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Circulating levels of vascular endothelial growth factor and post-stroke long-term functional outcome

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Objectives: Vascular endothelial growth factor (VEGF) acts in angiogenesis and neuroprotection, although the beneficial effects on experimental ischemic stroke (IS) have not been replicated in clinical studies. We investigated serum VEGF (s-VEGF) in the acute stage (baseline) and 3 months post-stroke in relation to stroke severity and functional outcome.

Methods: The s-VEGF and serum high-sensitivity C-reactive protein (hs-CRP) concentrations were measured in patients enrolled in the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS) at the acute time-point (median 4 days, N = 492, 36% female; mean age, 57 years) and at 3 months post-stroke (N = 469). Baseline stroke severity was classified according to the National Institutes of Health Stroke Scale (NIHSS), and functional outcomes (3 months and 2 years) were evaluated using the modified Rankin Scale (mRS), dichotomized into good (mRS 0-2), and poor (mRS 3-6) outcomes. Multivariable logistic regression analyses were adjusted for covariates.

Results: The baseline s-VEGF did not correlate with stroke severity but correlated moderately with hs-CRP (r = .17, P < .001). The baseline s-VEGF was 39.8% higher in total anterior cerebral infarctions than in lacunar cerebral infarctions. In binary logistic regression analysis, associations with 3-month functional outcome were non-significant. However, an association between the 3-month s-VEGF and poor 2-year outcome withstood adjustments for age, sex, cardiovascular covariates, and stroke

Alexander Wall and Olof Anger contributed equally to the paper.

severity (per 10-fold increase in s-VEGF, odds ratio [OR], 2.56, 95% confidence interval [CI] 1.12-5.82) or hs-CRP (OR 2.53, CI 1.15-5.55).

Conclusions: High 3-month s-VEGF is independently associated with poor 2-year functional outcome but not with 3-month outcome.

KEYWORDS

cerebrovascular diseases, functional outcome, inflammation, ischemic stroke, rehabilitation, stroke severity, strokes, vascular endothelial growth factor

1 | INTRODUCTION

Vascular endothelial growth factor (VEGF) is a 20-kDa homodimeric peptide hormone or growth factor that promotes angiogenesis, lymphangiogenesis, and vascular permeability (for review, see[1]). In the brain parenchyma, VEGF is not only expressed in endothelial cells, but also in astrocytes and neurons (for review, see[2]). Thus, VEGF and VEGF receptor (VEGF-R) isoforms are widely expressed in the brain and are upregulated in response to cerebral ischemia (for review, see[2]). Although post-stroke increases in the brain levels of VEGF or VEGF-R suggests the importance of VEGF signaling in relation to stroke, these responses may be secondary to injury and not neuroprotective. In the case of VEGF administration, two opposite types of responses complicate the understanding of VEGF action after stroke. In experimental stroke, local administration of VEGF within the brain exerts neuroprotective and plasticity-promoting effects,[2] whereas systemic administration of VEGF reduces recovery.[2-5] Thus, the actions of endogenous and exogenous VEGF might be different, and in addition, the mode of administration could influence the effects of VEGF in ischemic stroke (IS).

In patients with IS, in line with the experimental findings, endogenous serum VEGF (s-VEGF) has shown variable responses to IS, and the associations with functional outcomes have differed between studies.[6-14] These discrepancies may be attributable to the relatively small study cohorts with lower statistical power, biased selection of specific stroke types, or short follow-up periods. For example, s-VEGF was significantly increased in patients with acute IS (N = 29) at 0-7 days post-stroke compared to healthy controls without any clear day-to-day changes in s-VEGF.[6] A similar pattern of elevation of s-VEGF was shown in two relatively small studies that included both IS and hemorrhagic strokes (N = 52 and N = 33), with no association to improvement in the National Institutes of Health Stroke Scale (NIHSS) scores after 1 week,[12] or in the Stroke Impairment Assessment Set (SIAS) at discharge approximately 3 months post-stroke.[13] In another study (N = 30), the s-VEGF levels appeared to be more dynamic, with a plateau phase until Day 7 after an initial increase on Day 1-3.[10] Interestingly, in a recent study (N = 83), high s-VEGF levels 7 days after IS correlated significantly with better functional outcome (mRS 0-2) after 3 months,[8] although it was not examined whether baseline stroke severity or IS subtype affected these associations. Similarly, although the absolute functional outcome levels were not presented, high s-VEGF levels at 24 hours post-stroke were independently associated with a more marked improvement in NIHSS scores up to 3 months in IS patients with large vessel disease or small vessel disease (N = 180).[15] This is in contrast to another report, in which high-level plasma VEGF was associated with a worse 3-month functional outcome in cardioembolic IS, but not in other IS etiologies (N = 171).[9] A similar association was found for 90-day s-VEGF and the radiologic appearance of small vessel disease burden in IS patients (N = 263) having undergone thrombolysis, whereas functional outcome was not reported.[14] This underlines that stroke subtype and potential biases in the selection of patients need to be taken into account when evaluating the importance of s-VEGF in IS.

In summary, the role of s-VEGF in human IS remains unclear and there are conflicting data in terms of the association with IS outcome as previous studies have been relatively small and only reported functional outcomes up to 3 months post-stroke. To clarify these conflicting data, we related the acute and 3-month post-stroke levels of s-VEGF to functional outcomes after 3 months and 2 years in a relatively large group of IS patients and evaluated the importance of age, initial IS severity, and the major localization and etiology of IS. We used data from the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS, N = 492 for the acute time-point[16,17]), and the relative contributions of different mechanisms were evaluated using multivariate regression analyses. In these analyses, we included potential confounders or mediators, such as cardiovascular risk factors, IS severity, and as a marker for brain and systemic inflammation high-sensitivity C-reactive protein (hs-CRP).[18,19]

2 | SUBJECTS AND METHODS

The design of SAHLSIS has been reported elsewhere.[16,17,20] Briefly, patients aged <70 years with first-ever or recurrent acute IS were recruited consecutively at four Stroke Units in western Sweden between 1998 and 2003. The serum concentrations of VEGF were analyzed at the acute time-point (baseline, median 4 days post-stroke, N = 492) and after 3 months (N = 469), as specified below according to the *Strengthening the Reporting of Observational Studies in Epidemiology* (STROBE)[21] (Figure 1). Community controls without clinical manifestations of stroke or coronary artery disease from the same geographic area (N = 600, of which N = 513 had blood samples and successful analysis of s-VEGF) were

randomly selected from population registers to match the sex and average age of the patients. Stroke severity at acute stroke was initially recorded according to the Scandinavian Stroke Scale (SSS), as the maximum score during Days 0-10, and this was transformed into the more frequently used NIHSS according to the validated algorithm: NIHSS = 25.68-0.43 × SSS.[22] As NIHSS showed a skewed distribution, we also performed analyses in which NIHSS was transformed into quintiles (NIHSS-q) as described in the Appendix S1 and in a previous publication.[23] The recordings of hypertension, diabetes mellitus, and smoking had very few missing data-points (Table 1).[16] More data were lacking for low-density lipoprotein (LDL, mmol/L; N = 75, Table 1); the mean of the baseline LDL levels was imputed to have complete LDL datasets when used as a covariate in the regression models. The characteristics of the patients

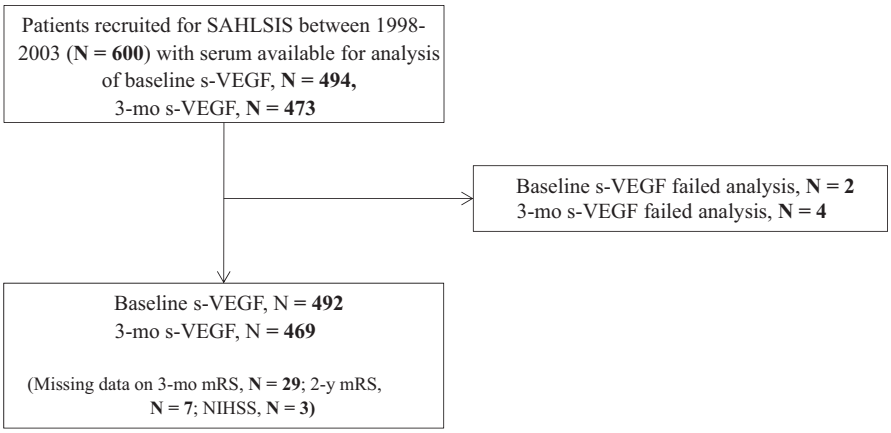

FIGURE 1 Flowchart showing the numbers of included subjects, as well as the criteria used to exclude other patients according to the *Strengthening the Reporting of Observational Studies in Epidemiology* (STROBE) guidelines[21]

TABLE 1 Baseline characteristics of the SAHLSIS participants (patients and controls)

Variable	Patients (P)	N	Controls (C)	N	P vs C [*P*-value]
Males, N (%)	315 (64.0)	492	329 (64.1)	513	1.00
Age (y)	57.1 (56.3-58.1)	492	57.2 (56.3-58.0)	513	.92
BMI (kg/m^2)	26.7 (26.3-27.1)	479	26.5 (26.2-26.9)	512	.51
Hypertension, N (%)	299 (61.8)	484	199 (38.9)	512	<.001
Diabetes, N (%)	94 (19.1)	492	30 (5.9)	511	<.001
Smoking, N (%)	193 (39.5)	489	89 (17.3)	513	<.001
Dyslipidemia (LDL, mmol/L)	3.35 (3.25-3.45)	417	3.33 (3.25-3.40)	510	.73
Baseline hs-CRP (mg/L)	10.8 (8.84-12.8)	492	3.06 (2.55-3.58)	512	<.001
3-mo hs-CRP (mg/L)	5.59 (4.57-6.61)	453	N/A	N/A	N/A
Baseline s-VEGF (pg/mL)	472.6 (432.1-513.0)	492	395.3 (369.6-421.1)[a]	513	.24
ΔVEGF (pg/mL)	−21.4 (−47.9-5.1)	449	N/A	N/A	N/A
3-mo S-VEGF (pg/mL)	444.4 (411.3-477.6)	469	395.3 (369.6-421.1)[a]	513	.14
NIHSS baseline	5.32 (4.82-5.81)	489	N/A	N/A	N/A

Note: Values are presented as means and 95% CI or percentage fraction. The *P*-values are based on Student's *t* test (continuous variables), the χ^2 test (fractions), or the Mann-Whitney *U* test (hs-CRP and s-VEGF). The Wilcoxon test showed that there was no significant difference in sS-VEGF levels between baseline and 3 mo (*P* = .24). N/A, Not available. ΔVEGF represents the intra-individual change in s-VEGF from baseline to 3 mo post-IS, which was tested with analysis of variance (ANOVA) against a hypothetical no change population with the same variation and number, and showed no statistical significance (*P* = .26).

Abbreviations: BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; N/A, not available; NIHSS, National Institutes of Health stroke scale; VEGF, vascular endothelial growth factor.

[a]Identical values.

and controls have been reported previously [16,20,24] (Table 1). For a more detailed description of the study design, and a description of a minor modification made to the Trial of Org 10172 in Acute Stroke Treatment (TOAST)[25,26] and major localization Oxford Community Stroke Project (OCSP) subtyping,[27] the reader is directed to the Appendix S1. The study was approved by the Ethics Committee of the University of Gothenburg.

2.1 | Biochemical analyses

The s-VEGF concentrations were measured using the commercially available Human Hypoxia Serum/Plasma Assay, MSD® 96-Well MULTI-SPOT® (Meso Scale Diagnostics) according to the instructions of the manufacturer, and using the QuickPlex SQ 120 reader and the Discovery Workbench software from the same company. In order to ensure a reliable analysis, we performed pre-analytical testing of variation and biomarker stability, which demonstrated high biological stability for up to 10 freeze-thaw cycles (data not shown). The intra-assay coefficient of variation (CV) of the measurements of s-VEGF was relatively low (3.6%). The inter-assay CV was 10.7%. In addition, a few samples (n = 18 for all analyzed samples, including controls) were below the detection limit; these were all set to 50% of the lower limit of the assay. Although the inter-assay variation for s-VEGF was relatively small, we chose to normalize the obtained values in each plate according to the three different quality control samples that were included in the runs.

2.2 | Statistical evaluation

Statistical analyses were performed using the SPSS ver. 25 software (SPSS Inc). The descriptive statistical results are given as means and 95% confidence intervals (CI) or standard deviations (SD). Comparisons between two groups were performed using Student's *t* test for continuous variables, the χ^2 test for categorical variables, and the Mann-Whitney *U* test for variables with skewed distributions (hs-CRP, NIHSS, and s-VEGF). Differences between multiple groups were evaluated using the Kruskal-Wallis test followed by a post hoc test (Mann-Whitney *U* test with Bonferroni correction). Paired sample comparisons (baseline vs. 3-month s-VEGF) were performed using the Wilcoxon signed rank test. To obtain *P*-values for ΔVEGF against a hypothetical no change, being normally distributed as opposed to baseline s-VEGF and 3-month s-VEGF, an ANOVA with the same variation and number was used. Correlations were explored using the Spearman rank order correlation test (rho values, *r*). Cox proportional hazards regression models were used to assess whether baseline s-VEGF levels above or below the median of 349.5 pg/mL were associated with the risk of all-cause mortality (presented as hazard ratios [HR], 95% CI, and *P*-values).

Our main outcome variable was functional outcome, as measured using mRS at 3 months and 2 years after IS. This variable was dichotomized to distinguish good (functional independence; mRS 0-2) from poor (death or functional dependency; mRS 3-6) outcomes. The baseline and 3-month s-VEGF levels were $\log_{10}$-transformed due to the marked skewness of the distributions. However, the changes in s-VEGF (ΔVEGF) were retained as original (non-transformed) values.

We used binary logistic regression to calculate the odds ratios (OR) and 95% CI, as well as the corresponding *P*-values (*P*-trends) for poor outcome (mRS 3-6) using the logarithmic values of s-VEGF as a continuous variable (showing the OR per 10-fold increase in s-VEGF). To adjust for potential confounders in the binary logistic regression analyses, we applied age and sex (Model 1), additionally, cardiovascular risk factors (hypertension, smoking, diabetes, and LDL) (Model 2), and finally, initial stroke severity (Model 3a). As stroke severity is related in part to stroke localization (OCSP,[27]) as well as inflammation (assessed by hs-CRP,[18,19]), these parameters were incorporated into Model 3b and 3c, respectively. When presenting the data, the hs-CRP values were converted into quintiles (q1 = 0-1.24; q2 = 1.25-2.66; q3 = 2.67-4.97; q4 = 4.99-11.8; q5 = 11.9-150 mg/l), whereas hs-CRP was used as continuous covariate in the regression analyses. Differences that gave *P*-values < .05 were considered statistically significant.

3 | RESULTS

3.1 | Baseline data, s-VEGF levels, and stroke characteristics

The baseline characteristics of the patients (N = 492, 36% female) and control subjects are presented in Table 1. Hypertension, diabetes, and smoking were observed more frequently among the IS patients. Although the baseline s-VEGF was numerically 19% higher in the patients than in the controls, this difference was not significant (Table 1). The 3-month s-VEGF levels were not significantly different from the baseline s-VEGF levels (*P* = .24, Table 1). ΔVEGF, which represents the intra-individual change in s-VEGF from baseline to 3 months post-stroke, tended non-significantly to be reduced (a mean decrease of −21.4 pg/mL, which corresponds to only 4.5% of the baseline level of s-VEGF, *P* = .26; Table 1). Furthermore, the baseline (*r* = .09, *P* = .04) but not the 3-month (*r* = .01, *P* = .84) s-VEGF levels correlated with age. However, both the baseline and 3-month s-VEGF levels differed between the age-decade groups, with gradually increasing values until the age range of 40-50 years followed by a plateau phase (Figure S1A). The baseline levels of s-VEGF did not differ in relation to the day after stroke onset on which it was sampled (Days 0-15, median Day 4, *P* = .9; data not shown).

The baseline s-VEGF level did not correlate with baseline stroke severity (s-VEGF vs. NIHSS, *r* = .05, *P* = .29), although the 3-month level of s-VEGF showed a weak correlation (*r* = .10, *P* = .04). These relations were only marginally changed if NIHSS was categorized into quintiles due to its marked skewness (baseline s-VEGF vs. NIHSS-quintiles, *r* = .05; *r* = .26; 3-month s-VEGF vs. NIHSS-q, *r* = .10, *P* = .06). Similarly, the ΔVEGF values did not correlate with the baseline NIHSS-q (*r* = .004, *P* = .9).

The baseline levels of hs-CRP had a weak but significant correlation with the baseline (r = .16, P < .001) and 3-month (r = .12, P = .01) levels of s-VEGF, and with ΔVEGF (r = −.13, P < .001). However, only the baseline s-VEGF differed between the acute hs-CRP quintiles (Figure S2B). Baseline s-VEGF was 39.8% higher, and 3-month s-VEGF was 28.9% higher, for the OCSP subtype of TACI compared to LACI, whereas there were no significant differences with regard to TOAST subtype (Table 2). The values of ΔVEGF across both OCSP and TOAST subtypes were statistically similar (data not shown). Furthermore, few patients with IS (N = 22) died during the 2-year follow-up period, and the age- and sex-adjusted Cox proportional hazards regression models did not show any significant association between high baseline levels of s-VEGF (values above the median of 349.5 pg/mL) and all-cause mortality (age- and sex-adjusted HR = 1.90, 95% CI 0.76-4.72, P = .17).

3.2 | Circulating VEGF levels and functional outcomes after 3 months and 2 years

The clinical data for patients with good or poor functional outcome 2 years after IS are given in Table 3. We performed binary logistic regression analysis using s-VEGF as a $\log_{10}$ continuous variable; these analyses showed that the baseline s-VEGF was not associated with poor outcome at either 3 months or 2 years post-IS, regardless of any adjustments made (Table 4, upper panels). ΔVEGF did not show any crude significant associations with functional outcome (not shown).

Next, we analyzed the 3-month level of s-VEGF with respect to functional outcome after 3 months and after 2 years (Table 4, lower panels). While the 3-month level of s-VEGF did not associate with functional outcome at 3 months post-stroke, there was a significant association between increasing 3-month s-VEGF and the risk of having poor functional outcome after 2 years. This association was retained after adjustment for traditional cardiovascular risk factors (Model 2) and additionally for baseline severity (Model 3a), localization (Model 3b), and level of hs-CRP (Model 3c). As the association between high 3-month s-VEGF and poor functional outcome remained significant in all models in the entire cohort, we performed subanalyses in which the study population was stratified according to etiology (OCSP)[25] or major localization (TOAST).[27] However, the subgroups were relatively small (N = 37-162), which restricted the possibility to include covariates in the analyses.[28] Therefore, in analyses only corrected for age and sex, the associations between 3-month s-VEGF and functional outcome were in the same direction (OR ≥ 1.20) as that of the entire cohort (OR = 2.76), with the exception of the arterial dissection group (non-significant OR < 1 for mRS 3-6). A significant association was only seen in the total anterior cerebral infarction group (Table S1).

Finally, as CRP is a marker of bacterial infections (typically at concentrations >10 mg/L) and inflammatory reactions related to the ischemic lesion size (typical range: 1-15 mg/L),[19] we excluded the cases with hs-CRP > 11.9 mg/L (quintile 5), so to assess the impact of CRP in patients without bacterial infections. This analysis of hs-CRP-q 1-4 (N = 358) did not attenuate the association for 2-year poor functional outcome, rather it marginally increased its strength (full adjustment, Model 3c; OR [per 10-fold increase in 3-month s-VEGF] = 3.01, 95% CI 1.15-7.85, P = .025).

4 | DISCUSSION

While there have been some studies of endogenous s-VEGF in IS, this is the first study to investigate both the acute (baseline) and 3-month levels of s-VEGF with respect to functional outcomes up to 2 years post-stroke. Although we did not identify any relationship between the baseline level of s-VEGF and stroke severity, the mean levels of both baseline and 3-month s-VEGF were higher in the subgroup with the largest IS (TACI). In contrast to the results of some previous studies, the baseline level of s-VEGF was not significantly higher in the patients with IS than in the age- and sex-matched healthy control population. Furthermore, both the baseline and 3-month levels of s-VEGF were slightly higher in older patients with IS, as well as in patients with IS who had higher levels of hs-CRP.

With regard to functional outcome, the level of s-VEGF (at baseline or after 3 months) did not associate with the 3-month functional outcome. In contrast, a high 3-month level of s-VEGF was associated with poor functional outcome 2 years after IS. Importantly, the association between the 3-month s-VEGF level and 2-year functional outcome withstood adjustments for age, sex, and cardiovascular risk factors, initial stroke severity, stroke localization, and hs-CRP. Taken together, these results suggest that convalescent s-VEGF is an independent prognostic marker for worse long-term (2-year) functional outcome. However, the 3-month s-VEGF was also moderately correlated with baseline stroke severity or to some more degree inflammation, as assessed by hs-CRP levels. This is in line with the finding that the association between the 3-month level of s-VEGF and the 2-year functional outcome was partially attenuated by these factors in the regression analysis.

4.1 | Methodological considerations

A strength of our study is that the study population (N = 492 for baseline levels and N = 469 for 3-month levels of s-VEGF, respectively) was considerably larger than those used in previous studies of VEGF in IS patients (N = 26,[11] N = 83,[8] and N = 171[9]). In addition, the follow-up time was 2 years, as compared to 3 months in comparable earlier studies.[8,9,11] Subsequently, some studies only investigated the sub-acute temporal profiles of s-VEGF in relation to stroke severity.[6,7] Patient enrollment in our study was consecutive and carried out at stroke centers in and around the Gothenburg area. Sweden has one of the highest hospitalization rates for IS in Europe,

TABLE 2 Baseline and 3-month s-VEGF levels in patients based on stroke subtype (localization and etiology)

	Baseline s-VEGF (pg/mL)	SD	N	3-month s-VEGF (pg/mL)	SD	N
OCSP subtypes (major localization)						
Lacunar cerebral infarction (LACI)	436.6*	411.8	177	434.1*	356.6	164
Total anterior cerebral infarction (TACI)	610.4	519.6	48	559.5	323	40
Partial anterior cerebral infarction (PACI)	480.9*	493.6	139	413.8*	304.3	139
Posterior cerebral infarction (POCI)	439.2	402.7	116	448.4	355.6	112
Other (undetermined)	N/A	N/A	12	N/A	N/A	14
All OCSP[a]	472.6	457.5	492	446.2	357.8	469
Missing			0			0
TOAST subtype (etiology)						
Large vessel disease	558.7	553.7	54	470.2	355.8	48
Small vessel disease	449.6	450.8	106	456.5	371.3	97
Cardioembolic	471.4	376.6	81	412.3	305.1	75
Other determined causes						
Arterial dissection	350.5	382.4	23	345.3	240.5	23
Other	N/A	N/A	14	N/A	N/A	15
Undetermined causes						
Cryptogenic	484.8	424.8	131	496.3	419.4	130
Other undetermined	N/A	N/A	83	N/A	N/A	81
All TOAST[a]	472.6	457.5	492	472.6	457.5	469
Missing			0			0

Note: Values are given as means and standard deviations (SD). TOAST, Trial of Org 10,172 in Acute Stroke Treatment, as slightly modified from Adams, Bendixen et al (1993); OCSP, Oxfordshire Community Stroke Project (Bamford, Sandercock et al 1991); N/A, not applicable, not given due to heterogeneity. The Kruskal-Wallis test showed that the baseline and 3-month s-VEGF levels differed between the OCSP groups ($P = .044$ and $P = .026$, respectively), but not between the TOAST groups ($P = .15$ and $P = .54$, respectively). A post hoc analysis was performed using the Mann-Whitney *U* test with Bonferroni correction (3×) for the OCSP groups.

[a]Identical values.

*$P < .05$, as compared with the TACI group at the same time-point).

at around 84%–95%,[29] and the highest rates are found among IS patients <70 years of age.[30] Our relatively young patient population (mean age, 57 years) was well-characterized, further contributing to a high-quality follow-up with low rates of co-morbidity and mortality, as well as few dropouts (eg, missing data were N = 7 for 2-year mRS and N = 23 for 3-month s-VEGF, Figure 1). For some of the subanalyses, the low number of events (eg, deaths; N = 22) reduced the statistical power, but we chose to present the HRs for future comparisons. Accordingly, despite a HR of 1.90, the 3-month levels of s-VEGF were not significantly associated with all-cause mortality. Furthermore, when our IS population was stratified based on major localization (OCSP)[27] and etiology (TOAST),[25] the subgroups were relatively small (N = 37-162). Although most of the ORs for the association between 3-month s-VEGF and 2-year functional outcome were not significant in the stroke subgroup analyses, the ORs were in the same direction as that in the entire cohort (except for the arterial dissection subgroup).

There are also some limitations, which deserve mention. While the inclusion of younger patients has certain advantages, it diminishes the generalizability to elderly IS patients. In addition, the participants were of Caucasian ethnicity and from a distinct geographic area, which might further reduce the generalizability of our findings. The variability of the first sampling time-point for the subjects (median, Day 4), which prevents the assessment of detailed individual day-by-day changes in s-VEGF levels, is a weakness, as is the lack of radiologic measurements of stroke volumes. However, there is a moderate-to-strong correlation between the baseline score on the NIHSS and stroke lesion volume ($r = .51$-$.71$).[31] The patients were enrolled in the period of 1998-2003, when few were treated with thrombolytic agents (only N = 5 in our cohort), and after this period, there have been some alterations in prevention therapies with, for example, extended indications for novel anticoagulants. Finally, although thrombolysis does not increase the level of s-VEGF,[11] it is of note that our study with a low rate of thrombolysis may be more

TABLE 3 Characteristics of the patients with good and poor functional outcomes after 2 y

Variable	Good outcome (mRS 0-2)	N	Poor outcome (mRS 3-6)	N	Good vs. poor outcome *P*-value
Males (N, %)	246 (65.3)	377	53 (60.2)	88	.38
Age (y)	57.1 (55.7-57.7)	377	59.9 (58.0-61.8)	88	.004
BMI (kg/m^2)	26.9 (26.5-27.4)	370	26.2 (25.2-27.2)	85	.19
Hypertension, N (%)	228 (60.5)	377	60 (68.2)	88	.18
Diabetes, N (%)	64 (17.0)	377	28 (31.8)	88	.002
Smoking, N (%)	145 (38.5)	377	35 (40.2)	87	.76
Dyslipidemia (LDL, mmol/L)	3.34 (3.23-3.45)	320	3.25 (3.03-3.47)	72	.37
Baseline hs-CRP (mg/L)	7.67 (5.95-9.4)	365	20.6 (13.9-27.3)	81	<.001
3-month hs-CRP (mg/L)	4.88 (4.03-5.74)	367	7.29 (3.79-10.8)	88	.001
Baseline s-VEGF (pg/mL)	442.0 (400.0-484.4)	365	573.5.0 (449.7-697.2)	80	.048
ΔVEGF (pg/mL)	−19.1 (−48.0-9.8)	365	−30.3 (−99.1-38.6)	80	.66
3-month s-VEGF (pg/mL)	421.2 (387.1-455.4)	377	553.7 (466.8-640.7)	88	.006
NIHSS baseline	3.77 (3.36-4.19)	376	10.7 (9.4-12.1)	88	<.001
NIHSS 3 mo	1.47 (1.33-1.62)	359	5.77 (5.89-6.66)	83	<.001
mRS 3 mo	1.47 (1.38-1.56)	367	2.87 (2.64-3.10)	84	<.001
mRS 2 y	1.32 (1.24-1.40)	377	3.81 (3.60-4.02)	88	<.001

Note: Values are presented as the means and 95% CI or percentage fraction. The *P*-values are based on Student's *t* test (continuous variables), χ^2 test (fractions), or Mann-Whitney *U* test (hs-CRP, s-VEGF, NIHSS, and mRS). Selection was based on the parameters of interest, mRS 2 y and 3-mo s-VEGF level (N = 465).

Abbreviations: BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; N/A, not available; NIHSS, National Institutes of Health stroke scale; VEGF, vascular endothelial growth factor.

representative of endogenous regulation of s-VEGF than studies with a higher rate of thrombolysis. As CRP is also a marker of bacterial infections (typically at concentrations >10 mg/L) and inflammatory reactions related to IS lesion size (typical range, 1-15 mg/L),[19] it is noteworthy that the exclusion of hs-CRP levels >11.9 mg/L (quintile 5) neither increased nor diminished the magnitude of the associations. This indicates that infections are probably not a confounder for functional outcome with regard to inflammation and s-VEGF levels.

4.2 | Previous studies on VEGF and IS

The baseline level of s-VEGF was numerically (19%), but non-significantly (*P* = .24), higher in the IS patients than in the age- and sex-matched controls, which is partly contrary to the results of previous studies showing higher s-VEGF levels in IS patients than in control subjects.[6,7] In the study by Slevin et al (N = 29), the mean s-VEGF concentration was about twofold higher in patients with IS than in age-matched controls, although the post-stroke fluctuations in s-VEGF levels observed in post-stroke Days 0-14 were only minor.[7] Similarly, Dassan et al[6] reported that s-VEGF levels in patients with IS (N = 29) were 2.5 times higher than those in non-matched healthy controls, albeit with no clear pattern of post-stroke s-VEGF changes. Furthermore, although we did not find any statistically significant correlation between s-VEGF at baseline and higher stroke severity (NIHSS), the baseline s-VEGF level was significantly higher (39%) in the group with the largest IS than in the group with the smallest IS (TACI vs. LACI). Our findings, derived from a considerably larger IS sample than in the previous studies, indicate relatively small differences in s-VEGF levels with respect to stroke severity and stroke localization, in contrast to the previous observations of 0.5- to 2.5-fold differences in s-VEGF levels between smaller and larger IS.[7]

Previous studies have shown partly divergent results with respect to the relationship between s-VEGF level and functional outcome after IS, possibly due to the small study populations and short durations of follow-up. One study (N = 83) observed that large increments of s-VEGF between Day 0 and Day 7 and the absolute level of s-VEGF on Day 7 post-IS correlated with a

TABLE 4 Odds ratios (OR) for the risk of poor functional outcome (mRS 3-6) after 3 mo and 2 y in patients with IS

	OR per log_{10} increase in baseline s-VEGF	*P*-value	N	OR per log_{10} increase in 3-month s-VEGF	*P*-value	N
mRS 3-6 after 3 mo						
Model 1 [A/S]	1.03 [0.62-1.73]	.91	463	1.84 [0.93-3.64]	.08	455
Model 2 [A/S/C]	0.98 [0.57-1.67]	.93	457	1.84 [0.91-3.68]	.09	454
Model 3a [A/S/C/I]	0.83 [0.42-1.62]	.58	456	1.41 [0.56-3.56]	.47	453
Model 3b [A/S/C/OCSP]	0.72 [0.39-1.32]	.28	445	1.33 [0.60-2.96]	.49	440
Model 3c [A/S/C/CRP]	0.74 [0.43-1.30]	.3	456	1.36[0.65-2.87]	.41	436
mRS 3-6 after 2 y						
Model 1 [A/S]	1.63 [0.95-2.80]	.079	485	2.76 [1.34-5.69]	.006	465
Model 2 [A/S/C]	1.58 [0.89-2.82]	.12	474	2.81 [1.34-5.88]	.006	464
Model 3a [A/S/C/I]	1.52 [0.80-2.87]	.2	473	2.56 [1.12-5.82]	.025	463
Model 3b [A/S/C/OCSP]	1.30 [0.72-2.37]	.39	462	2.29 [1.04-5.07]	.04	450
Model 3c [A/S/C/CRP]	1.30 [0.72-2.32]	.38	473	2.53 [1.15-5.55]	.021	445

Note: Baseline and 3-month s-VEGF levels and poor functional outcomes from IS. The odds ratios (OR) per log unit (10-fold) and the corresponding 95% confidence intervals (CI) were calculated by employing binary logistic regression using log_{10} s-VEGF as a continuous variable. Models 1-3 are shown with successively added adjustments for age (A), sex (S), traditional cardiovascular covariates (C, see *Methods*), initial stroke severity (I), major stroke localization according to the Oxford Community Stroke Project (OCSP) classification (Bamford, Sandercock et al 1991), and high-sensitivity C-reactive protein (hs-CRP).

favorable 3-month outcome,[8] that is, results that are discrepant with our findings. A weakness of the previous study is that adjustments for baseline stroke severity and cardiovascular covariates were not shown or not performed. Although thrombolysis was not presented separately with regard to s-VEGF levels, it is noteworthy that a large proportion of the latter study population was derived from patients who were treated at a rehabilitation center after thrombolysis or thrombectomy (77%), and the initial IS was quite severe (median NIHSS score of 17), as compared to the mean NIHSS score of 5.32 in our study. In contrast to these associations, another study (N = 171) presented results comparable to ours,[9] revealing either neutral (atherothrombotic IS) or inverse (cardioembolic) associations between high s-VEGF levels and functional outcome after 3 months. In another study (N = 43) including both IS and intracerebral hemorrhage (ICH), functional data were not reported, but an positive association was observed between 3-month s-VEGF and the radiologic appearance of post-stroke small vessel disease burden,[13] which indirectly would be in some accordance with our results. Thus, our findings, derived from a considerably larger cohort, indicate that a high 3-month level of s-VEGF is associated with a poor 2-year functional outcome.

We did not find any significant associations that withstood multivariate adjustments between s-VEGF levels and the 3-month functional outcome, which was the only functional outcome reported in the previous studies. The association between the 3-month level of s-VEGF and poor 2-year functional outcome remained statistically significant in all the models including adjustment for initial IS severity, and the ORs were rather high at 2.29-2.81 per 10-fold increase in s-VEGF. Finally, for another biomarker that we studied previously, IGF-1, the post-stroke serum level change was associated with functional outcome.[23,32] In the case of VEGF, when evaluating the change from the acute phase to 3 months post-IS, we found no such pattern.

4.3 | Significance of VEGF—local brain and serum VEGF levels

Previous reports on VEGF and experimental stroke have shown two diverging lines of treatment effects; local administration of VEGF within the brain was favorable with neuroprotective and plasticity-promoting effects (for review, see[2]), whereas systemic administration resulted in adverse or obscure effects.[2-5] This suggests that the neurobiology of VEGF is complicated; on one hand, it may be beneficial to achieve high local levels of VEGF to enhance plasticity-related recovery, and on the other hand, an unfavorable systemic response in serum associated with inflammation, the latter supported by the positive association in our study between hs-CRP and s-VEGF. Possibly, also the vascular damage on the blood-brain barrier (BBB) caused by the stroke may be associated with increasing VEGF in the penumbra zone,[33] which may reach the circulation via "backward" efflux due to the lack of an intact BBB. This has not been shown in experimental IS,[34] but may be present in the more severe BBB breakdown in tumors.[35] However, the widely accepted possibility is that a molecule or growth factor in the circulation can reach the brain via surpassing an injured BBB, for at least 4 weeks after experimental IS.[34] Thus, an elevated s-VEGF, due to for example systemic inflammation, may reach the

brain more readily due to BBB dysfunction in IS. Furthermore, there is a need to include control groups, preferably consisting of age- and sex-matched healthy subjects as well as measurements at several time-points to evaluate s-VEGF and its changes in IS, which has not been performed in some previous studies.[6,7,10,12,13] In contrast, our study has a large sample size, a population-based control group of similar age and gender distribution as the IS patients, and measurements of s-VEGF at two time-points. Therefore, the absence of statistically significant differences in s-VEGF in our study, both when comparing the two time-points and versus the controls, suggests that s-VEGF is not to any major degree dynamically regulated during the post-stroke period. Although the underlying mechanisms, like how BBB dysfunction influences VEGF distribution, are very difficult to evaluate, the deleterious association between high 3-month s-VEGF levels and 2-year functional outcome in our study suggests that a high circulating VEGF may have a negative impact on recovery. Therefore, s-VEGF could be an important target in further research. It would be of interest in future studies to investigate whether a high s-VEGF concentration is linked to high levels of VEGF in the cerebrospinal fluid (CSF), possibly with sampling at more time-points. It cannot be excluded that the CSF VEGF plays a positive role in recovery, as opposed to the role of endogenous s-VEGF.

5 | CONCLUSIONS

To the best of our knowledge, this is the first study to present results for VEGF levels in serum and long-term follow-up beyond 3 months with respect to functional outcome in IS patients. We show that s-VEGF is associated with poor functional outcome at 2 years post-stroke. The 2-year associations withstood adjustments for age, sex, cardiovascular risk factors, and initial stroke severity. In contrast to the acute baseline level of s-VEGF, the 3-month s-VEGF level appears to be an independent prognostic factor for stroke outcome, although the associations with outcome are linked in part to stroke severity and hs-CRP levels. The present study, having a higher statistical power than previous studies and with more extensive adjustments for relevant covariates, attaches a caveat to the associations between endogenous s-VEGF and functional outcome observed in some of the previous studies. Finally, although the observational nature of our study precludes any conclusion about causality, the results provide support that s-VEGF might be an important factor to target in further research. In further studies, the relationships between VEGF levels in the circulation and CSF with respect to stroke severity and functional outcome could be of special interest.

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CONFLICT OF INTEREST

The authors wish to disclose the following interests. HZ has served on scientific advisory boards for Roche Diagnostics, Wave, Samumed, and CogRx and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg (with no connection to this study). KB has served as a consultant or on scientific advisory boards for Roche Diagnostics, Alzheon, MagQu, Novartis, Biogen, Lilly, and CogRx and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg (all unrelated to this study). The other authors have no conflict of interests to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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