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RESEARCH ARTICLE

Endogenous granulocyte colony-stimulating factor: a biomarker in acute ischemic stroke

Shih-Chieh Yu[1], Chen-Ling Kuo[2], Ching-Shan Huang[2], Cheng-Shu Chang[2,3], Shey-Lin Wu[2,3], Shih-Li Su[2,4], and Chin-San Liu[2,3,5]

[1]Department of Neurology, Kuang-Tien General Hospital, Taichung, Taiwan, [2]Vascular & Genomic Center, [3]Department of Neurology, Changhua Christian Hospital, Changhua, Taiwan, [4]Division of Endocrinology and Metabolism, Department of Internal Medicine, Diabetes Education Center, Changhua, Taiwan, and [5]Graduate Institute of Integrated Medicine, China Medical University, Taichung, Taiwan

Abstract

Granulocyte colony-stimulating factor (G-CSF) may protect ischemic brain injury either in animal or human. No studies have reported that endogenous G-CSF (enG-CSF) level is related to the severity of ischemic stroke. This study was designed to assess the severity of ischemic patients correlated with the alteration of enG-CSF on the 1st day after an ischemic event. Patient's plasma enG-CSF and scoring of National Institute of Health Stroke Scale were measured on the 1st day after ischemic stroke. The acute ischemic stroke could significantly induce enG-GCF secretion as compared with healthy control group (16.77 vs. 22.86 μg/L, $p = 0.001$). Elevated enG-CSF concentration was positively correlated with the severity of stroke patients on day 1 after the event ($p = 0.006$; Spearman correlation coefficient = 0.268). The enG-CSF is a good biomarker for prediction of severity of acute ischemic stroke.

Keywords: Ischemia, neuroprotective agents, stroke recovery, endogenous granulocyte colony-stimulating factor, biomarker

Introduction

In acute ischemic stroke, the injured vessel wall produces systemic cytokines such as interleukin-1β, interleukin-6, and tumor necrosis factor α in response to injury (Rader 2000). Inflammatory markers such as high-sensitivity C-reactive protein (hs-CRP) and fibrinogen are stimulated by cytokines, and the atherosclerotic vessel walls produces soluble adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and P-selection. Growing evidence suggests that inflammatory markers can predict stroke recurrence (Vibo et al. 2007, Di Napoli et al. 2001 , Winbeck et al. 2002, Rost et al. 2001, Blum. et al. 2006, Whiteley et al. 2012, Welsh et al. 2009), volume of ischemic tissue (Youn et al. 2010, Thijs et al. 2000), and subtype diagnosis of acute ischemic stroke (Thijs et al. 2000, Tuttolomondo et al. 2010a,b). Inflammation plays an important role in the pathophysiology of acute brain ischemia (Sotgiu et al. 2006, Jefferis et al. 2009, Welsh et al. 2009, del Zoppo et al. 2000).

Endogenous granulocyte colony-stimulating factor (enG-CSF) is produced by monocytes, fibroblasts, mesothelial cells, and endothelial cells (Schneider et al. 2005, Schabitz et al. 2003). The granulocyte count will be increased during acute ischemic stroke, and the regulation of granulocyte is mediated by enG-CSF (Burgess et al. 1980), same as inflammatory marker. Human *in-vivo* studies suggest that acute ischemic stroke and acute myocardial infarction actually stimulate the endogenous release of CD34+ cells with serum levels peaking on day 2 and remaining elevated for at least 6 days after the event and then returning to the baseline (Paczkowska et al. 2005).

Upon review of the literature, it became apparent that the relationship between enG-CSF secretion and the severity of ischemic stroke had not been reported before.

Address for Correspondence: Chin-San Liu, Department of Neurology, Vascular and Genomic Research Center, Changhua Christian Hospital, 135 Nanhsiao Street, Changhua 500, Taiwan. Tel: 4752 886-4-7238595; Fax: 4063 886-4-7238595. E-mail: liu48111@gmail.com

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Our hypothesis is that human being can secrete enG-CSF during acute ischemic stroke and stimulate the endogenous release of CD34+ cells, and it raised the question whether higher plasma levels of enG-CSF are correlated with more stress of ischemic brain tissue (penumbra), whereas lower plasma levels of enG-CSF suggest less severity of ischemic penumbra.

The aim of this study was to evaluate the predictive role of enG-CSF for severity in patients with acute ischemic stroke. The expression of acute phase proteins can also be increased during acute ischemic stroke (Vibo et al. 2007, Di Napoli et al. 2001, Winbeck et al. 2002, Rost et al. 2001, Blum et al. 2006, Welsh et al. 2009, Whiteley et al. 2012), and adhesion molecular, hs-CRP, and fibrinogen were also measured and compared with the level of plasma enG-CSF.

Methods

Patients selection

Starting in March 2007, a total of 120 patients were recruited from the Kuang-Tien General Hospital, Taichung, Taiwan. Patients were included if (1) first event of stroke, (2) the clinical diagnosis was acute ischemic stroke, (3) onset was within 24 h, (4) the diagnosis was confirmed on brain computed tomography or magnetic resonance imaging (MRI), and (5) they were older than 18 years. Subjects were excluded if the diagnosis was intracranial hemorrhage, any sign of infection or inflammatory biological pattern. The National Institute of Health Stroke Scale (NIHSS) was used as stroke severity evaluation tool (Brott et al. 1989, The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group 1995). Inflammatory biomarkers evaluation was made on admission, including the measurement of plasma enG-CSF and inflammatory markers, and the NIHSS scores were recorded. We followed up the plasma level of enG-CSF, NIHSS, and modified Ranking Scale (mRS), scoring at 3 months later after acute event. Scoring was recorded by a qualified neurologist (Yu SC) with the international NIHSS certificate, who was blind to biochemical markers. The primary outcome was NIHSS scoring and its relationship to plasma enG-CSF, and other inflammatory biomarker concentrations. During the 3-month follow-up, three patients died and four patients lost follow-up.

A total of 121 subjects without cardiovascular history, without cardiovascular disease through physical examination, without any sign of infection or inflammatory biological pattern and aged ≥18 years were enrolled as our healthy control group during March 2007 and October 2009. The smoking history of the study subjects was obtained by questionnaire. Passive smoking exposure was not considered in this study, and our nonsmokers had no previous smoking experience. All of the human experimental procedures followed the ethical standards of Kuang-Tien General Hospital and was approved by the institutional review committee (KT-IRB: 9605). Informed consent for study enrollment was obtained from all subjects.

Plasma lipid profile measurement

Plasma total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were determined enzymatically as described in our previous study (Liu et al. 2005). The low-density lipoprotein (LDL) cholesterol concentration was calculated according to the formula developed by Friedewald et al. 1972.

$$LDL\ cholesterol = \frac{Total\ cholesterol - HDL\ cholesterol - Triglyceride}{5}$$

Assays for soluble ICAM-1(sICAM-1), soluble vascular VCAM-1 (sVCAM-1), and sE-selectin

The plasma concentrations of sICAM-1, sVCAM-1, and sE-selectin were assayed using commercially available enzyme-linked immunosorbent assay kits [ELISA] (R&D Systems, Minneapolis, MN, USA) in accordance with the manufacturer's instructions.

Measurement of plasma G-CSF, fibrinogen, and Hs-CRP

An ELISA assay kit (R&D Systems) was used for measuring plasma high-sensitivity G-CSF levels. Fibrinogen was measured by the Sysmex CA6000 coagulation analyzer with Dade Behring thrombin reagent (Dade Behring, Milton Keynes, UK). Intra-assay coefficients of variation were <4%. hs-CRP was measured with BN Prospec (Dade Behring). Inter-assay and intra-assay coefficients of variation were <4% and <2%, respectively, with a detection limit of 0.20 mg/L.

Measurement of stroke outcome

To evaluate the outcome after acute ischemic stroke, we used the mRS of 3 months after stroke event, absolute improvement in NIHSS (ΔNIHHS), and the relative improvement in NIHSS (Δ^{R}NIHSS) as our stroke outcome. The absolute improvement in NIHSS (ΔNIHHS) is defined as the percentage of NIHSS improvement = $NIHSS^{day\ 1} - NIHSS^{3\ months}$. The relative improvement in NIHSS (Δ^{R}NIHSS) is defined as the percentage of NIHSS improvement = $(NIHSS^{day\ 1} - NIHSS^{3\ months})/NIHSS^{day\ 1} \times 100\%$.

Statistical analyses

Because of the sample size limitation, tertile classification of NIHSS score for our patients with acute ischemic stroke included 1st tertile: lower (NIHSS 1–4), 2nd tertile: moderate (NIHSS 5–7), and 3rd tertile: higher (NIHSS 8–29) subgroups. We used analysis of variance F-test to examine the significance among NIHSS score groups, with an adjustment of age in our model. We conducted Spearman correlation coefficient (SCC) analysis to assess the linear dependence between serum biomarkers and NIHSS; multivariate linear regression analysis to assess the prediction of stroke improvement indices (ΔNIHSS/Δ^{R}NIHSS) by all potential biomarkers, age, sex, and stroke-etiology. All statistical procedures were

performed with the SPSS statistical software package (SPSS, Chicago, IL, USA). p values < 0.05 were taken to be statistically significant.

Results

The mean level of plasma enG-CSF was 16.77 μg/L in our healthy control group (data not shown) and 23.73 μg/L in acute ischemic stroke group, with p value = 0.001. Stroke severity is not normally distributed, and the severity of most ischemic stroke patients is mild to moderate. So, the NIHSS scoring of two-third patients in this study were between 1–7 points and the other one-third patients were between 8–29 points (Table 1). According to trial of ORG 10172 in acute stroke treatment (TOAST) classification (a subtype classification of acute ischemic stroke, Adams et al., 1993), we failed to find any significant correlation between enG-CSF level and each stroke subtype (data not shown). The three subgroups were made according to their NIHSS scores: 1st tertile (NIHSS 1–4), 2nd tertile (NIHSS 5–7), and 3rd tertile (NIHSS 8–29). Within these three groups, there was no significant variation in baseline demographic data (Table 1) including age, sex, body mass index, smoking index, and incidence of hypertension, hyperlipidemia, and diabetes mellitus.

Biochemical data from each NIHSS group were summarized in Table 2. There is no significant difference in blood urine nitrogen, creatinine, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, fasting sugar, fibrinogen, sE-selectin, sICAM-1, sVCAM-1, hs-CRP, white cell count (WBC), platelets, or hemoglobin across the three groups. However, only first day plasma level of enG-CSF was significantly increased in the 3rd tertile NIHSS group compared with the 1st tertile or 2nd tertile NIHSS groups (p = 0.012; Table 2). In Table 3, the plasma level of enG-CSF in first day of acute stroke was not only successfully predict the severe of stroke from the evidence of high NIHSS1st scores (p = 0.006, SCC = 0.268) but also

Table 1. Demographics in patients with acute ischemic strokes and healthy control subjects.

			Severity of ischemic stroke			
Group of patients	Healthy control subjects (n = 121)	Acute ischemic stroke (n = 120)	1st tertile (NIHSS 1–4) (n = 40)	2nd tertile (NIHSS 5–7) (n = 40)	3rd tertile (NIHSS 8–29) (n = 40)	p[a]
Age, y (M ± SD)	55 ± 10	67 ± 13	66 ± 12	67 ± 10	70 ± 13	0.062
Male (%)	55%	55%	61	66	45	0.472
BMI (kg/m^2) (M ± SD)	24 ± 2	25 ± 4	25 ± 4	25 ± 4	23 ± 3	0.312
Smoking index (M ± SD)	7 ± 13	10 ± 19	19 ± 20	20 ± 24	5 ± 14	0.866
Hyperlipidemia (%)	0	12	10	13	14	0.136
Hypertension (%)	0	88	89	91	83	0.183
Diabetes mellitus (%)	0	25	18	30	29	0.617

Abbreviations: NIHSS, National Institute of Health Stroke Scale; M ± SD, mean ± standard deviation; BMI, body mass index; Smoking index, pack per day × years.
[a]One way-ANOVA with age control.

Table 2. Alteration of serum biomarkers in different severity of ischemic stroke.

Group of patients	1st tertile (NIHSS 1-4) (n = 40)	2nd tertile (NIHSS 5–7) (n = 40)	3rd tertile (NIHSS 8–29) (n = 40)	p[a]	p[b]
Ac sugar (mg/dL)	131.16 ± 86.16	139.88 ± 77.37	166.06 ± 116.3	0.383	0.385
Creatinine (mg/dL)	1.12 ± 0.57	1.49 ± 1.31	1.19 ± 0.54	0.105	0.393
Total cholesterol (mg/dL)	181.58 ± 36.71	185.61 ± 39.29	179.38 ± 42.76	0.786	0.406
Triglyceride (mg/dL)	145.11 ± 87.69	153.12 ± 79.23	167.3 ± 168.3	0.658	0.138
HDL-C (mg/dL)	43.38 ± 11.86	43.86 ± 11.62	42.29 ± 9.03	0.848	0.267
LDL-C (mg/dL)	111.03 ± 34.07	109.25 ± 39.00	89.87 ± 38.43	0.055	0.265
RBC (×10,000/cmm)	4.46 ± 0.62	4.41 ± 0.57	4.41 ± 0.498	0.898	0.229
WBC (×1000/cmm)	7.36 ± 2.40	7.13 ± 2.47	8.31 ± 2.87	0.130	0.522
Platelet (×1000/cmm)	196.481 ± 56.54	211.76 ± 76.42	226.59 ± 89.86	0.237	0.905
sICAM-1 (mg/dL)	335.12 ± 167.20	332.04 ± 173.52	377.81 ± 165.70	0.580	0.707
sVCAM-1 (mg/dL)	604.2 ± 281.84	595.59 ± 323.04	631.97 ± 223.00	0.907	0.293
sE-seletin (mg/dL)	17.54 ± 14.55	14.15 ± 8.45	19.06 ± 11.14	0.359	0.459
Fibrinogen (mg/dL)	356.85 ± 99.08	343.26 ± 70.57	390.2 ± 88.29	0.232	0.247
hs-CRP (mg/dL)	1.08 ± 3.13	0.586 ± 0.99	0.838 ± 1.59	0.700	0.451
enG-CSF (μg/L)	21.78 ± 8.34	21.20 ± 8.54	28.21 ± 12.71	0.012*	0.029*

Abbreviations: NIHSS, National Institute of Health Stroke Scale; hs-CRP: high-sensitivity C-reactive protein; enG-CSF, endogenous granulocyte colony-stimulating factor; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1.
*p <0.05 as compared with mild or moderate group of ischemic patients by [a]multicovariance ANOVA with age control and by [b]multicovariance ANOVA with age, gender, BMI, smoking index control.

predict the NIHSS$^{3\ month}$ scores ($p = 0.047$, SCC = 0.162) which revealed the significantly determinate of the outcome of stroke event even after 3 months. Fibrinogen and WBC were also the predictors of stroke severity at day 1, the p values were 0.042 and 0.035 and the SCC were 0.209 and 0.182, respectively. Other biomarkers including WBCs, fasting sugar, platelet, hs-CRP, sICAM-1, sVCAM-1, and sE-selectin were not significantly associated; the p values were between 0.242 and 0.678 and the SCC were between –0.049 and 0.144. From Table 3, we can find that plasma enG-CSF have significant correlation ($p = 0.047$, correlation coefficient = 0.162) and other biomarkers were not significantly associated. Figure 1 shows the data of plasma enG-CSF level and NIHSS^{1st} scores of all acute ischemic stroke patients. The correlation coefficient was 0.16. We use mRS of 3 months after acute ischemic stroke as stroke outcome evaluation: mRS < 3 as good outcome and mRS ≥ 3 as bad outcome.

Table 3. Prediction of serum biomarkers to the severity of stroke at day 1 and 3 months by correlation analysis.

Variables	NIHSS$^{1st\ day}$ SCC/P	NIHSS$^{3\ month}$ SCC/P
WBC (×1000/cmm)	0.182/0.035*	0.129/0.170
Ac sugar (mg/dL)	0.077/0.459	0.107/0.264
Platelet (×1000/cmm)	0.100/0.302	0.170/0.840
Fibrinogen (mg/dL)	0.209/0.042*	0.157/0.098
hs-CRP (mg/dL)	0.055/0.639	0.136/0.163
sICAM-1 (mg/dL)	–0.049/0.678	0.125/0.299
sVCAM-1 (mg/dL)	0.144/0.242	0.114/0.364
sE-selectin (mg/dL)	0.131/0.288	0.196/0.118
enG-CSF (μg/L)	0.268/0.006*	0.162/0.047*

Abbreviations: NIHSS, National Institute of Health Stroke Scale; SCC/P, Spearman correlation coefficient/p value; hs-CRP, high-sensitive C-reactive protein; enG-CSF, endogenous granulocyte colony-stimulating factor; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1.
*$p < 0.05$.

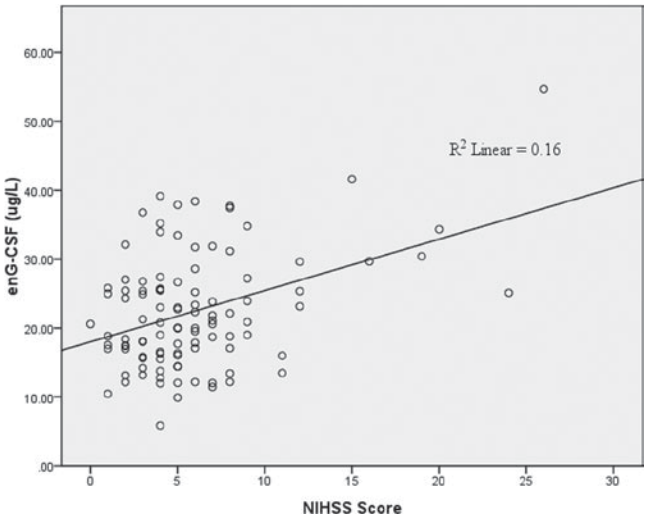

Figure 1. The linear correlation between day 1 plasma enG-CSF level and NIHSS^{1st} score of all acute ischemic stroke patients.

Figure 2 shows the correlation between three NIHSS subgroup of first day's NIHSS scoring and percentage of good outcome (mRS < 3 in 3 months later evaluation). The percentage of good outcome in the three subgroups were 93.62% (1st tertile), 87.50% (2nd tertile), and 42.31% (3rd tertile) ($p < 0.05$ as 1st tertile *vs.* 3rd tertile and 2nd tertile *vs.* 3rd tertile subgroup).

As shown in Table 4, only enG-CSF was significantly and negatively correlated with the absolute and relative improvement in NIHSS (β = –0.115 and –0.331; $p = 0.0002$ and 0.027). The fasting sugar, fibrinogen, age, and sex could not be predictive of outcome after acute ischemic stroke. Initial NIHSS could be significantly and negatively correlated with the relative improvement of NIHSS (β = –3.153; $p = 0.003$) but not for absolute improvement of NIHSS.

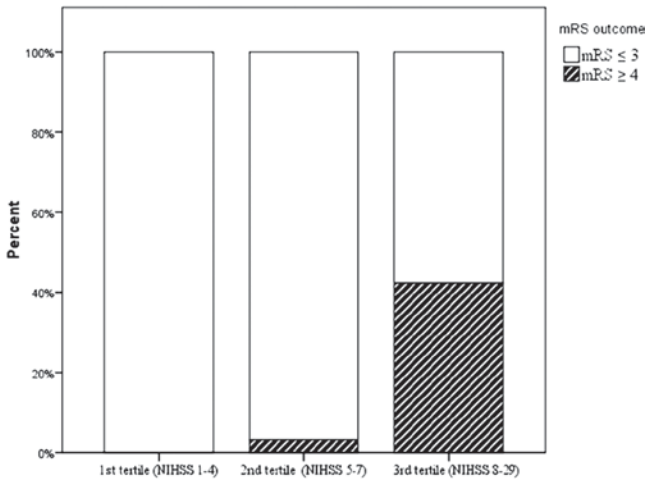

Figure 2. The percentage of good outcome (mRS < 3) in 3 months later evaluation in three subgroups of NIHSS scoring in first day according to tertile methods of SPSS software. *$p < 0.05$. The percentage of good outcome in the three subgroups were 93.62% (1st tertile), 87.50% (2nd tertile), and 42.31 % (3rd tertile) ($p < 0.05$ as 1st tertile *vs.* 3rd tertile and 2nd tertile *vs.* 3rd tertile subgroup).

Table 4. Prediction of stroke improvement indices (ΔNIHSS/Δ^{R}NIHSS) by all potential biomarkers, age, sex, and stroke-etiology with multivariate linear regression analysis.

Dependent variable: Index of outcome improvement	ΔNIHSS (the absolute improvement)		Δ^{R}NIHSS (the relative improvement)	
	β	P	β	P
enG-CSF (μg/L)	–0.115	0.0002*	–0.331	0.027*
Fibrinogen (mg/dL)	0.267	0.131	0.217	0.164
Ac sugar (mg/dL)	0.097	0.481	0.122	0.314
Age	0.079	0.590	1.230	0.225
Sex	0.045	0.742	0.022	0.858
NIHSS (1st day after stroke)	0.178	0.261	–3.153	0.003*

$\Delta NIHHS = NIHSS^{day\ 1} - NIHSS^{3\ months}$.
$\Delta^{R}NIHSS = (NIHSS^{day\ 1} - NIHSS^{3\ months})/NIHSS^{day\ 1} \times 100\%$.
Abbreviations: NIHSS, National Institute of Health Stroke Scale; β/P, regression coefficient/p value; enG-CSF, endogenous granulocyte colony-stimulating factor.
*$p < 0.05$.

Discussion

Our data suggest that acute ischemic stroke can significantly induce enG-CSF secretion, and plasma enG-CSF concentration during acute ischemic stroke may be used to predict severity of clinical stroke at admission.

enG-CSF, an inflammatory marker, is secreted by monocytes, fibroblasts, mesothelial cells, and endothelial cells involved in the acute stress response. In clinical practice, severity fluctuation and evolution are frequently seen during acute phase of ischemic stroke (Jongbloed 1986, Chambers et al. 1987, Johnston et al. 2000), which makes outcome prediction difficult. Our finding of enG-CSF level may be clinically useful and improve the predictive value of clinical measures such as NIHSS. If the NIHSS scoring is lower but the plasma level of enG-CSF is higher, this mismatch may be a warning sign to clinical physicians that the patient's brain may have more ischemic stress than present clinical evaluation, and they should be observed for stroke-in-evolution.

In this study, various inflammatory markers were assessed for the prediction of stroke severity, but we failed to reveal any significant change including fibrinogen, sICAM-1, and sVCAM-1 except enG-CSF . Therefore, this study suggests that plasma levels of enG-CSF are a highly sensitive predictive biomarker in the prediction of stroke severity, but not the fasting sugar, hs-CRP, fibrinogen, sE-selection, sICAM-1, and sVCAM-1. In previous studies of hs-CRP and acute ischemic stroke, hs-CRP is an independent marker for the risk of ischemic stroke and prognosis after stroke (Vibo et al. 2007, Di Napoli et al. 2001, Winbeck et al. 2002, Rost et al. 2001, Blum et al. 2006, Welsh et al. 2009, Whiteley et al. 2012). But in this study, hs-CRP was not a sensitive biomarker for stroke severity. In this study, we did not find the significance between enG-CSF and hsCRP, which was a powerful and well-known predictor in the process of atherosclerosis but not in the prediction of stroke severity (data not shown). Thus, the plasma level of enG-CSF can be considered as a response cytokine, other than hsCRP, during the stroke event.

The higher NIHSS scoring data were successful in the prediction of poor outcome of stroke patient with a high score of mRS (Figure 2), and higher enG-CSF had significantly decreased improvement of NIHSS between 1st day and 3 months after a stroke event (Table 4). Accumulating evidence suggests that subcutaneous injection of exogenous G-CSF (exG-CSF) to an occluded middle cerebral artery in recent rodent models of ischemic stroke both reduced infarction volume in the hyperacute stage and enhanced functional recovery in subsequent subacute stages (Zhao et al. 2006, Shyu et al. 2004, Kawada et al. 2006). Some authors report that higher PB-MNC-CD34+ counts in high responders of en-GCSF had good outcome and better recovery than low responders of en-GCSF in a small case-control prospective study of human stroke (Dunac et al. 2007, England et al. 2012). In a small randomized control clinical trial, the exogenous use of G-CSF within the first week following an ischemic stroke led to a better prognosis and functional outcome than those in the placebo group (Shyu et al. 2006).

During stroke, higher plasma level of enG-CSF did not indicate better short-term outcome. Why did endogenous plasma enG-CSF not yield a neuroprotective effect in our study? We think there may be at least two possible reasons. First, plasma enG-CSF concentrations do not reflect the local concentrations in the brain (Schneider et al. 2005). Second, in the murine models, the given dose in exG-CSF is around 40–60 μg/kg. However, in this study, the elevation in enG-CSF was only approximately 7 μg/dL. Self-production of enG-CSF may be inadequate for mobilization of CD34+ cell to ischemic brain tissue for neogenesis.

If our data is valid, the mismatch of initial NIHSS scoring and plasma level of enG-CSF (low NIHSS score and high enG-CSF) will raise a red flag to clinical neurologist: "the patient is at risk stroke progression and need for more aggressive monitor and treatment".

In future studies, we plan to recruit more number of stroke patients for evaluating the relationship among enG-CSF level, MRI-infarction volume, and stroke subtype.

Conclusion

In conclusion, enG-CSF level during acute ischemic stroke may be a good biochemical marker of stroke severity. However, our finding needs further studies to confirm its validity. In the future, we are planning to increase the case number and investigate more deeply the causal relationship or mechanism between enG-CSF generation and neuron repair/damage.

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Declaration of interest

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References

Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE 3rd. (1993) Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke* 24:35-41.

Blum A, Khazim K, Merei M, Peleg A, Blum N, Vaispapir V. (2006). The stroke trial - can we predict clinical outcome of patients with ischemic stroke by measuring soluble cell adhesion molecules (CAM)? *Eur Cytokine Netw* 17:295–298.

Brott T, Adams HP Jr, Olinger CP, Marler JR, Barsan WG, Biller J, Spilker J, Holleran R, Eberle R, Hertzberg V. (1989). Measurements of acute cerebral infarction: a clinical examination scale. *Stroke* 20:864–870.

Burgess AW, Metcalf D. (1980). Characterization of a serum factor stimulating the differentiation of myelomonocytic leukemic cells. *Int J Cancer* 26:647–654.

Chambers BR, Norris JW, Shurvell BL, Hachinski VC. (1987). Prognosis of acute stroke. *Neurology* 37:221–225.

del Zoppo G, Ginis I, Hallenbeck JM, Iadecola C, Wang X, Feuerstein GZ. (2000). Inflammation and stroke: putative role for cytokines, adhesion molecules and iNOS in brain response to ischemia. *Brain Pathol* 10:95–112.

Di Napoli M, Papa F, Bocola V. (2001). Prognostic influence of increased C-reactive protein and fibrinogen levels in ischemic stroke. *Stroke* 32:133–138.

Di Napoli M, Papa F, Bocola V. (2001). C-reactive protein in ischemic stroke: an independent prognostic factor. *Stroke* 32: 917–924.

Dunac A, Frelin C, Popolo-Blondeau M, Chatel M, Mahagne MH, Philip PJ. (2007). Neurological and functional recovery in human stroke are associated with peripheral blood CD34+ cell mobilization. *J Neurol* 254:327–332.

England TJ, Abaei M, Auer DP, Lowe J, Jones DR, Sare G, Walker M, Bath PM. (2012). Granulocyte-colony stimulating factor for mobilizing bone marrow stem cells in subacute stroke: the stem cell trial of recovery enhancement after stroke 2 randomized controlled trial. *Stroke* 43:405–411.

Friedewald WT, Levy RI, Fredrickson DS. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18:499–502.

Jefferis BJ, Whincup PH, Welsh P, Wannamethee SG, Rumley A, Lennon LT, Thomson AG, Carson C, Ebrahim S, Lowe GD. (2009). Circulating TNFalpha levels in older men and women do not show independent prospective relations with MI or stroke. *Atherosclerosis* 205:302–308.

Jongbloed L. (1986). Prediction of function after stroke: a critical review. *Stroke* 17:765–776.

Johnston KC, Connors AF Jr, Wagner DP, Knaus WA, Wang X, Haley EC Jr. (2000). A predictive risk model for outcomes of ischemic stroke. *Stroke* 31:448–455.

Kawada H, Takizawa S, Takanashi T, Morita Y, Fujita J, Fukuda K, Takagi S, Okano H, Ando K, Hotta T. (2006). Administration of hematopoietic cytokines in the subacute phase after cerebral infarction is effective for functional recovery facilitating proliferation of intrinsic neural stem/progenitor cells and transition of bone marrow-derived neuronal cells. *Circulation* 113:701–710.

Liu CS, Chiang TH, Kuo CL, Ou CC, Lii CK, I SH., Wei YH, Chen HW. (2005). Contribution of plasma folic acid and homocysteine levels to the mean carotid intima media thickness in smokers. *Nutr Res* 25: 835–845.

Paczkowska E, Larysz B, Rzeuski R, Karbicka A, Jalowinski R, Kornacewicz-Jach Z, Ratajczak MZ, Machalinski B. (2005). Human hematopoietic stem/progenitor-enriched CD34(+) cells are mobilized into peripheral blood during stress related to ischemic stroke or acute myocardial infarction. *Eur J Haematol* 75:461–467.

Rader DJ. (2000). Inflammatory markers of coronary risk. *N Engl J Med* 343:1179–1182.

Rost NS, Wolf PA, Kase CS, Kelly-Hayes M, Silbershatz H, Massaro JM, D'Agostino RB, Franzblau C, Wilson PW. (2001). Plasma concentration of C-reactive protein and risk of ischemic stroke and transient ischemic attack: the Framingham study. *Stroke* 32:2575–2579.

Schneider A, Krüger C, Steigleder T, Weber D, Pitzer C, Laage R, Aronowski J, Maurer MH, Gassler N, Mier W, Hasselblatt M, Kollmar R, Schwab S, Sommer C, Bach A, Kuhn HG, Schäbitz WR. (2005). The hematopoietic factor G-CSF is a neuronal ligand that counteracts programmed cell death and drives neurogenesis. *J Clin Invest* 115:2083–2098.

Schäbitz WR, Kollmar R, Schwaninger M, Juettler E, Bardutzky J, Schölzke MN, Sommer C, Schwab S. (2003). Neuroprotective effect of granulocyte colony-stimulating factor after focal cerebral ischemia. *Stroke* 34:745–751.

Shyu WC, Lin SZ, Yang HI, Tzeng YS, Pang CY, Yen PS, Li H. (2004). Functional recovery of stroke rats induced by granulocyte colony-stimulating factor-stimulated stem cells. *Circulation* 110:1847–1854.

Shyu WC, Lin SZ, Lee CC, Liu DD, Li H. (2006). Granulocyte colony-stimulating factor for acute ischemic stroke: a randomized controlled trial. *CMAJ* 28;174:927–933.

Sotgiu S, Zanda B, Marchetti B, Fois ML, Arru G, Pes GM, Salaris FS, Arru A, Pirisi A, Rosati G. (2006). Inflammatory biomarkers in blood of patients with acute brain ischemia. *Eur J Neurol* 13:505–513.

The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. (1995). Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med* 333:1581–1587.

Thijs VN, Lansberg MG, Beaulieu C, Marks MP, Moseley ME, Albers GW. (2000). Is early ischemic lesion volume on diffusion-weighted imaging an independent predictor of stroke outcome? A multivariable analysis. *Stroke* 31:2597–2602.

Tuttolomondo A, Di Raimondo D, Pecoraro R, Serio A, D'Aguanno G, Pinto A, Licata G. (2010a). Immune-inflammatory markers and arterial stiffness indexes in subjects with acute ischemic stroke. *Atherosclerosis* 213:311–318.

Tuttolomondo A, Di Sciacca R, Di Raimondo D, Serio A, D'Aguanno G, Pinto A, Licata G. (2010b). Arterial stiffness indexes in acute ischemic stroke: relationship with stroke subtype. *Atherosclerosis* 211:187–194.

Vibo R, Kõrv J, Roose M, Kampus P, Muda P, Zilmer K, Zilmer M. (2007). Acute phase proteins and oxidised low-density lipoprotein in association with ischemic stroke subtype, severity and outcome. *Free Radic Res* 41:282–287.

Winbeck K, Poppert H, Etgen T, Conrad B, Sander D. (2002). Prognostic relevance of early serial C-reactive protein measurements after first ischemic stroke. *Stroke* 33:2459–2464.

Welsh P, Barber M, Langhorne P, Rumley A, Lowe GD, Stott DJ. (2009). Associations of inflammatory and haemostatic biomarkers with poor outcome in acute ischaemic stroke. *Cerebrovasc Dis* 27:247–253.

Whiteley W, Wardlaw J, Dennis M, Lowe G, Rumley A, Sattar N, Welsh P, Green A, Andrews M, Sandercock P. (2012). The use of blood biomarkers to predict poor outcome after acute transient ischemic attack or ischemic stroke. *Stroke* 43:86–91.

Zhao Y, Luo YM, Xiao BG, Lu CZ. (2006). Fibronectin and neuroprotective effect of granulocyte colony-stimulating factor in focal cerebral ischemia. *Brain Res* 1098:161–169.

Youn CS, Choi SP, Kim SH, Oh SH, Jeong WJ, Kim HJ, Park KN. (2012). Serum highly selective C-reactive protein concentration is associated with the volume of ischemic tissue in acute ischemic stroke. *Am J Emerg Med* 30:124–128.