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Serum erythropoietin and outcome after ischaemic stroke: a prospective study

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ABSTRACT

Objectives: Erythropoietin (EPO), which is inversely associated with blood haemoglobin (Hb), exerts neuroprotective effects in experimental ischaemic stroke (IS). However, clinical treatment trials have so far been negative. Here, in patients with IS, we analysed whether serum EPO is associated with (1) initial stroke severity, (2) recovery and (3) functional outcome.

Design: Prospective. Controls available at baseline.

Setting: A Swedish hospital-initiated study with outpatient follow-up after 3 months.

Participants: Patients (n=600; 64% males, mean age 56 years, controls n=600) were included from the Sahlgrenska Academy Study on IS (SAHLSIS).

Primary and secondary outcome measures: In addition to EPO and Hb, initial stroke severity was assessed by the Scandinavian Stroke Scale (SSS) and compared with SSS after 3 months (follow-up) as a measure of recovery. Functional outcome was evaluated using the modified Rankin Scale (mRS) at follow-up. Serum EPO and SSS were divided into quintiles in the multivariate regression analyses.

Results: Serum EPO was 21% and 31% higher than in controls at the acute phase of IS and follow-up, respectively. In patients, acute serum EPO was 19.5% higher in severe versus mild IS. The highest acute EPO quintile adjusted for sex, age and Hb was associated with worse stroke severity quintile (OR 1.70, 95% CI 1.00 to 2.87), better stroke recovery quintile (OR 1.93, CI 1.09 to 3.41) and unfavourable mRS 3–6 (OR 2.59, CI 1.15 to 5.80). However, the fourth quintile of EPO increase (from acute to follow-up) was associated with favourable mRS 0–2 (OR 3.42, CI 1.46 to 8.03). Only the last association withstood full adjustment.

Conclusions: The crude associations between EPO and worse stroke severity and outcome lost significance after multivariate modelling. However, in patients in whom EPO increased, the association with favourable outcome remained after adjustment for multiple covariates.

Strengths and limitations of this study

- A large study population including 600 patients with ischaemic stroke (IS) with initial (acute) and follow-up (3 months) evaluation.
- Six hundred population-based controls were included at baseline.
- High participation rate and structured follow-up in the Sahlgrenska Academy Study on IS (SAHLSIS) study.
- Patients with IS were well characterised in terms of multiple covariates, allowing multivariate regression analyses to determine the independency of associations.
- More early intraindividual sampling points and the addition of cerebrospinal fluid samples could have given more information regarding temporal changes in, and origin of, erythropoietin levels.

INTRODUCTION

Erythropoietin (EPO) is a peptide known to promote brain plasticity.[1 2] Circulating serum EPO originates from the kidneys and it increases net erythropoiesis by suppressing erythroid precursor apoptosis in the bone marrow to counteract anaemia.[3] EPO and EPO receptors are also expressed within the brain, being upregulated in response to ischaemic stroke (IS).[4] Additionally, increases in circulating EPO have been observed after medial cerebral artery occlusion in patients.[5] Furthermore, EPO has been shown to cross the human blood-brain barrier (for review, see ref. 1). With these findings in mind, EPO administration was shown to have positive effects in animal models of IS;[2] for review and meta-analysis.[6 7] Surprisingly, although a pilot study on 27 patients showed promising results,[5] following larger clinical trials on intravenous EPO administration[8 9] have failed to show improvement in stroke outcome. It has been proposed that the negative results in later clinical studies could have been due to an unfavourable interaction of tissue plasminogen activator and EPO in patients receiving thrombolysis,[10] as compared with the earlier studies with much fewer thrombolysed patients. Furthermore, serum EPO is inversely related to anaemia or low haemoglobin (Hb).[3] Indeed, anaemia is

associated with worse outcome after IS.[11] Therefore, it would be important to consider effects of serum EPO on IS in relation to Hb. Another factor that may be important is whether the brain injury per se may induce significant amounts of local brain EPO expression. This has been suggested by human[4] and animal studies, predominantly via astrocyte hypoxia-induced factor (HIF)-1α and more robustly HIF-2α.[12] In these mice, brain EPO expression and, to a lesser degree, circulating EPO could be altered by genetic manipulation of HIF. In humans, serum EPO levels peaked 2.6-fold at day 7 after medial cerebral artery occlusion, remaining elevated until day 30 poststroke as analysed in a subsample of nine placebo-treated patients.[5] Altogether, this adds rationale for a broader clinical investigation of both the actual serum EPO levels and changes in EPO levels after human IS. If endogenous serum EPO is independently associated with better poststroke outcome, it would support the need to revisit the timing and dosing of clinical EPO administration.

Our primary hypothesis was that high acute serum EPO levels, after correction for Hb, would promote recovery and outcome after IS. Our secondary hypothesis was that increasing serum EPO levels during the first 3 months after stroke would also promote recovery and improve outcome. We studied serum EPO in relation to stroke severity, recovery and functional outcome in a relatively large group of patients with IS from the Sahlgrenska Academy Study on IS (SAHLSIS).[13 14] In the multivariate statistical analyses, adjustments were made for Hb and C reactive protein (CRP), cardiovascular risk factors (diabetes, hypertension, smoking, body mass index (BMI), dyslipidaemia), as well as age and gender.

METHODS

Subjects and methods

The design of SAHLSIS has been reported previously[13–15] but is also compiled in more detail in online supplementary information. Briefly, a selection of 600 patients (<70 years) with serum samples available with first-ever or recurrent acute IS and matched controls was recruited consecutively at four stroke units in Western Sweden between 1998 and 2003.

The original study was thus a case–control study estimated to have reasonable power to detect genetic polymorphisms with respect to common stroke risk and stroke subtypes.[16] In the present study, case–control comparison was not used other than for comparison with baseline serum EPO. In the patient group, the design was prospective with two longitudinal sampling time points in the same cohort. Both in patients and controls, CRP, EPO and Hb were analysed in blood samples obtained between 0830 and 1030 before any morning meal from overnight fasting participants. The samples were drawn early poststroke (median 4 days), designated 'acute' and after 3 months (median 101 days). Stroke severity at inclusion was scored using the Scandinavian Stroke Scale (SSS), a scale similar to the National Institutes of Health (NIH) stroke scale, but with the highest score (58) for no clinical deficit. To facilitate statistical analysis and based on the skewed distribution of data, we divided the SSS scale into quintiles (table 1). Assessment by SSS was repeated 3 months poststroke and functional outcome was evaluated by the modified Rankin Scale (mRS). Stroke recovery was defined as the change in SSS from acute stroke to 3-month poststroke (ΔSSS quintiles, table 1). Anthropometric parameters (BMI) and data on hypertension, diabetes mellitus, smoking and low-density lipoprotein (LDL) levels were recorded (see online supplementary information). Participants or next of kin provided written informed consent.

Biochemical analysis

Serum EPO (mIU/mL) from controls (baseline only) and from patients with IS during the acute phase and at 3-month follow-up was determined using the MSD human hypoxia kit (K15122C, Meso Scale Discovery, Rockville, Maryland, USA, see online supplementary information). Hb and CRP were analysed at the Department of Clinical Chemistry at the Hospital (see online supplementary table S1).

Statistical evaluation

Statistical evaluation was performed using SPSS V.21 (SPSS Inc, Chicago, Illinois, USA). Regarding descriptive data, independent or pairwise Student t tests were used to compare the respective groups and χ^2 tests were used to evaluate differences in proportions. The change in serum EPO from the acute phase to 3-month follow-up was determined (ΔEPO). While Hb showed an approximately normal distribution, EPO was relatively skewed. Therefore, EPO and ΔEPO levels were transformed into quintiles (table 1). In figures 1A,B and 2A–C, differences in stroke severity, stroke recovery and functional outcome with respect to quintiles of EPO were analysed by analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Crude correlation analysis with unadjusted coefficients (r) was performed according to Pearson. Stroke severity (SSS quintiles) and stroke recovery (ΔSSS quintiles) were evaluated by ordinal logistic regression. Functional outcome (unfavourable, mRS 3–6 vs favourable, mRS 0–2) was evaluated by binary logistic regression. Regression yielded specific OR and 95% CIs for each quintile of EPO as a factor. To evaluate the trend (p trend), EPO quintiles were also analysed as a continuous variable. Different models of adjustments are shown with numbers (n) of complete records. Relatively few participants had missing data,[13] and possible bias was not assessed with regard to missing data. Effects of bias can, however, be deduced partly from the presentation of included observations for each model. Sensitivity analysis was not performed. Adjustments were also made

Table 1 Definitions of quintiles

Variable	Unit	New variable	Name in text
SSS	Units (0–58)	SSS quintile	SSS quintile
ΔSSS	Units (3 months-acute)	ΔSSS quintile	Stroke recovery
EPO (acute)	mIU/mL	Acute EPO quintile	Acute EPO quintile
EPO (3 months)	mIU/mL	3-month EPO quintile	3-month EPO quintile
ΔEPO	mIU/mL (3 months-acute)	ΔEPO quintile	ΔEPO quintile

Variable	Information	q1	q2	q3	q4	q5	Interpretation
SSS	Ranges	1–36	37–50	51–54	55–57	58	Low values=severe stroke
	Severity	Severe	Major	Moderate	Minor	Mild	
	n	117	126	120	109	128	
ΔSSS	Ranges	−15 to −1	0	1–3	4–10	11–53	High values=more recovery
	n	15	148	114	146	123	
EPO (acute)	Ranges	1.20–5.399	5.4–6.799	6.8–8.499	8.5–11.339	11.34–78.1	High values=more EPO
	n	95	94	103	102	98	
EPO (3-month)	Ranges	1.6–6.099	6.1–7.699	7.7–9.599	9.6–12.699	12.7–57	High values=more EPO
	n	88	91	101	95	94	
ΔEPO	Ranges	−71 to −1.60	−1.60 to 0.30	0.30–1.80	1.80–3.80	3.80–31.1	High values=increase of EPO
	n	87	91	92	87	92	

EPO, erythropoietin; SSS, Scandinavian Stroke Scale.

for the cardiovascular risk factors (see introduction) as well as for Hb and CRP. A two-tailed p value <0.05 was considered statistically significant.

RESULTS

Baseline data

Baseline demographics of controls and patients of SAHLSIS have been reported previously[13–15] and are summarised in table 2. The traditional risk factors, hypertension, diabetes and smoking, were more common in patients, whereas LDL levels and BMI did not differ. CRP levels were higher in patients than in healthy controls. The fraction of anemic patients at baseline was not different from that in healthy controls. Table 2 also presents data on these factors according to quintiles of initial stroke severity. Only the frequency of diabetes and levels of CRP were significantly higher in severe versus mild IS.

Serum EPO and Hb levels

Serum EPO was 21% higher in patients in the acute phase, and 31% higher after 3 months as compared with healthy controls (table 2). Furthermore, the 3-month serum EPO was 9% higher than the acute level (p=0.025). Acute EPO did not correlate with the time (days) of the first blood draw (r=0.036, p=0.433, n=472) and there was only a statistically non-significant increase of 5.7% from days 0–2 to 9–15 (see online supplementary figure S1). However, there was a correlation between acute EPO and CRP (r=0.14, p=0.003, n=463), a prognostic marker of infarct size.[17] There were no correlations between either acute or 3-month EPO and age (data not shown).

Acute Hb levels were higher in patients (145±1.2 g/dm^3) than in controls (139±0.9 g/dm^3, p<0.001). After 3 months, Hb levels (140±1.0 g/dm^3) had decreased as compared with the acute levels (p=0.001). There was no correlation between acute Hb and time (days) of the blood draw after IS onset (r=−0.01, p=0.8). Serum EPO and Hb were negatively correlated (r=−0.219, p<0.001, n=469). Anaemia is defined as Hb <120 in females and Hb <130 in males ((World Health Organization, WHO, 1997), and, as expected, acute EPO was higher in anemic patients as compared with those with normal Hb (13.6±3.0 vs 8.6±0.7 mIU/mL in males, and 17.5±7.6 vs 9.6±1.1 mIU/mL in females, both p≤0.001). However, the anaemia was relatively mild with a mean Hb of 123 ±2.5 g/dm^3 in males and 107±6.7 g/dm^3 in females.

Serum EPO and stroke severity and recovery

Serum EPO was 19.5% higher in severe IS as compared with mild IS during the acute phase (table 2). We next assessed associations between acute EPO levels, stroke severity and 3-month stroke recovery (ΔSSS). Quintiles of acute EPO correlated with worse initial stroke severity (figure 1A), as well as a significantly better stroke recovery after 3 months (figure 1B). There was no significant correlation between acute EPO and 3-month SSS (r=−0.056, p=0.235, n=448).

We performed ordinal logistic regressions with different models to correct for Hb, cardiovascular covariates and CRP (figure 1C–D). In the ordinal regressions

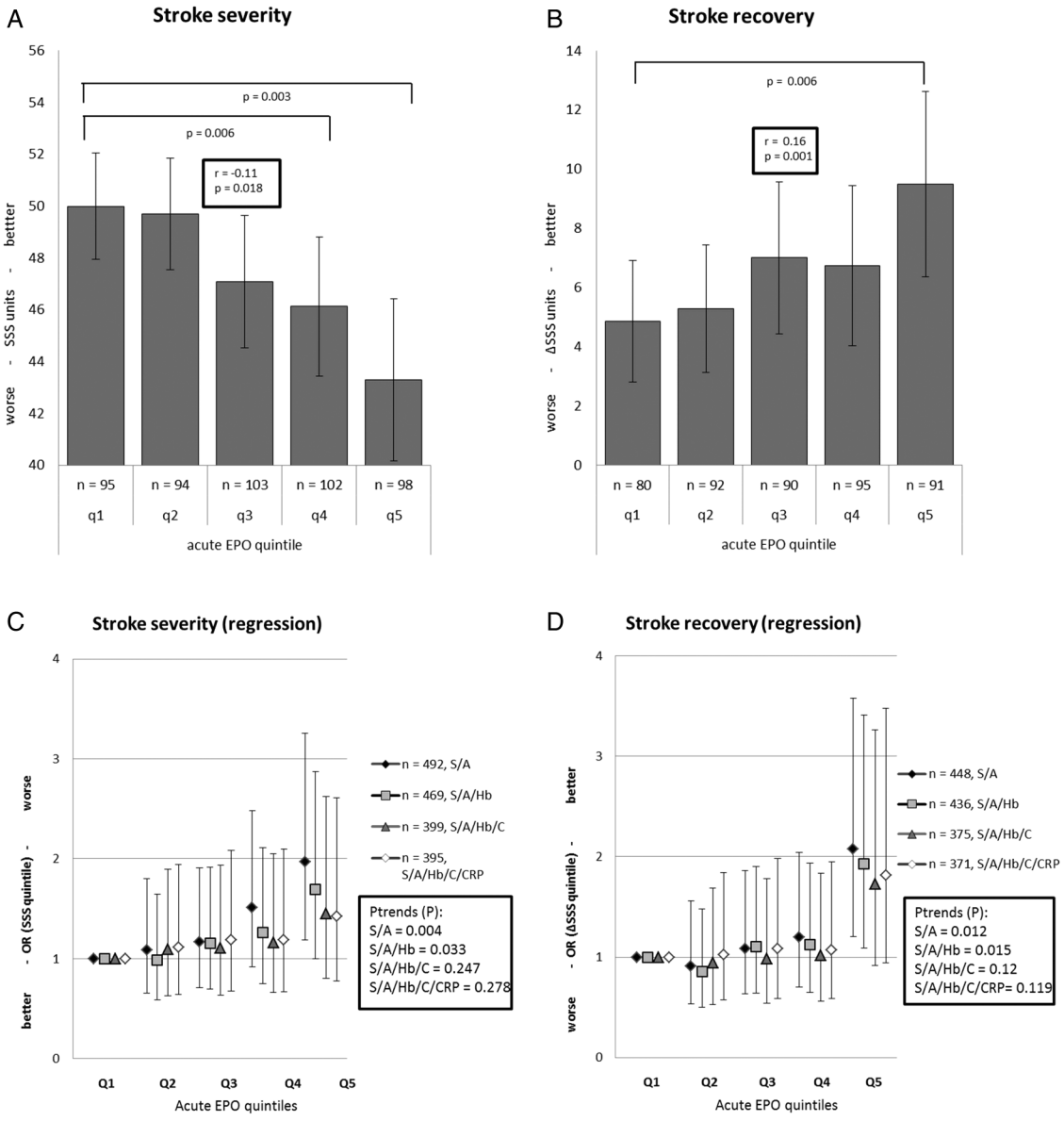

Figure 1 Stroke severity and recovery according to quintiles (q1–q5) of serum EPO during the acute phase. (A) Initial stroke severity according to the SSS units. (B) Stroke recovery (ΔSSS) after 3 months (q1 indicating deterioration and q5 improvement). (C) ORs and 95% CIs for the associations (ordinal logistic regression) between acute EPO quintiles and initial stroke severity, as measured by SSS quintiles. (D) As in panel C, but for stroke recovery (ΔSSS quintiles). For (A–D), numbers of included patients are shown above each quintile (q1–q5). Statistically significant differences between groups (as evaluated by ANOVA followed by Tukey's post hoc test) are shown with brackets. In A and B, the boxes show the overall correlation coefficients according to Pearson including p values. In C and D, the boxes show the overall association using acute EPO quintiles as a continuous variable (p trends). Different models of adjustment in which sex (S), age (A), cardiovascular factors (C) and CRP are included as indicated. ANOVA, analysis of variance; CRP, C reactive protein; EPO, erythropoietin; Hb, haemoglobin; SSS, Scandinavian Stroke Scale.

adjusted for sex and age, the association of acute EPO with worse stroke severity was confirmed (OR 1.97, CI 1.19 to 3.26 for the fifth quintile, figure 1C). However, adjustments for Hb, cardiovascular confounders and CRP weakened the associations and statistical significance was lost.

With regard to 3-month recovery (ΔSSS, figure 1D), participants in the highest quintile of acute EPO had a twofold greater chance (OR 2.08, CI 1.20 to 3.58) of having a better recovery 3 months after stroke than those in the lowest quintile. Again, associations weakened and statistical significance was lost after adjustments for multiple covariates (figure 1D).

Serum EPO and 3-month functional outcome

Next, we assessed whether serum EPO was associated with functional outcome indexed as unfavourable (mRS 3–6) and favourable (mRS 0–2). As could be expected, higher quintiles of acute EPO were associated with worse functional outcome of stroke (figure 2A).

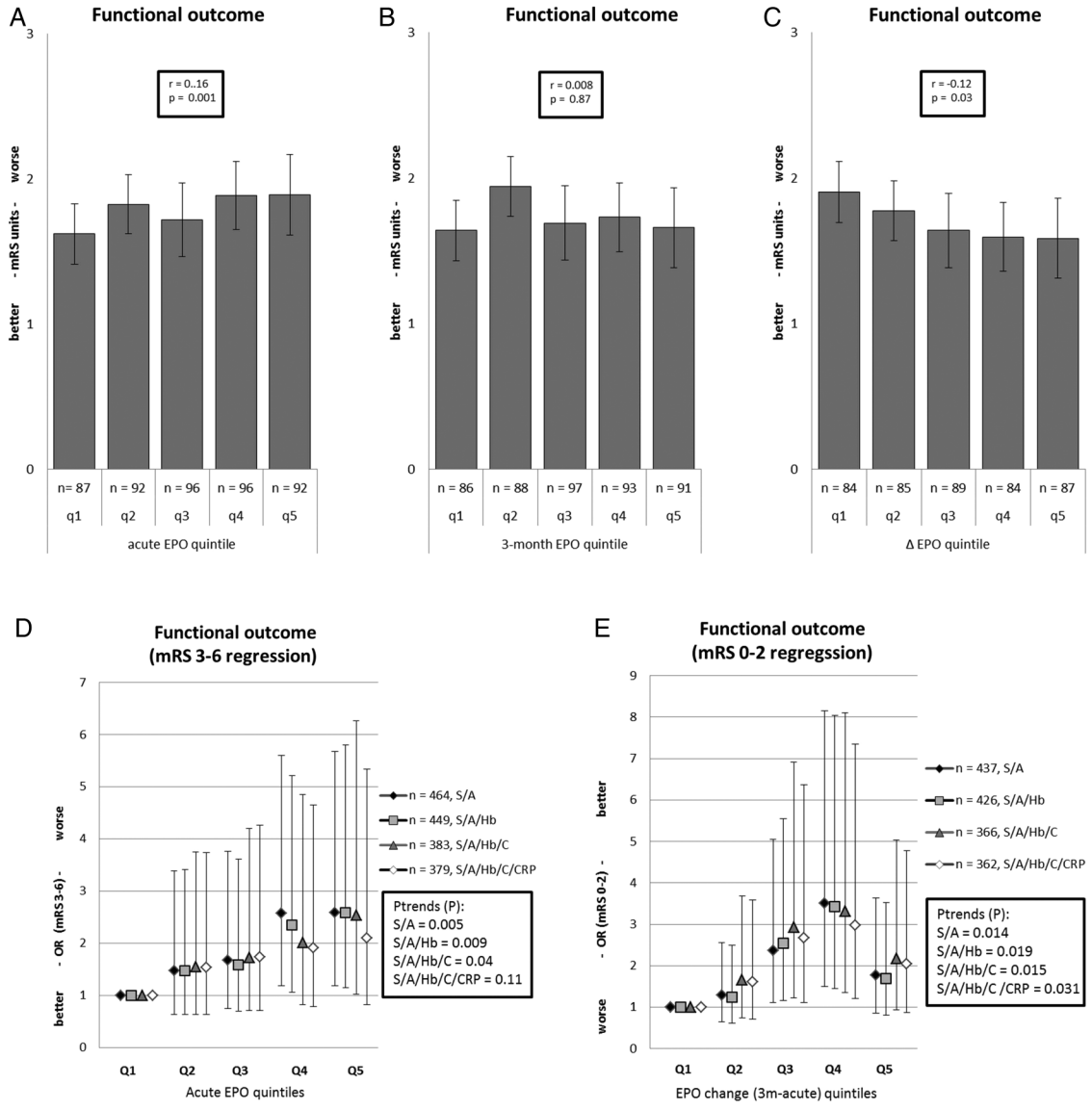

Figure 2 Functional outcome after stroke as indexed by the modified Rankin Scale (mRS) and regressions of favourable mRS according to quintiles (q1–q5) of acute serum erythropoietin (EPO), 3-month serum EPO and changes in EPO (ΔEPO). (A) Functional outcome (mRS units) according to acute EPO quintiles. (B) Functional outcome (mRS units) according to 3-month EPO quintiles. (C) Functional outcome (mRS units) according to ΔEPO quintiles. (D) ORs and 95% CIs for the associations (binary logistic regression) of mRS (values 3–6) over favourable (mRS 0–2) functional recovery according to quintiles of acute EPO. (E) Same as in D but mRS 0–2 over mRS 3–6 according to ΔEPO quintiles. Numbers of included patients are shown above each quintile (q1–q5). No statistically significant differences were found between groups as evaluated by analysis of variance. In A–C, the boxes show the overall correlation coefficients according to Pearson and the corresponding p values. In D and E, the boxes show the overall association using acute EPO quintiles as a continuous variable (p trends). Different models of adjustment with abbreviations as in 'C'.

Quintiles of EPO at 3 months, on the other hand, did not show any significant association with outcome (figure 2B). Furthermore, analyses of the change in serum EPO from the acute phase to 3-month follow-up (ΔEPO quintiles) demonstrated that in participants with increases in EPO, there were significantly more patients with better outcome (figure 2C).

Multivariate binary logistic regression analyses were next performed to evaluate the influence of serum EPO on unfavourable versus favourable mRS scores. Since serum EPO did not change significantly between different days of blood sampling in the acute phase, this factor was not added into the models. Participants in the highest acute EPO quintile showed a 2.5-fold higher risk of having an unfavourable functional outcome after 3 months (OR 2.59, CI 1.18 to 5.68, figure 2D). This was independent of Hb and cardiovascular risk factors but not of CRP. Furthermore, participants in the higher quintiles of ΔEPO (indicating increased EPO overtime) were more likely to have a better/favourable functional outcome (figure 2E). The associations remained in all models, including the addition of CRP. Of note is that

Table 2 Baseline characteristics of SAHLSIS participants and healthy controls

Variable	All patients (P) Mean±CI	n	Healthy controls (C) Mean±CI	n	P vs C p
Total number (% of all, n)	100	600	100	600	
Females (n)	215		215		
Males (n)	385		385		
Males (fraction of all)	0.64		0.64		>0.15
Age (years)	56.7 (55.9 to 57.5)	600	56.8 (56 to 57.6)	600	>0.15
BMI (kg/m^2)*	26.5 (26.1 to 26.9)	585	26.5 (26.2 to 26.8)	599	>0.15
Hypertension (fraction)*	0.6	592	0.37	599	<0.001
Diabetes (fraction)*	0.19	600	0.06	598	<0.001
Smoking (fraction)*	0.39	597	0.18	600	<0.001
Dyslipidaemia (LDL level)*	3.3 (3.2 to 3.4)	506	3.3 (3.2 to 3.4)	597	>0.15
CRP (mg/L)	12.2 (10.3 to 14.1)	563	5.9 (5.7 to 6.1)	589	<0.001
EPO (acute)	9.3 (8.7 to 9.9)	492	7.7 (7.4 to 8.0)	513	<0.001
EPO (3 months)	10.1 (9.6 to 10.6)	469	See acute values		>0.15
Anaemia (fraction)	0.072	41	0.079	47	>0.15
SSS (acutely)	47.1 (46 to 47.2)	600	NA		
SSS (3 months)	54.3 (53.7 to 55)	546	NA		
mRS (3 months)	1.9 (1.8 to 2)	568	NA		

Patients according to quintiles of severity of SSS score

Variable	Severe (1–36, A) Mean±CI	n	Major (37–50, B) Mean±CI	n	Moderate (51–54, C) Mean±CI	n	Minor (55–57, D) Mean±CI	n	Mild (58, E) Mean±CI	n	A vs E p Value
Total number (% of all, n)	19.5	117	21.0	126	20.0	120	18.2	109	21.3	128	NA
Females (n)	41		45		42		43		44		NA
Males (n)	76		81		78		66		84		NA
Males (fraction of all)	0.65		0.64		0.65		0.61		0.66		>0.15
Age (years)	55.9 (54.0 to 57.8)	117	59.3 (57.8 to 60.8)	126	56.1 (54.1 to 58.1)	120	56.6 (54.9 to 58.3)	109	55.4 (53.4 to 57.4)	128	>0.15
BMI (kg/m^2)*	26.0 (25.1 to 26.9)	111	26.9 (26.1 to 27.7)	122	27.2 (26.4 to 28.0)	119	26.5 (25.7 to 27.3)	107	26.1 (25.3 to 26.9)	126	>0.15
Hypertension (fraction)*	0.54	111	0.65	125	0.64	119	0.64	109	0.52	128	>0.15
Diabetes (fraction)*	0.2	117	0.25	126	0.21	120	0.16	109	0.14	128	0.025
Smoking (fraction)*	0.41	114	0.41	126	0.32	120	0.43	109	0.38	128	>0.15
Dyslipidaemia (LDL level)*	3.1 (2.9 to 3.3)	92	3.3 (3.1 to 3.5)	103	3.4 (3.2 to 3.6)	100	3.4 (3.2 to 3.6)	96	3.4 (3.2 to 3.6)	115	0.11
CRP (mg/L)	17.6 (12.3 to 22.9)	105	12.0 (7.9 to 16.1)	119	12.2 (7.1 to 17.2)	114	9.2 (5.0 to 13.4)	103	10.3 (7.3 to 13.3)	122	0.02
EPO (acute)	10.6 (9.4 to 11.8)	93	9.6 (7.9 to 11.3)	106	8.4 (7.7 to 9.1)	105	9.4 (7.6 to 11.2)	87	8.8 (7.6 to 10.0)	101	0.043
EPO (3 months)	9.9 (8.8 to 11.0)	81	9.5 (8.6 to 10.4)	104	10.5 (9.4 to 11.6)	103	11.0 (9.5 to 12.5)	81	10.0 (8.5 to 11.5)	100	>0.15

Continued

Table 2 Continued

Patients according to quintiles of severity of SSS score												
Variable	Severe (1–36, A) Mean±CI	n	Major (37–50, B) Mean±CI	n	Moderate (51–54, C) Mean±CI	n	Minor (55–57, D) Mean±CI	n	Mild (58, E) Mean±CI	n	A vs E p Value
Anaemia (fraction)	0.010	11	0.058	7	0.069	8	0.067	7	0.066	8	>0.15
SSS (acutely)	23.4 (21.7 to 25.1)	117	45.4 (44.8 to 46.2)	126	52.7 (52.5 to 52.9)	120	55.8 (55.6 to 55.9)	109	58 (58 to 58)	128	<0.001
SSS (3 months)	45.2 (43.3 to 47.1)	98	53.6 (52.6 to 54.6)	117	56.5 (56.2 to 56.8)	111	57.3 (57.1 to 57.5)	102	57.9 (57.9 to 58.0)	118	<0.001
mRS (3 months)	3.2 (3.0 to 3.4)	106	2.1 (1.9 to 2.1)	121	1.5 (1.4 to 1.6)	115	1.3 (1.1 to 1.5)	103	1.2 (1.1 to 1.3)	115	<0.001

Data on male sex, age, BMI, presence of diabetes, hypertension, smoking, LDL, CRP, stroke severity (SSS) and stroke outcome (mRS) are presented for patients and controls as shown.
Patients were categorised into quintiles using the SSS.
Data are shown as indicated in the columns with 95% CIs, or number (n) and percentage (%). Comparisons are made with Student t test or χ^2 tests (for fractions).
*Defined as cardiovascular risk factor.
BMI, body mass index; CRP, C reactive protein; EPO, erythropoietin; LDL, low-density lipoprotein; mRS, modified Ranking scale; NA, not applicable; SAHLSIS, Sahlgrenska Academy Study on Ischaemic Stroke; SSS, Scandinavian Stroke Scale.

quintile 4 of ΔEPO (model with full adjustment OR 2.98, CI 1.21 to 7.36) showed the largest association with favourable outcome. Although the values of acute EPO varied relatively little with respect to the day of sampling (see online supplementary figure S1), day of sampling could nevertheless affect the magnitudes of associations with respect to ΔEPO. We therefore performed an analysis using the acute samples of EPO obtained days 3–5 after IS (n=195). Statistical significance was maintained at a somewhat lower level, but the ORs were somewhat greater (see online supplementary information, results, text), supporting that the association of ΔEPO with favourable outcome was not largely obscured by the day on which the acute sample was taken.

DISCUSSION

Principal findings

This is the first large study to investigate serum EPO corrected for Hb levels in relation to human IS. After 3 months, serum EPO was higher than at the acute time point and also higher in patients than in healthy controls, indicating that EPO increased. However, in comparison to a previous smaller study,[5] in which serum EPO increased 2–3 fold with a peak at 7 days after IS, our findings indicate much smaller increases. Although there was a crude association between acute EPO and worse stroke severity and worse functional outcome, in the multivariate models, these associations weakened and lost statistical significance. Furthermore, 3-month EPO levels did not associate with functional outcome. In contrast, an increase in EPO levels between the two time points (ΔEPO) was associated with better functional outcome in all models.

Strengths and limitations

Although there is a methodological strength of this study including consecutive recruitment of well-characterised IS participants, the age of the study group is relatively young IS cases (mean age 56 years). Thus, the associations found may be different in a population of older IS participants which more often exhibits anaemia of various causes. Since the hospitalisation rate is high (84–95%) for strokes in Sweden,[18] selection bias is unlikely. Also, the study includes detailed information on cardiovascular confounders, as well as CRP, an infarction size marker,[17] and Hb levels, which is crucial to the interpretation of EPO levels.[3] Although there was a good record of most parameters, there were data missing for some patients (table 2), especially with regard to dyslipidaemia (LDL levels). Overlap of missing data generated approximately 20–25% missing observations in the final models of multivariate analysis. However, we believe that the specific bias of these missing values is minimal, especially in the light of the fact that LDL levels differed very little across the severities of IS. The study includes measurement of within-participant change (acute phase and after 3 months), which is in contrast to many studies

having sampling points within the first week after IS only, and few patients were lost to follow-up. Weaknesses include the relatively small sample size and lack of replication in another geographic area. Furthermore, regression analysis is a poor method to assess causality. More sampling points and predisease samples would have been helpful to address causal relationships. Although our study indicates relatively small acute changes in serum EPO, the previous study by Ehrenreich *et al*[5] showed larger increases. It would therefore have been preferable to have consecutive intraindividual sampling in the acute phase, in order to definitely establish how endogenous serum EPO is regulated. Finally, although EPO is able to cross the blood-brain barrier,[1 5] the proportion of EPO that actually does so is unknown. To address that issue, cerebrospinal fluid (CSF) sampling would have been preferable.

Why is there only a positive effect on outcome via increasing levels of EPO?

Acute serum EPO is associated with worse stroke severity. While acute EPO did not associate with time of blood draw (days after stroke onset), the crude association with CRP levels indicates that EPO levels may be influenced by the size of the infarct (or vice versa). The association between EPO and worse stroke severity may also reflect the fact that serum EPO is mainly a direct indicator of previous anaemia, which is a known negative factor for stroke severity and recovery.[11 19] This is indeed what we observed for initial stroke severity (SSS) and stroke recovery (ΔSSS) with regard to initial acute EPO, where statistical significance was decreased (but not lost) when adding Hb into the models. With full adjustment for cardiovascular covariates and CRP, statistical significance was, however, lost. In contrast, with regard to changes in EPO (ΔEPO), statistical significance remained in all models. Therefore, the associations between increases in serum EPO and favourable outcome are only partly explained by effects of Hb/anaemia. There are other possible bystander effects, that is, the possibility of local brain upregulation of EPO[4 5 12] in larger infarctions. Also, vigorous physical activity has been shown to increase serum EPO.[20] Our results do not allow us to discriminate between these two possibilities.

Further on, acute serum EPO was associated with better recovery 3 months after stroke, independent of low Hb but not of cardiovascular confounders or CRP (figure 1B). To some degree, this probably reflects a regression towards the mean, that is, those with greater initial injuries have a greater recovery in terms of improved SSS units. Altogether, our data reject the idea that the actual EPO levels will have a net beneficial effect on early recovery and functional independence 3 months poststroke. This would be in line with the results of a phase II/III clinical study which failed to show positive effects of intravenously given EPO within 48 h after stroke onset.[8] However, it should be pointed out that the concentrations of EPO achieved in a pilot study with similar design were much higher (~250-fold) than the endogenous circulating EPO,[5] which hampers comparison to our results. In addition, apart from the different concentrations of EPO in the mentioned clinical trial and our study, the negative results[8] were largely associated with increased secondary haemorrhagic transformations, possibly via interactions between EPO and tissue plasminogen activator.[10] In our study, haemorrhagic transformations were not recorded, but they are usually of low frequency in cohorts not being thrombolysed (thrombolysed patients were only n=5 in our study).

Interestingly, 3-month serum EPO levels were neutral with respect to association with functional outcome, while increasing EPO levels between the two time points were associated with favourable outcome. The last association firmly withstood confounder adjustments of Hb, cardiovascular factors and CRP. Therefore, it appears that changes (increases) in serum EPO are associated with better stroke outcome, which in turn preserves the idea that EPO has a positive role in human IS recovery. It might even be the case that the increase in serum EPO may derive from the local upregulation of brain EPO that has been reported after stroke injuries in autopsies 4 and in animal experiments on hypoxia 12. This suggests that individuals having a higher potential of upregulating local brain EPO, which may be reflected in elevated serum EPO, exhibit better recovery and outcome. As above, additional sampling points including analysis of CSF could elucidate this more clearly.

Implications and unanswered questions

In a well-characterised study population consisting of 600 patients with IS and 600 controls, we have shown that unadjusted serum EPO in the acute phase of IS is associated with worse stroke severity as well as with better recovery using the SSS scale. Furthermore, the increase in serum EPO (ΔEPO) from the acute phase to the 3-month follow-up was associated with favourable outcome using the mRS instrument, and the latter association was the only one that withstood full adjustment (Hb, cardiovascular confounders and CRP) in the multiple regression analyses. Therefore, our results suggest that an increasing level of EPO is a predictor of positive functional outcome after IS, although the associations between serum EPO and IS are complicated and to a large degree dependent on coexisting morbidities. Specifically, our study indicates that rather small increases in EPO concentration (fourth quintile of ΔEPO was defined as 1.9–3.8 mIU/mL, which corresponds to a relative increase of approximately 20–40% as compared with the mean acute serum EPO), in contrast to larger changes (fifth quintile), are associated with optimal outcome. In terms of the SSS scale, the differences in SSS units between acute EPO quintiles were relatively small and the associations between acute EPO quintiles and SSS measures were of moderate strength (ORs ≤2; figure 1). Furthermore, the associations

between acute EPO quintiles and SSS measures lost statistical significance when adding covariates into the models, and therefore the importance of these findings is unclear. Our study has not investigated early intraindividual (<2 to 3 weeks) temporal changes in serum EPO in relation to infarction size, giving room for further studies with more sampling points. Finally, there is also a need for studies measuring CSF EPO levels to determine whether changes in EPO are primarily originating from the brain or kidneys.

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