Low Circulating Acute Brain-Derived Neurotrophic Factor Levels Are Associated With Poor Long-Term Functional Outcome After Ischemic Stroke

Tara M. Stanne, PhD; N. David Åberg, MD, PhD; Staffan Nilsson, PhD; Katarina Jood, MD, PhD; Christian Blomstrand, MD, PhD; Ulf Andreasson, PhD; Kaj Blennow, MD, PhD; Henrik Zetterberg, MD, PhD; Jörgen Isgaard, MD, PhD; Johan Svensson, MD, PhD; Christina Jern, MD, PhD

Background and Purpose—Brain-derived neurotrophic factor (BDNF) plays important roles in brain plasticity and repair, and it influences stroke outcomes in animal models. Circulating BDNF concentrations are lowered in patients with traumatic brain injury, and low BDNF predicts poor recovery after this injury. We sought to investigate whether circulating concentrations of BDNF are altered in the acute phase of ischemic stroke and whether they are associated with short- or long-term functional outcome.

Methods—Serum concentrations of BDNF were measured in the Sahlgrenska Academy Study on Ischemic Stroke. The main outcomes were modified Rankin Scale (mRS) good (mRS score of 0–2) versus poor (mRS score of 3–6) at 3 months and 2 years after stroke, and good (mRS score of 0–2) versus poor (mRS score of 3–5) at 7 years after stroke.

Results—Acute concentrations of BDNF were significantly lower in ischemic stroke cases (n=491) compared with controls (n=513). BDNF concentrations were not significantly associated with 3-month outcome. However, patients with BDNF in the lowest tertile had an increased risk of experiencing a poor outcome both at 2-year and 7-year follow-up, and these associations were independent of vascular risk factors and stroke severity (odds ratio, 2.6; confidence intervals, 1.4–4.9; P=0.002 and odds ratio, 2.1; confidence intervals, 1.1–3.9; P=0.028, respectively).

Conclusions—Circulating concentrations of BDNF protein are lowered in the acute phase of ischemic stroke, and low levels are associated with poor long-term functional outcome. Further studies are necessary to confirm these associations and to determine the predictive value of BDNF in stroke outcomes. (Stroke. 2016;47:1943-1945. DOI: 10.1161/STROKEAHA.115.012383.)

Key Words: brain-derived neurotrophic factor | pathogenesis | risk factors | serum | stroke

Brain-derived neurotrophic factor (BDNF) has a role in neurogenesis and influences functional motor recovery after an ischemic brain lesion in animal models. Recently, the prognostic value of circulating BDNF levels has received attention in some brain disorders, including traumatic brain injury and poststroke depression. Acute serum concentrations of BDNF predicted severity and outcome of a traumatic brain injury, and patients with the lowest BDNF concentrations had the highest odds of incomplete recovery. Similarly, patients with stroke who developed poststroke depression had low admission levels of serum BDNF. No study has yet looked at acute BDNF protein levels in relation to functional outcome after ischemic stroke (IS).

Levels of circulating BDNF correlate with several vascular risk factors, and low BDNF concentrations have recently been found to associate with an increased risk of incident stroke/transient ischemic attack. We hypothesized that circulating BDNF concentrations are lowered in the acute phase of IS and that low BDNF concentrations associate with poor short- (3 months) or long-term (2 and 7 years) functional outcome after stroke.

Methods

Additional details can be found in the online-only Data Supplement.

Study Population, BDNF, and Outcome Measurements

Participants were from the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS), which includes patients and controls aged 18 to 69 years, the design of which has been reported. Serum samples...
were collected from 514 patients in the acute phase and from 514 matched controls. BDNF levels were measured using the BDNF E₄₄ ImmunoAssay System (Promega, Madison, WI). Functional outcome was assessed by the modified Rankin Scale (mRS). During the first month after IS, death is often due to stroke-related complications and can be hypothesized to associate with recovery. Therefore, outcome was defined as good (mRS score of 0–2) versus poor (mRS 3–6) at the 3-month time point and to take a conservative approach also at the 2-year time point. With regard to recovery in the long-term perspective, there are many confounding causes of death, such as malignancies, diabetes mellitus and cardiac causes. Thus, for the 7-year follow-up, good versus poor outcome was defined as mRS score of 0 to 2 versus 3 to 5, respectively.

Statistical Analysis

Associations between BDNF and IS were investigated using unconditional logistic regression analysis. Model A was adjusted for age and sex, Model B adjusted for traditional risk factors. CE indicates cardioembolic stroke; Crypt, cryptogenic stroke; LVD, large vessel disease; and SVD, small vessel disease. It can be hypothesized to associate with recovery. Therefore, outcome was defined as good (mRS score of 0–2) versus poor (mRS 3–6) at the 3-month time point and to take a conservative approach also at the 2-year time point. With regard to recovery in the long-term perspective, there are many confounding causes of death, such as malignancies, diabetes mellitus and cardiac causes. Thus, for the 7-year follow-up, good versus poor outcome was defined as mRS score of 0 to 2 versus 3 to 5, respectively.

Baseline characteristics for SAHLSIS have been described previously. Acute serum concentrations of BDNF were significantly lower in patients with IS compared with controls (geometric mean 18.3 [95% confidence intervals, 17.0–19.7] ng/mL versus 23.9 [95% confidence intervals, 23.1–24.8] ng/mL; P<0.001). In multivariable analyses, lower BDNF was independently associated with IS and each of the 4 main pathogenic subtypes (Figure 1).

In the analysis of functional outcome, patients in the lowest tertile of BDNF (T1) were compared with patients in the 2 upper tertiles (T2+T3). The analysis of cutoffs for BDNF and the distribution of clinical characteristics based on BDNF tertiles are presented in the online-only Data Supplement (Results section and Table I in the online-only Data Supplement). Participants in T1 were significantly younger, had higher triglyceride levels, higher prevalence of atrial fibrillation, lower low-density lipoprotein cholesterol, lower blood pressure, and lower prevalence of hypertension.

The majority of patients who experienced poor outcome was in T1 (Figure 2A). In regression analyses, BDNF concentrations were not associated to functional outcome in the short-term (Figure 2B). However, low BDNF concentrations were associated with poor functional outcome both at 2- and 7-year follow-up, and these associations were independent of stroke severity and traditional risk factors (Figure 2B). When patients who died were included in the 7-year analysis the association was attenuated (Model A: odds ratio, 1.51 [1.00–2.32]; P=0.051 and Model C: odds ratio, 1.58 [0.96–2.60]; P=0.069). The additional predictive value of BDNF was next measured over clinical parameters by c-statistics, IDI, and NRI. Addition of baseline BDNF yielded marginal improvement in the clinical risk prediction models in all 3 measures of discrimination at both 2- and 7-year follow-up (2-year: c-statistic, 0.838 [0.795 to 0.883] to 0.848 [0.806 to 0.892]; P=0.311, Figure SII in the online-only Data Supplement; IDI, 1.3% [0 to 2.5]; P=0.035 and NRI, 18.9% [-3.0 to 40.8]; P=0.091 and 7-year: c-statistic, 0.811 [0.754 to 0.867] to 0.820 [0.765 to 0.875]; P=0.347, Figure SII in the online-only Data Supplement; IDI, 1.4% [-0.2 to 3.0]; P=0.099 and NRI, 21.3% [-5.0 to 47.5]; P=0.112).

Figure 2. A. Percentage of patients in each brain-derived neurotrophic factor (BDNF) tertile with good (modified Rankin Scale [mRS] score of 0–2) or bad functional outcome (defined as mRS score of 3–6 at 3-month and 2-year follow-up and mRS score of 3–5 at 7-year follow-up). B. Odds ratios and 95% confidence intervals for the association of BDNF protein levels (T1 vs T2–T3) with poor functional outcome at 3 months, 2 years, and 7 years post stroke. *P<0.05 compared with good outcome after stroke. †P<0.01.
Discussion

Here, we report that circulating concentrations of BDNF are lower in the acute phase of IS compared with healthy controls. Low BDNF concentrations have been observed in patients with metabolic syndrome, atrial fibrillation, and acute coronary syndromes. Furthermore, low concentrations of BDNF were recently demonstrated to be associated with an increased risk of incident stroke/transient ischemic attack when adjusting for age, sex, and traditional risk factors. Although these studies support our findings, the mechanism of decreased serum BDNF in IS requires further study.

We also report that low acute concentrations of BDNF are associated with an increased risk of suffering poor functional outcome 2 and 7 years after IS. These findings are in line with a study on traumatic brain injury that assessed recovery after 6 months. However, in this study there was no significant association between BDNF and 3-month outcome. One potential explanation may be that for several impairments, such as aphasia, recovery continues much beyond 3 months. In the 7-year analysis, when patients who died were included, the association was attenuated and no longer significant. However, death in the long-term prospective is because of many different causes, and it is reasonable to assume that many of these do not associate with recovery.

Although the logistic regression models showed that BDNF was an independent predictor of poor functional outcome 2 and 7 years post stroke, the additional predictive value of BDNF was modest over clinical data in terms of c-statistic, IDI, and NRI. This suggests that BDNF on its own may have limited clinical use as a prognostic biomarker. Provided replication in other studies, it would be of interest in the future to evaluate BDNF together with other biomarkers related to recovery in a multimarker panel.

In conclusion, in this study low acute concentrations of BDNF are associated with poor long-term functional outcome after IS, and further studies are necessary to seek replication.

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Disclosures

None.

References

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Author affiliations:
1The Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden
2Chalmers University of Technology, Mathematical Sciences, Gothenburg, Sweden
3UCL Institute of Neurology, Queen Square, London WC1N 3BG, United Kingdom

Corresponding author*: Tara M. Stanne, Institute of Biomedicine, Department of Medical and Clinical Genetics, Box 445, Gothenburg SE-40530. Tel: +46-31-343 65 26, Fax: +46-31-842 160, E-mail: tara.stanne@gu.se

Cover title: BDNF levels are associated with stroke outcome

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SUPPLEMENTAL METHODS

Study population
The study population comprised participants in the case-control study the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS), the design of which has been reported. Briefly, Caucasians aged 18-69 years (n=600) who presented with ischemic stroke (IS) were recruited consecutively at four stroke units in western Sweden. Controls (n=600) included in SAHLSIS were matched to cases for age (+/- 1 year), sex and geographic area of residence. The controls were randomly selected from the community during the same time period as the cases. All patients underwent neuroimaging. Initial stroke severity was assessed with the Scandinavian Stroke Scale (SSS) on admission. Assessment was performed by a physician trained in stroke medicine. Patients were classified by the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria into the IS etiologic categories large-vessel disease (LVD), small-vessel disease (SVD), cardioembolic (CE) stroke, cryptogenic stroke, other determined cause of stroke, and undetermined stroke, using a local specified protocol as described previously. Cryptogenic stroke was defined for cases in which no cause was identified despite extensive investigation. The undetermined stroke group included patients for whom more than one cause was identified or for whom the evaluation was cursory (n=92). All participants or their next-of-kin gave written informed consent. The study was approved by the Ethics Committee of the University of Gothenburg and performed in accordance with institutional guidelines.

Blood sampling
The isolation of serum was included in the SAHLSIS protocol from patient 86 and onwards. Therefore, serum samples were collected for n=514 patients and matching controls. Among cases, blood was collected at two time points as described previously: within 10 days of the index stroke event (median 4 days; acute-phase samples); at 3-month follow-up (median 101, range 85–125 days; convalescent-phase samples). For the controls, blood sampling was performed once. On both occasions, as well as in controls, venous blood was collected between 08:30 and 10:30 AM after an overnight fast. Sera were isolated within 2 hours by centrifugation 2000 × g at 4 °C for 20 minutes, aliquotted, and stored at −80 °C.

BDNF antigen measurement
BDNF levels were measured with an ELISA kit (BDNF Emax® ImmunoAssay System; Promega, WI, USA), according to the manufacturer’s specifications. Serum from stroke patients at the various time points and the matched controls were tested concurrently on the same microtiter plate. BDNF measurements were carried out by a single operator using the same instrumentation and reagents throughout. For the present study, 491 acute phase, 470 3-month follow-up and 513 control samples were available for analysis.
Definition of vascular risk factors
Hypertension was defined by pharmacological treatment for hypertension, systolic blood pressure (SBP) ≥ 160 mm Hg, and/or diastolic blood pressure (DBP) ≥ 90 mm Hg. Diabetes mellitus was defined by diet or pharmacological treatment, fasting plasma glucose ≥ 7.0 mmol/L, or fasting blood glucose ≥ 6.1 mmol/L. Hyperlipidemia was defined as pharmacological treatment, total fasting serum cholesterol level > 5.0 mmol/L, and/or LDL > 3.0 mmol/L. Smoking habit was coded as current versus never or former (smoking cessation at least one year before inclusion in the study). Information regarding vascular risk factors was registered once in controls and at the 3-month follow-up in patients.

Functional outcome, mRS
Functional outcome was assessed at 3 months, 2 years and 7 years post-stroke by the modified Rankin Scale (mRS). At the 3-month follow-up, mRS was scored by one stroke neurologist (n=7 patients had died and are coded mRS=6; missing scores for 31 patients). At the 2-year follow-up, all surviving patients (or when relevant their next-of-kin) were interviewed by one study nurse who was trained in stroke medicine and also specifically trained to score mRS (n=24 patients had died; missing scores for 7 patients). Surviving patients were also interviewed by the research nurse at 7-year follow-up (n=98 patients had died; missing scores for n=115).

Missing data
Serum samples were missing for 1 control, 23 patients in the acute phase, and 37 patients at 3 month follow-up. The number of individuals for whom the values for risk factors and other covariates were missing, have been reported previously. In the logistic regression, missing values were replaced by dummy variables for the covariates being categorical variables.

Statistical Analyses
Differences in study characteristics were examined with the $\chi^2$-test for proportions and with Student’s t-test or Mann–Whitney U-test for continuous variables. BDNF concentrations were analyzed as continuous variables in analyses for association to stroke or subtypes. BDNF values were log-transformed to normalize the skewed distribution. Associations between BDNF and overall IS or TOAST subtypes were investigated using unconditional logistic regression analysis. All of the reported ORs were scaled to estimate the ORs associated with an increase of one standard deviation (SD) in the log BDNF concentration of the controls.

For prediction of outcome, receiver operating characteristic (ROC) analysis was used to scan the continuous BDNF scale to determine the optimal BDNF cut-off values. The differences in the area under the curve (AUC/ c-statistic) between the baseline clinical model (model C) to a model also including BDNF were assessed to determine whether the addition of BDNF improved the discrimination between the favorable and poor outcome groups. The accuracy of the prediction was estimated by the c-statistic. Additionally, we calculated the net reclassification improvement (NRI) index and integrated discrimination index (IDI) using the R-Package PredictABEL.
SUPPLEMENTAL RESULTS AND DISCUSSION

There were no significant differences among the TOAST subtypes with regard to the time of blood sampling (Kruskal-Wallis, \(P=0.48\)). Furthermore, there was no correlation between acute phase BDNF levels and the time of the first blood draw (Spearman’s rho -0.08, \(P = 0.10\)), or between 3-month levels and day of blood draw (Spearman’s rho 0.07, \(P=0.16\)). The concentration of BDNF in the acute-phase was not correlated with stroke severity at admission as measured by the SSS (Spearman’s rho: 0.04, \(P=0.41\)) and did not differ by clinical subtype according to the Oxfordshire Community Stroke Project (\(P=0.39\)).

ROC analyses were used to identify the optimal BDNF cut-off for functional outcome prediction after 2 and 7 years. For 2-year follow-up the best cut-off for maximizing the sum of sensitivity and specificity is 21.8 ng/ml which coincides with the lowest BDNF tertile (T1, sensitivity and specificity both 80%). For 7-year follow-up the best cut-off is 16.8 ng/ml (sensitivity 82% and specificity 74%). The difference in cut-offs was not statistically significant and thus, the cut-off corresponding to the lowest BDNF tertile was used at both time points. This cut off (T1 vs T2+T3) was also used to summarize the distribution of clinical characteristics (Table I).

To our knowledge, this is the first study to explore associations between BDNF levels and TOAST subtypes. Results from multivariate analysis showed an independent association between acute BDNF and overall ischemic stroke as well as all four major etiologic subtypes. BDNF concentrations were lower in all subtypes compared to controls. The acute levels of BDNF may be influenced by the stroke itself. We therefore also explored associations between convalescent BDNF levels (isolated 3 months after index stroke) and overall ischemic stroke and TOAST subtypes. We found an attenuated but significant association between low BDNF and overall ischemic stroke and to the subtype CE stroke (Figure SI). These data are in agreement with a recent prospective study, that showed low concentrations of BDNF are associated with increased risk of incident stroke/TIA when adjusting for age, sex and traditional risk factors. Our observation that BDNF is lowest in the cardioembolic stroke subtype is also supported in the literature. Low concentrations of BDNF have been observed in patients with atrial fibrillation, the main cause of CE stroke.

Finally, it should be noted that the population in the present study is relatively young (<70 years of age), which means that it is not possible to extrapolate these results to older populations.
SUPPLEMENTAL REFERENCES


**SUPPLEMENTAL TABLE**

*Table I:* Baseline characteristics of SAHLSIS based on BDNF tertiles.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>BDNF tertiles</th>
<th>T1 v T2-3</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>402</td>
<td>603</td>
<td></td>
</tr>
<tr>
<td>BDNF, range, ng/ml</td>
<td>&lt;22</td>
<td>≥22</td>
<td></td>
</tr>
<tr>
<td>BDNF, mean ± SD, ng/ml</td>
<td>15.4 ± 5.9</td>
<td>30.2 ± 10.6</td>
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</tr>
<tr>
<td>Nr of subjects with stroke</td>
<td>231</td>
<td>260</td>
<td></td>
</tr>
<tr>
<td>Age, median (IQR)</td>
<td>58 (51-63)</td>
<td>59 (54-65)</td>
<td>0.002</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>258 (64)</td>
<td>385 (64)</td>
<td>ns</td>
</tr>
<tr>
<td>BMI, mean ± SD, kg/m²</td>
<td>26.4 ± 4.1</td>
<td>26.7 ± 4.3</td>
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</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>180 (45)</td>
<td>317 (53)</td>
<td>0.041</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>46 (11)</td>
<td>78 (13)</td>
<td>ns</td>
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<tr>
<td>Current smoking, n (%)</td>
<td>122 (30)</td>
<td>160 (26)</td>
<td>ns</td>
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<tr>
<td>Hyperlipidemia, n (%)</td>
<td>271 (67)</td>
<td>416 (69)</td>
<td>ns</td>
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<td>Systolic BP*, mean ± SD, mmHg</td>
<td>137 ± 20</td>
<td>141 ± 21</td>
<td>0.001</td>
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<tr>
<td>Diastolic BP, mean ± SD, mmHg</td>
<td>81 ± 11</td>
<td>83 ± 11</td>
<td>0.001</td>
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<td>HDL cholesterol, mean ± SD, mg/dL</td>
<td>1.38 ± 0.41</td>
<td>1.43 ± 0.39</td>
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<tr>
<td>LDL cholesterol, mean ± SD, mg/dL</td>
<td>3.05 ± 0.87</td>
<td>3.21 ± 0.94</td>
<td>0.009</td>
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<tr>
<td>Triglycerides, mean ± SD, mg/dL</td>
<td>1.47 ± 0.96</td>
<td>1.36 ± 0.72</td>
<td>0.037</td>
</tr>
<tr>
<td>Atrial fibrillation, n (%)</td>
<td>35 (9)</td>
<td>28 (5)</td>
<td>0.034</td>
</tr>
<tr>
<td>SSS†, median (IQR)</td>
<td>53 (44-56)</td>
<td>51 (38-56)</td>
<td>ns</td>
</tr>
</tbody>
</table>

*BP, blood pressure; †SSS, Scandinavian Stroke Scale*
**Figure SI.** Odds ratios and 95% confidence intervals for ischemic stroke and TOAST subtypes per 1 SD increase in BDNF concentration at 3-month follow-up. Model A adjusted for age and sex and Model B adjusted for traditional risk factors. LVD, large vessel disease; SVD, small vessel disease; CE, cardioembolic stroke; Crypt, cryptogenic stroke. *P*<0.05, †*P*<0.01, ‡*P*<0.001
**Figure SII:** Receiver operator curve for distinguishing between good (mRS 0-2) or bad functional outcome (defined as mRS 3-6 at 2-year follow-up, and mRS 3-5 at 7-year follow-up) in a model with clinical variables and a model that also includes BDNF. A) 2-year follow-up. B) 7-year follow-up.