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Neurological Research

A Journal of Progress in Neurosurgery, Neurology and Neurosciences

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/yner20

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To cite this article: Alexander Wall, Olof Anger, Katarina Jood, Christian Blomstrand, Ulf Andreasson, Kaj Blennow, Henrik Zetterberg, Jörgen Isgaard, Christina Jern, N. David Åberg & Johan Svensson (2021): Circulating granulocyte colony-stimulating factor and functional outcome after ischemic stroke: an observational study, Neurological Research, DOI: 10.1080/01616412.2021.1948766

To link to this article: https://doi.org/10.1080/01616412.2021.1948766

NEUROLOGICAL RESEARCH
https://doi.org/10.1080/01616412.2021.1948766

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Circulating granulocyte colony-stimulating factor and functional outcome after ischemic stroke: an observational study

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ABSTRACT

Objectives: While granulocyte colony-stimulating factor (G-CSF) has shown beneficial effects in experimental ischemic stroke (IS), these effects have not been reproduced clinically. Small-to-medium-sized observational studies have reported varying associations for G-CSF with stroke severity and post-stroke functional outcome, prompting their investigation in a larger study.

Methods: Endogenous serum G-CSF (S-GCSF) was measured in the acute phase and after 3 months in patients with IS (N = 435; 36% females; mean age, 57 years) from the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS). Stroke severity was scored according to the National Institutes of Health Stroke Scale (NIHSS), and the modified Rankin Scale (mRS) assessed functional outcomes at 3-month and 2-year post-stroke. Correlation and logistic regression analyses with confounder adjustments assessed the relationships.

Results: The acute S-GCSF level was 23% higher than at 3-month post-stroke ($p < 0.001$). Acute G-CSF correlated weakly with stroke severity quintiles ($r = 0.12$, $p = 0.013$) and with high-sensitivity C-reactive protein ($r = 0.29$, $p < 0.001$). The association between S-GCSF (as quintiles, q) and poor functional outcome at 3 months (mRS 3–6; S-GCSF-q5 vs. S-GCSF-q1, age- and sex-adjusted odds ratio: 4.27, 95% confidence interval: 1.82–9.99; $p = 0.001$) withstood adjustment for cardiovascular risk factors and stroke subtype, but not additional correction for stroke severity. Post-stroke changes in S-GSCF and absolute 3-month S-GCSF were not associated with 3-month or 2-year functional outcomes.

Discussion: Early post-stroke S-GCSF is increased in severe IS and associated with 3-month poor functional outcomes. The change in S-GCSF and the 3-month S-GCSF appear to be less-important, and S-GCSF likely reflects inflammation in large infarctions.

ARTICLE HISTORY
Received 28 October 2020
Accepted 22 June 2021

KEYWORDS
ischemic stroke; granulocyte colony-stimulating factor; stroke severity; functional outcome; inflammation

1. Introduction

The 19.6-kDa glycoprotein granulocyte colony-stimulating factor (G-CSF), which is produced by monocytes, mesothelial cells, fibroblasts, and endothelial cells, stimulates the mobilization and differentiation of hematopoietic stem cells [1,2]. Accordingly, for more than two decades, G-CSF has been used clinically to treat neutropenia induced by chemotherapy or hematologic malignancies [3].

G-CSF receptors are highly expressed in specific parts of the central nervous system (CNS), such as the cortex, hippocampus, and cerebellum [4,5]. In rats, peripheral administration of G-CSF inhibited apoptosis and increased the numbers of newly generated neurons in the hippocampal dentate gyrus in both ischemic and non-ischemic animals [4]. Furthermore, a meta-analysis has demonstrated that G-CSF administration reduces infarct size and ameliorates sensorimotor deficits in ischemic stroke (IS) models [6]. However, randomized controlled trials have produced varying results regarding the acute phase of IS; a meta-analysis did not find any effect of G-CSF treatment on stroke outcome in the clinical setting [7].

Concomitant with the G-CSF administration studies, observational studies have reported discrepant levels of endogenous serum G-CSF (S-GCSF) for different study populations. For example, S-GCSF has been monitored in IS cases receiving tissue plasminogen activator (tPA) treatment [8], cases of intracerebral hemorrhage (ICH) [9], and cases of general IS

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#Equal contribution to the Manuscript.

Supplemental data for this article can be accessed here.

[10,11]. Specifically, for IS patients (N = 79) who received tPA treatment, the level of S-GCSF was significantly higher in the patients with favorable functional outcomes, i.e. modified Rankin Scale (mRS) 0–2 [8]. A similar association was found for general IS (N = 83), in which S-GCSF was measured 7 days after symptom onset [10]. In addition, in 95 patients with ICH, patients who experienced a good functional outcome had increased levels of S-GCSF in samples drawn upon admission, as well as in those drawn at 24 and 72 hours post-admission [9]. Furthermore, the S-GCSF levels correlated with neurologic improvement and a smaller lesion volume [9]. However, contrary to these findings, a Chinese study (N = 120) found that higher S-GCSF levels correlated with higher acute and 3-month National Institutes of Health Stroke Scale (NIHSS) scores [11].

In summary, while the S-GCSF level in the blood may be increased after IS, the associations with outcome and stroke severity are not clear. Moreover, none of the previous studies investigated the relationship between S-GCSF level and functional outcome beyond 3-month post-IS. As G-CSF has been linked to beneficial effects in experimental IS and bearing in mind the limited and varying associations observed in previous clinical studies, we determined the role of S-GCSF in the acute phase of IS, at 3-month post-stroke, and with regard to the changes in S-GCSF levels in the post-stroke phase in a large observational study. The S-GCSF levels in the patients with IS were compared with those in control subjects, and also related to initial stroke severity, high-sensitivity C-reactive protein (hsCRP) levels, and etiologic stroke subtypes. Finally, in logistic regression models, we investigated whether the S-GCSF levels were associated with poor functional outcome (mRS 3–6) at 3 months and 2 years after the index IS.

2. Materials and methods

2.1. Ethical approval

Participants or next-of-kin provided written informed consent for participation in the study. The study was approved by the Ethics Committee of the University of Gothenburg

2.2. Participants

The design of the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS) has been described elsewhere [12–15]. SAHLSIS was designed as an observational study, combining analyses of genetic variants and serum factors and extensive clinical characterization at admission to hospital and during follow-up. Briefly, patients (<70 years of age) with first-ever or recurrent acute IS were recruited consecutively at four Stroke Units in western Sweden between 1998 and 2003 (N = 489 patients with serum samples drawn in the acute phase). For this group, the levels of S-GCSF were analyzed in the acute phase (N = 435) and at 3-month post-stroke (N = 418), as indicated in Figure 1, according to Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) [16].

Age- and sex-matched Caucasian controls (N = 600) from the same geographic area were randomly chosen from population registers (N = 450 with available blood samples and completed S-GCSF analysis). The NIHSS scores were recalculated from the original Scandinavian Stroke Scale (SSS) scores using the following algorithm: NIHSS = 25.68–0.43× SSS [17]. The acute values were thereafter transformed into the following five NIHSS quintiles (NIHSS-q): q1, 0–0.74 (mild); q2, 0.74–2.03 (minor); q3, 2.03–3.75 (moderate); q4, 3.75–10.2

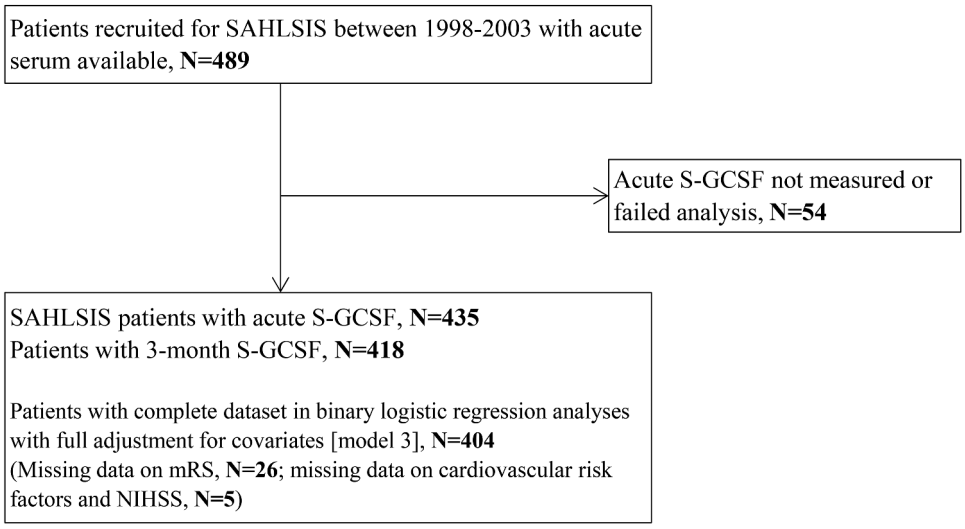

Figure 1. Flowchart showing the numbers (N) of included patients and exclusions according to Strengthening the Reporting of Observational Studies in Epidemiology (STROBE). Abbreviations: mRS, Modified Rankin Scale; S-GCSF, endogenous serum granulocyte colony-stimulating factor; NIHSS, National Institutes of Health Stroke Scale; SAHLSIS, Sahlgrenska Academy Study on Ischemic Stroke.

(major); and q5, 10.2–42 (severe), as described previously [18]. The index frequencies of previous hypertension, diabetes mellitus, smoking, and the acute levels of low-density lipoprotein (LDL) were considered as cardiovascular risk factors [13].

Among our samples, there were some missing data (mostly, acute LDL values) for the variables used as covariates (N = 59; Figure 1). However, by imputing the baseline LDL values based on the overall mean level of LDL, we were able to reduce the number of missing cases (N = 5; Figure 1) in the adjusted models for the logistic regression analysis.

The characterization of each distinct IS subgroup according to the *Trial of Org 10172 in Acute Stroke Treatment* (TOAST) [19] and the *Oxford Community Stroke Project* (OCSP) classification [20] has been reported previously [12,15]. For further details, see the *Supplementary material*.

2.3. Biochemical analysis

The S-GCSF concentration was measured using an immunoassay in accordance with the instructions of the manufacturer (Quantikine HS, #HSFB00D; R&D Systems, Minneapolis, MN). Among the patients, blood sampling was performed acutely within 19 days of the stroke event (with a median of 4 days), and an additional sample was drawn at the follow-up 3 months after the index stroke occurred. Blood sampling was performed only once in the control group. Blood samples were drawn between 08:30 AM and 10:30 AM after overnight fasting. Serum was isolated within 2 hours by centrifugation at 2000 × *g* at 4°C for 20 minutes and then stored at −80°C for 5–10 years before being assayed. Prior to the major analysis, validation experiments were performed. The intra-assay coefficient of variation (CV) was 8.3%, and the inter-assay CV was 8.7% (see Supplementary Table 1). Serum hsCRP levels were analyzed using a solid-phase chemiluminescent immunometric assay (Immulite 2000; Diagnostic Products Corp., Los Angeles, CA). The analytical lower limit was 0.1 mg/l, and the intra-assay CV was 3.4%.

2.4. Statistical analysis

Statistical analyses were performed using the SPSS ver. 25 software (SPSS Inc., Chicago, IL). The descriptive statistical results are given as means and 95% confidence intervals (CI) or standard deviations (SD). Comparisons of the two groups were performed using the Student's *t*-test for continuous variables, the χ^2 test for categorical variables, and the Mann–Whitney *U*-test for variables with skewed distribution (hsCRP, NIHSS, and S-GCSF). Differences between multiple subgroups were tested using the Kruskal–Wallis test followed by a post-hoc test (Mann Whitney *U*-test with Bonferroni correction). Paired sample comparison (acute vs. 3-month S-GCSF) was performed using the Wilcoxon test. Correlations were derived with Spearman's rank-order correlation test (rho values, r).

Our main outcome variable was functional outcome, as measured with 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 acute and 3-month S-GCSF values (pg/ml) were transformed into quintiles (S-GCSF-q) due to the marked skewness of the distributions. For the acute S-GCSF-q, these were: q1, 7.1–20.5; q2, 20.5–25.3; q3, 25.3–31.2; q4, 31.2–42.9; and q5, >42.9. For the 3-month S-GCSF-q, these were: q1, 7.1–17.5; q2, 17.5–23.0; q3, 23.0–28.2; q4, 28.2–34.9; and q5: >34.9. For the individual change in S-GCSF level from the acute to the 3-month timepoint, the quintiles were: q1, <−11.98; q2, −11.98 to −5.68; q3, −5.68 to −0.001; q4, 0–6.01; and q5, >6.1. Acute hsCRP (mg/l) was transformed into quintiles hsCRP-q, these were: q1, 0–1.24; q2, 1.25–2.66; q3, 2.67–4.97; q4, 4.99–11.8; and q5: >11.9. We used binary logistic regression models, including adjustments for covariates, to calculate the odds ratios (OR) with 95% CI and corresponding p-values for poor outcome (mRS 3–6), using S-GCSF-q as a categorical variable with S-GCSF-q1 as the reference. To adjust for potential confounders in the multivariate analyses, we applied age and sex (model 1), and additionally: cardiovascular risk factors (hypertension, smoking, diabetes, and LDL) (model 2); history of previous stroke (yes/no) and stroke subtype according to the OCSP classification [20] (model 3); and initial stroke severity (model 4). P-values <0.05 were considered statistically significant.

3. Results

3.1. Baseline characteristics and S-GCSF distributions based on hsCRP levels and stroke severity

The baseline characteristics of the patients and controls are listed in Table 1. As expected, in the patient group, a significantly larger proportion had cardiovascular risk factors, including smoking, diabetes, and hypertension, as compared to the control group (all $p < 0.001$, Table 1). In addition, the mean acute (+54%) and 3-month (+26%) S-GCSF levels were higher in the patient group compared to baseline values in the healthy control group (all $p < 0.001$). Furthermore, the acute levels of S-GCSF were significantly 23% higher than the 3-month levels of S-GCSF ($p < 0.001$). In the patients, there was a mean individual decrease of 5.16pg/ml in S-GCSF from the acute

Table 1. Baseline characteristics of the SAHLSIS participants.

Variable	Patients (P)	N	Controls (C)	N	P vs. C, p-value
Age (years)	57.3 [56.4–58.3]	435	57.2 [56.3–58.1]	450	0.84
Males (N, %)	280 (64.4)		288 (64.0)		0.94
BMI (kg/m^2)	26.8 [26.3–27.2]	425	26.6 [26.2–26.9]	449	0.45
Hypertension (N, %)	269 (62.7)	429	173 (38.5)	449	<0.001
Diabetes (N, %)	80 (18.4)	435	27 (6.0)	448	<0.001
Smoking (N, %)	165 (38.2)	432	75 (16.7)	450	<0.001
Previous stroke (N, %)	87 (20.0)	435	NA		
Dyslipidemia (LDL level)	3.35 [3.24–3.45]	370	3.34 [3.26–3.43]	447	0.97
Acute hsCRP (mg/l)	10.6 [8.58–12.7]	434	3.13 [2.55–3.71]	449	<0.001
3-month hsCRP (mg/l)	5.56 [4.48–6.63]	403	3.13 [2.55–3.71]‡	449	<0.001
Acute S-GCSF (pg/ml)	37.0 [31.8–42.2]	435	24.1 [22.7–25.6]	450	<0.001
3-month S-GCSF (pg/ml)	30.1 [27.6–32.5]	418	24.1 [22.7–25.6]‡	450	<0.001
S-GCSF change (pg/ml)$	−5.16 [−10.47 – −0.14]]	402	NA	NA	
NIHSS acutely	5.37 [4.84–5.90]	433	NA	NA	
NIHSS 3 months	2.27 [2.02–2.54]	397	NA	NA	
mRS 3 months	1.78 [1.67–1.90]	409	NA	NA	
mRS 2 years	1.91 [1.78–2.05]	429	NA	NA	
Deaths 2 years (n)	21		NA		

Values are presented as means and 95% CI or percentage fractions. The p-values are based on the Student's *t*-test (continuous variables), χ2 test (fractions), or the Mann–Whitney *U*-test (variables with skewed distribution; hsCRP and S-GCSF). The Wilcoxon test showed that the S-GCSF levels were significantly higher in the acute phase than after 3 months ($p < 0.001$). NA, Not available; ‡, identical to the above values. $ S-GCSF change was defined as the average individual difference between acute and 3-month S-GCSF, a negative value representing a decrease.

Abbreviations: SAHLSIS, Sahlgrenska Academy Study on Ischemic Stroke; BMI, body mass index; LDL, low-density lipoprotein; hsCRP, high-sensitive C-reactive protein; S-GCSF, endogenous serum granulocyte colony-stimulating factor; NIHSS, National Institute of Health Stroke Scale; mRS, modified Rankin Scale; CI, confidence interval.

time-point to the 3-month post-stroke (Table 1). In addition, analysis of the baseline characteristics for S-GCSF-q1 and S-GCSF-q5 showed higher levels of hsCRP and NIHSS in S-GCSF-q5 (all $p < 0.01$), whereas the other characteristics were similar (see Supplementary Table 2).

In the included patients, we examined the acute and 3-month S-GCSF levels with respect to initial stroke severity (Figure 2A), hsCRP level (Figure 2B), age group, and sampling day. In general, the acute S-GCSF levels showed more marked crude correlations than the 3-month S-GCSF levels or the changes in S-GCSF levels. The acute S-GCSF levels differed significantly among the NIHSS-q ($p = 0.025$; Figure 2A), and this was confirmed in a crude correlation analysis of acute S-GCSF to NIHSS-q ($r = 0.12$, $p = 0.013$). However, the 3-month values for S-GCSF and acute NIHSS-q did not correlate ($r = -0.002$, $p = 0.97$) nor did the changes in S-GCSF and NIHSS-q ($r = -0.066$, $p = 0.19$). Furthermore, the acute S-GCSF levels differed significantly among the acute hsCRP-q ($p < 0.001$; Figure 2B). There were equally significant, positive correlations between both the acute ($r = 0.29$, $p < 0.001$) and 3-month ($r = 0.19$, $p < 0.001$) hsCRP-q and S-GCSF levels, whereas the correlation between the changes in S-GCSF and hsCRP-q was weaker ($r = -0.11$, $p = 0.028$). The acute NIHSS-q also correlated with the acute levels of hsCRP-q ($r = 0.30$, $p < 0.001$). However, there were no significant crude correlations between the acute S-GCSF and age decade ($r = -0.046$, $p = 0.34$); 3-month S-GCSF and age decade ($r=-0.047$, $p=0.34$); or the changes in S-GCSF and age decade ($r = -0.001$, $p = 0.98$). The post-stroke day of blood sampling did not correlate with either the acute S-GCSF levels ($r = -0.07$, $p = 0.16$) or the changes in S-GCSF ($r = -0.037$, $p = 0.47$). Similarly, there were no significant differences in the mean levels of the acute S-GCSF or changes in S-GCSF according to the day of sampling (data not shown).

The baseline characteristics of the patients with good or poor 3-month functional outcomes are compiled in Table 2. The patients with poor outcomes after 3 months exhibited higher acute levels of hsCRP ($p < 0.001$), higher acute NIHSS scores ($p < 0.001$), and increased acute S-GCSF levels ($p = 0.003$), whereas the 3-month S-GCSF levels and the changes in S-GCSF from the acute to the 3-month time-points were unchanged. With respect to the 2-year outcomes, the baseline values were somewhat different, with higher levels of hsCRP and higher NIHSS scores in the poor outcome group, whereas the acute and 3-month S-GCSF levels as well as the changes in S-GCSF levels were not significantly related to functional outcome (data not shown).

Neither the acute nor the 3-month S-GCSF levels were significantly associated with stroke subtype, either with respect to the major localization of the IS according to the OCSP classification [20] or the etiology of IS according to TOAST [19] (see Supplementary Table 3). The acute S-GCSF values were significantly higher in the patients with IS, as compared to the healthy controls, for all stroke subtypes with the exception of arterial dissection (see Supplementary Table 3). However, 3-month post-stroke S-GCSF levels did not differ significantly between the patients and controls in most of the OCSP and TOAST categories, with the exceptions of lacunar cerebral infarction (LACI), partial anterior cerebral infarction (PACI), and small vessel disease, all of which displayed somewhat higher levels of S-GCSF compared to the controls.

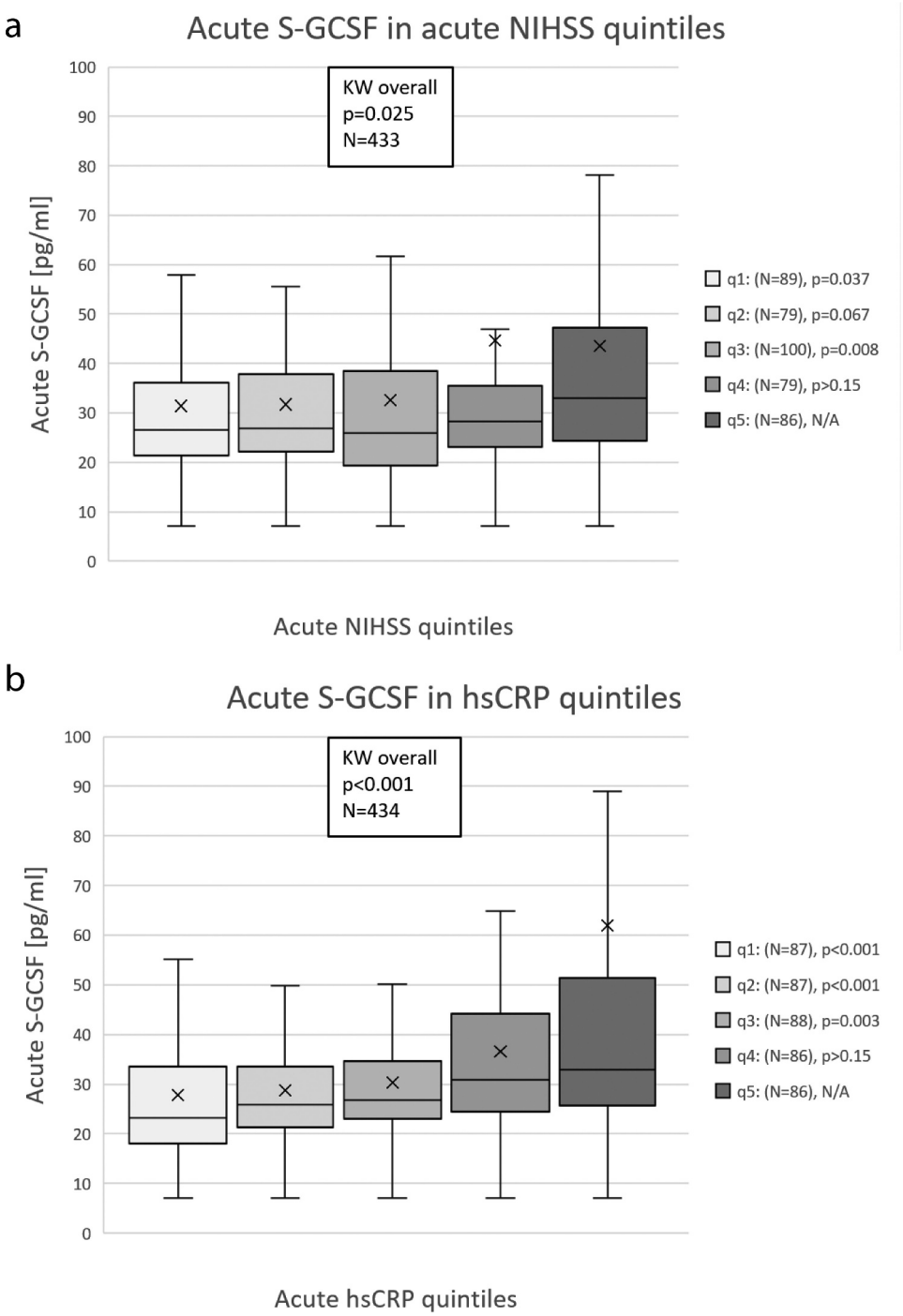

Figure 2. **A**: Acute S-GCSF levels in relation to initial stroke severity (NIHSS) presented in a Tukey boxplot. The acute NIHSS scores were transformed into quintiles, with a higher quintile corresponding to a higher NIHSS score. Values in the box plots are given as means (crosses), medians (horizontal lines), 25th–75th percentiles (boxes), and ranges (whiskers) along with the number (N) of patients included in the analysis. All data-points that exceeded 1.5× interquartile range (IQR) added to the 75th percentile were defined as outliers and are not shown. The p-value presented in the box, calculated using the Kruskal–Wallis test, shows that the acute S-GCSF levels differed across the NIHSS-q ($p = 0.025$). Significant differences between the acute S-GCSF levels in NIHSS-q5 vs. those in the lower NIHSS-q, as calculated in a post-hoc analysis using the Mann–Whitney *U*-test with Bonferroni correction (×4), are presented for the corresponding NIHSS-q. Abbreviations: S-GCSF, endogenous serum granulocyte colony-stimulating factor; NIHSS, National Institutes of Health Stroke Scale; KW, Kruskal–Wallis test. **B:** The acute S-GCSF levels in relation to the acute hsCRP levels presented in a Tukey boxplot. The values for acute hsCRP were transformed into quintiles, with a higher quintile corresponding to a higher hsCRP level. Values in the box plots as in 'A'. The p-value presented in the box, calculated using the Kruskal–Wallis test, shows that the acute S-GCSF levels differed across the hsCRP-q ($p < 0.001$). Significant differences between the acute S-GCSF levels in hsCRP-q5 vs. those in the lower hsCRP-q, as calculated in post-hoc analysis using the Mann–Whitney *U*-test with Bonferroni correction (×4), are presented for the corresponding hsCRP-q. Abbreviations are as in 'A', and additionally hsCRP, high-sensitivity C-reactive protein.

Table 2. Baseline characteristics of the patients with good and poor functional outcomes after 3 months.

Variable	Good outcome (mRS 0–2)	N	Poor outcome (mRS 3–6)	N	Good vs. Poor, p-value
Age (years)	57.1 [56.0–58.2]	325	59.0 [56.9–61.0]	84	0.12
Males (N, %)	206 (63.4)	325	57 (67.9)	84	0.52
BMI (kg/m^2)	26.8 [26.3–27.3]	318	26.6 [25.6–27.7]	81	0.75
Hypertension (N, %)	204 (62.8)	325	53 (65.4)	81	0.70
Diabetes (N, %)	53 (16.3)	325	24 (28.6)	84	0.018
Smoking (N, %)	128 (39.4)	325	26 (31.7)	82	0.25
Previous stroke (N, %)	62 (19.1)	325	21 (25.0)	84	0.23
Dyslipidemia (LDL level)	3.37 [3.25-3.49]	281	3.22 [2.98-3.47]	71	0.29
Acute hsCRP (mg/l)	6.15 [4.91–7.39]	324	28.8 [20.4–37.1]	84	<0.001
3-month hsCRP (mg/l)	5.02 [4.09–5.94]	318	7.12 [3.00–11.2]	73	0.052
Acute S-GCSF (pg/ml)	34.3 [28.6–40.0]	325	47.6 [32.1–63.2]	84	0.003
3-month S-GCSF (pg/ml)	29.9 [27.2–32.7]	317	29.4 [23.8–35.0]	73	0.90
S-GCSF change (pg/ml)$	−4.21 [−10.2–1.8]	317	−10.3 [−23.2–2.55]	73	0.26
NIHSS acutely	3.40 [3.02–3.78]	325	12.7 [11.4–14.0]	83	<0.001
NIHSS 3 months	1.29 [1.20–1.38]	316	6.28 [5.49–7.07]	78	<0.001

Values are presented as means and 95% CI or percentage fraction. The p-values are based on the Student's *t*-test (continuous variables), χ2 test (fractions), or the Mann–Whitney *U*-test (variables with skewed distribution; hsCRP, S-GCSF, and NIHSS). NA, not available. Abbreviations as in Table 1. $ S-GCSF change was defined as the average individual difference between acute and 3-month S-GCSF, a negative value representing a decrease.

3.2. Circulating G-CSF levels and functional outcomes 3 months and 2 years post-stroke

Next, to assess the integrity of the associations, we carried out binary logistic regression analyses, which included adjustments for multiple covariates. These analyses demonstrated that the highest quintile of acute S-GCSF was associated with an increased risk of poor functional outcome (mRS 3–6) after 3 months (S-GCSF-q5 vs. S-GCSF-q1, age- and sex-adjusted OR: 4.27, 95% CI: 1.82–9.99; p = 0.001) (Table 3A). This association withstood adjustment for cardiovascular risk factors (model 2), as well as for history of previous stroke and stroke subtype (model 3), although it lost statistical significance after an additional adjustment for initial stroke severity (model 4) (Table 3A).

Acute S-GCSF-q5 (vs. q1) showed a tendency for association with poor functional outcome (mRS 3–6) after 2 years (age- and sex-adjusted OR: 2.10, 95% CI: 1.00–4.43; p = 0.051; Table 3B, left columns), although this borderline association was attenuated by further adjustments. There was no significant association between the S-GCSF level after 3 months and poor functional outcome (Table 3A, right columns). Similarly, there were no significant differences with regard to the changes in S-GCSF and good or poor 3-month or 2-year functional outcome (data not shown).

Table 3A. Odds ratios for the risk of poor functional outcome (mRS 3–6) after 3 months in IS patients, using the lowest quintile (Q1) of S-GCSF as reference.

	OR (95% CI) for acute S-GCSF-q	p-value	OR (95% CI) for 3-month S-GCSF-q	p-value
Model 1 [A/S]				
Q2	1.63 [0.67–4.01]	0.28	1.13 [0.51–2.50]	0.77
Q3	2.05 [0.86–4.92]	0.11	1.65 [0.76–3.55]	0.20
Q4	1.81 [0.74–4.39]	0.19	0.81 [0.35–1.89]	0.63
Q5	4.27 [1.82–9.99]	0.001	1.23 [0.55–2.77]	0.61
Model 2 [A/S/C]				
Q2	1.64 [0.65–4.11]	0.30	1.21 [0.54–2.74]	0.65
Q3	2.43 [1.00–5.95]	0.051	1.76 [0.80–3.91]	0.16
Q4	1.66 [0.67–4.14]	0.27	0.87 [0.37–2.06]	0.75
Q5	3.94 [1.63–9.51]	0.002	1.33 [0.58–3.08]	0.50
Model 3 [A/S/C/H/O]				
Q2	1.81 [0.64–5.13]	0.26	1.70 [0.66–4.39]	0.27
Q3	2.65 [0.94–7.47]	0.066	2.41 [0.94–6.17]	0.066
Q4	1.74 [0.61–4.94]	0.30	1.01 [0.37–2.72]	0.99
Q5	3.27 [1.19–9.00]	0.022	1.83 [0.69–4.83]	0.22
Model 4 [A/S/C/H/O/I]				
Q2	1.15 [0.33–4.04]	0.82	2.10 [0.62–7.05]	0.23
Q3	1.96 [0.59–6.50]	0.27	2.76 [0.82–9.23]	0.10
Q4	1.03 [0.30–3.58]	0.96	1.18 [0.32–4.38]	0.80
Q5	1.57 [0.45–5.45]	0.48	2.16 [0.62–7.46]	0.22

Quintiles of acute and 3-month S-GCSF levels and 3-month poor functional outcomes of IS. The OR and corresponding 95% CI were calculated by binary logistic regression using the lowest quintile of S-GCSF (Q1) as reference. Models 1–4 are shown with successively added adjustments for: age (A); sex (S); traditional cardiovascular covariates (C); history of previous stroke (H); stroke subtype according to Oxfordshire Community Stroke Project (OCSP) [O]; and initial stroke severity (I).

Abbreviations: mRS, modified Rankin Scale; IS, ischemic stroke; S-GCSF, endogenous serum granulocyte colony-stimulating factor; OR, odds ratio; CI, confidence interval

Table 3B. Odds ratios for the risk of poor functional outcome (mRS 3–6) after 2 years in IS patients, using the lowest quintile (Q1) of S-GCSF as reference.

	OR acute S-GCSF-q	p-value	OR in 3-month S-GCSF-q	p-value
Model 1 [A/S]				
Q2	1.09 [0.50–2.38]	0.82	1.20 [0.54–2.67]	0.66
Q3	1.21 [0.56–2.60]	0.63	1.57 [0.72–3.43]	0.26
Q4	0.83 [0.37–1.87]	0.66	1.26 [0.57–2.78]	0.57
Q5	2.10 [1.00–4.43]	0.051	0.93 [0.40–2.17]	0.86
Model 2 [A/S/C]				
Q2	1.18 [0.52–2.66]	0.70	1.26 [0.55–2.88]	0.58
Q3	1.51 [0.68–3.37]	0.31	1.59 [0.71–3.56]	0.26
Q4	0.80 [0.34–1.87]	0.60	1.30 [0.58–2.95]	0.52
Q5	1.87 [0.84–4.14]	0.12	0.91 [0.38–2.20]	0.84
Model 3 [A/S/C/H/O]				
Q2	1.15 [0.48–2.74]	0.75	1.64 [0.67–3.99]	0.28
Q3	1.45 [0.62–3.40]	0.40	1.95 [0.82–4.66]	0.13
Q4	0.75 [0.30–1.86]	0.54	1.54 [0.65–3.69]	0.33
Q5	1.49 [0.64–3.46]	0.36	1.08 [0.42–2.74]	0.87
Model 4 [A/S/C/H/O/I]				
Q2	0.86 [0.34–2.20]	0.75	2.03 [0.74–5.57]	0.17
Q3	1.24 [0.50–3.04]	0.65	2.05 [0.76–5.54]	0.16
Q4	0.53 [0.20–1.41]	0.20	1.86 [0.68–5.11]	0.23
Q5	0.88 [0.34–2.23]	0.78	1.13 [0.40–3.22]	0.82

Acute and 3-month S-GCSF levels and 2-year poor functional outcomes of IS. The OR and corresponding 95% CI were calculated by binary logistic regression using the lowest quintile of S-GCSF (Q1) as reference. Models 1–4 are shown with successively added adjustments for: age (A); sex (S); traditional cardiovascular covariates (C); history of previous stroke (H); stroke subtype according to Oxfordshire Community Stroke Project (OCSP) [O]; and initial stroke severity (I). Abbreviations as in Table 3A.

4. Discussion

To our knowledge, this is the first large study that has examined the relationship between the circulating levels of S-GCSF and long-term functional outcome after IS, with the goal of resolving some of the discrepancies noted between previous studies. We found that higher S-GCSF levels in the acute stage of IS correlated with higher initial stroke severity, which is in agreement with the finding of one previous study [11]. Furthermore, patients with higher acute S-GCSF levels had worse 3-month functional outcomes, although this association lost statistical significance after adjustment for IS severity. Thus, although S-GCSF may represent a biomarker of IS severity, it is not an independent prognostic marker for functional outcome. This was confirmed by the finding of a lack of a significant association between acute S-GCSF and functional outcome after 2 years after adjustment for covariates in models 2–4. Moreover, neither the change in S-GCSF from the acute timepoint to 3 months nor the 3-month level of S-GCSF predicted functional outcome after 3 months or 2 years, which negates the prognostic benefit of following G-CSF after the acute phase. Furthermore, as there was a significant positive correlation between the S-GCSF and hsCRP levels, it seems likely that G-CSF reflects the inflammatory response to either large infarctions or possibly infections, as discussed in detail below.

4.1. Methodologic aspects

The methodologic strengths of this observational study include the consecutive recruitment, in Gothenburg and surroundings, of well-characterized IS patients aged <70 years. Another advantage is the high rate of hospitalization (84%–95%) of stroke patients in Sweden [21], which is among the highest in Europe [22]. On the one hand, the relatively young age of our participants (mean age, 57 years), as compared to the mean age of all IS patients in Sweden (approximately 76 years [23]), resulted in a high rate of follow-up, with very few dropouts and low fatality rate. On the other hand, this might reduce the generalizability of our results in older age groups with IS. Finally, the fact that few patients were lost to follow-up makes other selection biases unlikely.

As our patients were recruited between 1998 and 2003, few received thrombolytic therapy (local arterial, N = 5; intravenous, N = 0) and some of the patients received warfarin (N = 43). Another limitation is that the exact stroke volumes were not determined, although baseline stroke severity can be used as a marker of stroke lesion volumes, with correlation coefficients in the range of 0.62–0.64 [24]. Furthermore, we did not replicate our results in other populations or in different geographic areas.

Another potential limitation is that only 22% of the acute samples of G-CSF were taken within 48 hours of hospital admission, which could be important if the S-GCSF level is highly dynamic in the acute phase of stroke. In one study of 95 patients with ICH, the S-GCSF level after 72 hours was significantly higher (by 50%–54%) than it was after 24 hours or at baseline [9]. However, a comparison with baseline may be difficult to interpret given that the admission times after symptom onset could differ. Furthermore, there was no evidence of dynamic

changes in the acute S-GCSF levels when S-GCSF was measured daily for 1 week in a small series (N = 4) of IS patients, with no day-to-day differences being observed [25]. In the present study, the sampling dates for the acute samples did not significantly affect the S-GCSF levels, although the acute levels of S-GCSF in all the patients were higher than those after 3 months. These findings appear to support the notion that there are, to a moderate degree, post-stroke dynamic changes in S-GCSF levels during the acute phase of IS.

G-CSF stimulates neutrophil proliferation and mobilization. The latter effect of G-CSF has been exploited in the treatment of chemotherapy-induced neutropenia [2]. Potentially, the S-GCSF level in the bloodstream increases in response to infections. In our study, it was not recorded whether the high acute level of hsCRP was secondary to infection. However, the correlation analysis showed that the positive correlation between the acute S-GCSF and hsCRP-q in the entire cohort ($r = 0.27$, $p < 0.001$) was preserved even when the highest hsCRP-q was excluded (exclusion of hsCRP >11.8; $r = 0.25$, $p < 0.001$). This indicates that inflammation rather than infection is the major causal factor behind the observed associations.

4.2. Comparisons with previous studies of G-CSF in ischemic stroke

Following on the reported effect of G-CSF administration on reducing infarction size in ischemic stroke models [6], the lack of a clear clinical effect, as reported in a meta-analysis, came as a disappointment [7]. Furthermore, observational studies of stroke, albeit small in terms of size and power, have reported different associations between G-CSF levels and stroke severity and functional outcome. For example, a previous study of 83 patients with IS showed results discrepant from ours in terms of the relationship between S-GCSF level and functional outcome; in the previous study, higher S-GCSF levels were observed in patients who had good functional outcomes (mRS 0–2) [10]. Interestingly, this was only seen in the samples collected on Day 7 after the stroke and not in the samples collected upon admission [10], which contrasts with the findings of the present study with a considerably earlier admission sampling day (median of Day 4). Moreover, two additional studies of patients with ICH and tPA-treated patients with IS, respectively, found significantly higher levels of S-GCSF in those patients who showed good functional outcomes after 3 months [8,9]. In addition, the mean levels of acute S-GCSF in these studies were generally higher than our values (mean values for patients with good functional outcomes in the two previous studies: 614 pg/ml and 350–663 pg/ml, respectively) [8,9]. This may be due to the fact that other patient groups were studied (with higher mean age and higher initial stroke severity), and also that the studies involved patients with ICH and tPA-treated IS, which are rather different conditions to those seen in our clinical setting [8,9].

Only one of the previous studies included a healthy control group (N = 121) [11]. These controls had significantly lower S-GCSF levels than the patients with IS, suggesting that G-CSF plays an active role in the acute phase of IS [11]. Moreover, our results indicate that the levels of S-GCSF are significantly higher in patients with IS than in healthy controls, not only during the acute phase (54%) but also after 3 months (26%). Yet, comparisons with healthy controls are complicated by the fact that patients with IS have more cardiovascular risk factors that might influence their S-GCSF levels. Furthermore, the observed association between acute S-GCSF levels and more severe IS implies that G-CSF is a pre-stroke risk factor for IS. While we cannot assess these alternatives, our data primarily suggest that S-GCSF is part of the inflammation related to stroke severity. However, this does not exclude the possibility that G-CSF expressed locally in the brain has other effects that are of importance for neuroprotection or long-term repair after IS. Thus, while endogenous S-GCSF has been characterized in several contradictory observational studies, the present study of a larger cohort leads us to conclude that acute S-GCSF is associated with worse initial stroke severity, more-severe post-stroke inflammation, and worse functional outcome, whereas the changes in post-stroke S-GCSF and the 3-month S-GCSF levels lack prognostic value. As we report several significant associations, we believe the power of the study was adequate and higher than those in previous studies.

4.3. Conclusions

This observational study is considerably more comprehensive than the previously performed studies evaluating subsamples of stroke (ICH, tPA-treated IS or general IS). Our present study establishes that the acute level of S-GCSF is increased in more-severe (or large) IS and is associated with poor functional outcomes for the patients. However, the association between acute S-GCSF and poor outcome is attenuated by adjustment for initial stroke severity. This indicates that S-GCSF is primarily a component of the inflammatory response after IS and is not an independent prognostic marker of long-term functional outcome. The association of acute S-GCSF with worse functional outcome after 3 months suggests that S-GCSF is a candidate target for inhibition, at least in the bloodstream, in patients with IS. It is worth pointing out that the potential benefit of lowering the level of peripheral S-GCSF does not preclude the potential accrual of benefits from central administration (e.g. intrathecally or intracerebroventricularly) of GCSF in relation to specific stroke types. Finally, we conclude that measurements of S-GSCF in the acute post-stroke phase are of limited

value to predict functional outcome as compared with already known clinical parameters such as initial infarction size and comorbidities. In addition, several key issues remain to be resolved. Further research is needed with stricter timing of blood sampling during the acute phase of IS, to investigate the dynamics of S-GCSF in acute IS, as well as its clinical role.

Acknowledgments

The authors thank research nurse Ingrid Eriksson for her excellent work and assistance in recruiting the participants and conducting the follow-up study and Vincent Collins for language corrections.

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 the nature of information, which could compromise the privacy of research participants.

Disclosure statement

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, 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.

Funding

This study was supported by grants the Swedish State under the agreement between the Government of Sweden and the County Councils (the ALF-agreement (ALFGBG-720081, ALFGBG-147771, ALFGBG-722371, ALFGBG-715986, ALFGBG-719761, ALFGBG-751111, and ALFGBG-720931)), the Swedish Research Council (2018-02542, 2019-02543), the Swedish Heart Lung Foundation (20160316), the Swedish Stroke Association, the European Research Council (681712), Hjärnfonden, Sweden (FO2017-0243), the Torsten Söderberg Foundation, the Göteborg Foundation for Neurological Research, and the Handlanden Hjalmar Svensson (HJSV2020022), Yngve Land, Rune and Ulla Amlöv, Edit Jacobson, Magnus Bergvall, Emelle, Lars Hierta, and John and Brit Wennerström foundations; FP7 Ideas: European Research Council [681712]; STROKE-Riksförbundet [N/A].

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