounding/bias cannot fully explain our results. In addition, standard follow-up time points for functional outcomes including mRS in clinical trials is 90 days. We had data only on poststroke functional outcome including mRS at 1 year that might limit the comparability. It should be noted that ≈15% were lost to follow-up. We decided against imputation methods for the primary end point because interpretation of results would be even more difficult and introduce even more bias.[46] In addition, follow-up rates of 80% to 90% are common in stroke cohorts with long-term follow-up.

Furthermore, autoantibody levels were only measured once in the acute phase. Therefore, we cannot exclude the possibility that autoantibody levels were altered by the acute stroke or may change over time as reported for complement levels after stroke.[47]

In conclusion, our study showed that high levels of autoantibodies against the VEGFR2 and the C3a receptor and autoantibodies against VEGF-B are associated with poor functional outcome in patients with first-ever ischemic stroke. Furthermore, patients with ischemic stroke with high vasoregulatory autoantibody levels in the acute phase were not at higher risk for secondary vascular events or death. Further research is needed to confirm these associations in independent cohorts, determine the underlying pathophysiological mechanisms, and identify potential treatment to improve outcomes.

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Supplemental Material

Tables S1–S4
Figure S1

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