Changes of serum uric acid and total bilirubin in elderly patients with major postischemic stroke depression

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Background: This was a longitudinal study which investigated the relationship between serum uric acid (SUA) and total bilirubin (Tbil) upon admission in elderly stroke patients and the occurrence of postischemic stroke depression (IPSD) at 3, 6, and 9 months of post-stroke follow-up.

Subjects and methods: Data were analyzed for 525 acute ischemic stroke patients. Beck Depression Inventory (BDI) scores $\geq 17$ and Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) were used separately to screen and diagnose IPSD at 3, 6, and 9 months post-stroke. Once IPSD was diagnosed, follow-up activities were terminated.

Results: High levels of SUA (odds ratio [OR]=2.08, $P<0.01$) and Tbil (OR=2.31, $P<0.01$) in the first 3 months post-stroke and low levels of SUA (OR=2.05, $P=0.03$) and Tbil (OR=2.79, $P<0.01$) from 3 to 6 months post-stroke were identified as risk factors for major IPSD. At 3 months, patients with SUA levels $\geq 406.5$ mmol/L (males with SUA levels of $\geq 409.5$ mmol/L and females with SUA levels $\geq 385.5$ mmol/L) and Tbil levels $\geq 23.65$ mmol/L were more likely to develop major IPSD. At 6 months, both SUA (area under curve [AUC]=0.625, $P=0.005$, cutoff=194.0 mmol/L) and Tbil (AUC=0.681, $P=0.004$, cutoff=6.75 mmol/L) had minor diagnostic values (AUC $<0.700$), although SUA levels $\leq 214.5$ mmol/L (AUC=0.756, $P=0.001$) in female patients had a good diagnostic value (AUC=0.722, $P=0.006$) for major IPSD. At 9 months, major IPSD showed no statistical relationship with either SUA ($\chi^2=2.33$, $P=0.13$) or Tbil ($\chi^2=0.41$, $P=0.84$).

Conclusion: Higher levels of SUA and Tbil on admission were closely related to the occurrence of major IPSD within 3 months of stroke. Lower levels of these two biomarkers on admission were characteristic for the occurrence of major IPSD between 3 and 6 months post-stroke, while 6 months after stroke, there was no relationship between major IPSD and these two biomarkers.

Keywords: depression after stroke, stroke, total bilirubin, uric acid, longitudinal study

Introduction

Postischemic stroke depression (IPSD) is an important post-stroke complication and a chronic disorder that seriously affects the prognosis of patients. The biological processes and pathways of depression generally include inflammatory, oxidative, and nitrosative stress pathways; neurotransmitter systems; neurotrophins; and the regulation of neurogenesis and modulation of the hypothalamic–pituitary–adrenal (HPA) axis. Post-stroke depression (PSD) may have a similar pathological process to depression. Previous research has shown that the levels of some molecular markers are changed in the peripheral system of PSD patients, including C-reactive protein (CRP) and...
homocysteine, \textsuperscript{5–7} brain-derived neurotrophic factor (BDNF), \textsuperscript{8} and triiodothyronine. \textsuperscript{9} Of these pathophysiological factors, oxidative and nitrosative stress pathways are believed to be the most crucial pathophysiological factors, \textsuperscript{10} since the brain has high metabolic rates and low antioxidant levels. \textsuperscript{11}

Serum uric acid (SUA) is the ultimate product of purine metabolism and undertakes 60\% of the body’s antioxidant reactions. \textsuperscript{12} Uric acid (UA) can inhibit inflammatory cascades, reduce the permeability of the blood–brain barrier, and protect central nervous tissue. \textsuperscript{13} Similar to the situation reported for cardiovascular disease, \textsuperscript{14} hyperuricemia appears to be common in post-stroke patients and is suggestive of a better prognosis. \textsuperscript{15} However, low SUA levels after ischemic stroke have also been reported in previous experimental data and were indicative of poor outcomes. \textsuperscript{16} UA is also a known scavenger of peroxynitrite, which is the main oxidizing congener of nitric oxide and is involved in the biochemical pathogenesis of depression. \textsuperscript{17} However, both high \textsuperscript{18} and low \textsuperscript{19,20} SUA levels have been previously shown to be associated with depression.

Bilirubin is a powerful antioxidant and is the final catabolite of the heme fragmentation pathway. \textsuperscript{21} Some studies have shown that decreased serum bilirubin level could be an independent predictor of stroke incidence, \textsuperscript{22,23} and a high serum bilirubin level is related to the severity of stroke and a poor prognosis. \textsuperscript{24} However, other studies have shown that a high level of serum bilirubin is used as a mechanism which just only reflects the intensity of initial oxidative stress rather than outcome of stroke in acute ischemic stroke. \textsuperscript{25} The clear inconsistency between these earlier studies is interesting and has led to confusion with regard to our understanding of the specific relationship between bilirubin and stroke. Similar effects have been observed in studies which have evaluated the relationship between bilirubin and depression. For example, Miyaoka et al showed that high levels of biopyr- rin (bilirubin oxidative metabolite) in the urine of psychiatric patients were correlated with depressive symptoms. \textsuperscript{26} In contrast, there is also strong evidence suggesting that low bilirubin levels represent a risk factor for depression. \textsuperscript{27}

The precise relationship between IPSD and levels of SUA and Tbil has yet to be studied in depth, particularly in terms of the occurrence of IPSD at different phases after stroke. To our knowledge, only a limited number of studies have attempted to explain why IPSD may occur over different time periods following stroke. \textsuperscript{28,29} We hypothesized that levels of SUA and Tbil were implicated in IPSD. Consequently, the key purpose of the present study was to investigate the specific relationship between levels of SUA and Tbil at admission and the occurrence of IPSD.

**Subjects and methods**

**Baseline participants and assessment**

This was a prospective cohort study undertaken at China Medical University Affiliated Hospital between October 2014 and March 2016 involving 525 patients with ischemic stroke. Data regarding the incidence of postischemic stroke depression and physical outcomes were collected from stroke survivors every 3 months following their initial hospital admission.

**Demographic and clinical data**

For all subjects, a trained research assistant collected the demographic data (gender, age, marital status, and educational level) and evaluated stroke severity using the National Institute of Health Stroke Scale (NIHSS) \textsuperscript{30} within 48 hours of admission. The following morning, fasting blood was collected and levels of SUA and Tbil were determined.

Our specific inclusion criteria were as follows: 1) a first occurrence of an ischemic stroke that met the standards of the World Health Organization diagnostic criteria (not exceeding 2 weeks post-stroke); 2) a duration of clinical neurological function deficit lasting over 24 hours; 3) radiological magnetic resonance imaging confirmation; \textsuperscript{11} and 4) over 55 years of age. Patients were excluded from the study if: 1) they refused to participate in the study; 2) there was apparent nonvascular etiologies (primary or metastatic neoplasms); 3) they were suffering from pre-stroke disability or depression, or antidepressant history; 4) they had a history of liver disease, hemolytic disease (such as Rh hemolytic disease and thalassemia), serious renal disease, or gout; 5) they were suffering from severe aphasia (language score \textsuperscript{2} using the NIHSS), total blindness, deafness, or disturbance of consciousness; 6) they had a Barthel Index score <10; \textsuperscript{31} 7) they had an NIHSS score >20; or 8) their blood indices were insufficient upon admission, such as liver function, renal function, SUA, or Tbil.

**SUA and Tbil assay**

Levels of SUA and Tbil were determined by Roche kit (Hoffman-La Roche Ltd., Basel, Switzerland) with the use of a Hitachi 7170 automatic biochemical analyzer test (Hitachi High-Technologies, Tokyo, Japan). In our hospital department, normal serum SUA level is known to range from 208 to 428 \(\mu\text{mol/L} \) in male patients and from 155 to 357 \(\mu\text{mol/L} \) in females, while normal Tbil levels range from 3.0 to 22.0 \(\mu\text{mol/L} \). As raw SUA and Tbil data were skewed, we recorded and divided these data into three tertiles: (tertile 1: \(=245.0 \mu\text{mol/L} \), 245.0 < tertile 2 < 330.0 \(\mu\text{mol/L} \), tertile 3 \(\geq 330.0 \mu\text{mol/L} \)) and (tertile 1 \(=8.0 \mu\text{mol/L} \), 8.0 < tertile 2 < 12.8 \(\mu\text{mol/L} \), tertile 3 \(\geq 12.8 \mu\text{mol/L} \), respectively.
Follow-up
Patients meeting our specific inclusion criteria were invited to participate in the study and were followed up until 9 months post-stroke. Follow-up measurements were taken at 3, 6, and 9 months post-stroke. All assessments were performed by trained research assistants.

Ethical statement
The study was approved by the Regional Medical Research Ethical Committee of China Medical University. All subjects, or their legal representatives, provided informed written consent.

Assessment of IPSD
Subjects were invited to complete the BDI\(^2\) by telephone interview at 3, 6, and 9 months of follow-up as the primary screening standard. Patients with scores \(>17\) were recruited in order to consider a final diagnosis of major IPSD in accordance with the DSM-IV\(^3\) by two professional clinicians who were blinded to laboratory results.

Statistical analysis
The SPSS 19.0 statistical package (SPSS Inc., Chicago, IL, USA) was used to analyze all data arising from the study. A \(\chi^2\) test was used to assess differences in categorical variables data. For continuous, normally distributed data, the Student’s \(t\)-test was used. Risk factors \((P \leq 0.10)\) were further analyzed by univariate and multivariate logistic regression. Relationships between gender and the levels of SUA and Tbil were analyzed using Spearman correlation analysis. Receiver operating characteristic curves were drawn to depict the diagnostic performance of SUA and Tbil levels in the occurrence of IPSD. All \(P\)-values were calculated as two-tailed, and significance level was set at \(P < 0.05\).

Results
Patients
A total of 1,006 ischemic stroke patients were screened and 760 met the specific entry criteria. During the follow-up process, 235 patients withdrew; consequently, the final data analysis included 525 patients. The follow-up procedure is depicted in Figure 1.

Patients who withdrew from the study had a significantly higher mean age (mean \(\pm\) SD: 68.26\(\pm\)7.48 years vs 64.00\(\pm\)13.19 years; \(t = 5.28, P < 0.01\)), and were more likely to have less than 9 years of formal education (46.6% vs 37.3%, \(P = 0.03\)). Baseline characteristics of patients who completed the follow-up period are shown in Table 1.

Correlation analysis between the levels of SUA and Tbil upon admission and the occurrence of IPSD
When no confounding factors were considered, a significant difference was observed in terms of Tbil levels when compared between patients with major IPSD and non-major IPSD \((\chi^2 = 10.89, P < 0.01;\) Table 1). Next, we entered all risk factors with \(P = 0.10\) (Table 1) into a multivariate logistic regression model (ie, female gender, age, Barthel Index [BI], NIHSS, frontal lobe, thalamus, and Tbil level [tertile 3]). Of these risk factors, multivariate logistic regression confirmed that Tbil levels (tertile 3) were independently related to the occurrence of IPSD with an odds ratio (OR) of 1.93 \((P < 0.01)\) (Table 2).

The relationship between the occurrence of major IPSD and the levels of serum SUA and Tbil after adjustment for other risk factors
There were 105 patients with major IPSD within 3 months of stroke, 48 patients from 3 to 6 months post-stroke, and 34 patients between 6 and 9 months post-stroke. The incidence of major IPSD was significantly different when compared between these different time periods \((\chi^2 = 24.96, P < 0.01)\). Therefore, the analysis of relationships between different levels of SUA and Tbil and major IPSD was stratified according to different time periods following stroke (3 months, 3–6 months, and 6–9 months post-stroke, as shown in Table 3).

When we compared patients with major IPSD and non-major IPSD across the different time periods in terms of gender, age, education, hypertension, diabetes, NIHSS, BI, location, 17-item Hamilton Depression Rating Scale, blood urea nitrogen, creatinine, alkaline phosphatase, and alanine aminotransferase, we identified several risk factors where \(P < 0.1\): age \((\chi^2 = 5.47, P = 0.02)\), frontal lobe \((\chi^2 = 4.57, P = 0.03)\), and NIHSS \((\chi^2 = 1.86, P = 0.07)\) in the first 3 months post-stroke; female gender \((\chi^2 = 5.81, P = 0.02)\), age \((\chi^2 = 1.68, P = 0.10)\), and NIHSS score \((\chi^2 = 2.78, P = 0.01)\) from 3 to 6 months post-stroke; female gender \((\chi^2 = 4.53, P = 0.03)\), age \((\chi^2 = 2.43, P = 0.02)\), and living alone \((\chi^2 = 6.13, P = 0.01)\) from 6 to 9 months post-stroke. Next, we used univariate and multivariate analyses to investigate major IPSD, SUA, and Tbil levels and the significant risk factors identified earlier (Table 2). The results of these analyses are shown in Table 4.

The sensitivity and specificity of SUA and Tbil levels to predict major IPSD in the first 6 months post-stroke
As shown in Table 5, a negative correlation was found between female gender and SUA level \((r = 0.30, P < 0.01)\). The predictive
values of SUA and Tbil levels are shown in Table 6. In the first 3 months post-stroke, SUA levels $\geq 330.0 \, \mu \text{mol/L}$ (area under curve [AUC]=0.834, $P<0.001$, Figure 2A) and Tbil levels $\geq 12.8 \, \mu \text{mol/L}$ (AUC=0.782, $P<0.001$, Figure 2B) showed significant diagnostic value. Furthermore, patients with SUA levels $\geq 406.5 \, \mu \text{mol/L}$, or Tbil levels $\geq 23.65 \, \mu \text{mol/L}$, were more likely to be depressive. Meanwhile, male patients with SUA levels $\geq 409.5 \, \mu \text{mol/L}$ (AUC=0.920, $P<0.001$, Figure 2C) and females with SUA levels $\geq 385.5 \, \mu \text{mol/L}$ (AUC=0.756, $P=0.001$, Figure 2D) had a greater risk of suffering from major IPSD.

From 3 to 6 months post-stroke, the diagnostic performance of SUA levels $\leq 245 \, \mu \text{mol/L}$ (AUC=0.676, $P=0.007$, Figure 3A) and Tbil levels $\leq 8.0 \, \mu \text{mol/L}$ (AUC=0.681, $P=0.004$, Figure 3B) was statistically significant, although the diagnostic value was not very high. However, compared with male patients (AUC=0.580, $P=0.465$, Figure 3C), female patients with SUA levels $\leq 214.5 \, \mu \text{mol/L}$ (AUC=0.722, $P=0.006$, Figure 3D) were more likely to develop major IPSD.

**Discussion**

Unlike previous studies, the present investigation focused on the occurrence of major IPSD across different time periods and attempted to link this information with levels of Tbil and SUA. The common characteristic of SUA and Tbil is
that both these factors exhibit antioxidant reactions within the human body. Our study also confirmed that the changes observed on the levels of these two biomarkers showed excellent consistency in major IPSD patients. While the precise mechanism underlying IPSD has yet to be established, our present study provided significant evidence of the important pathological roles of both SUA and Tbil in IPSD.

Patients developing major IPSD in the first 3 months after stroke also showed higher levels of SUA and Tbil upon admission. In a previous study, Nanetti et al showed

Table 1 Baseline subject characteristics with hemispheric lesion verified by radiologic magnetic resonance imaging

<table>
<thead>
<tr>
<th>Variables</th>
<th>No IPSD n=338 Mean ± SD or n (%)</th>
<th>IPSD n=187 Mean ± SD or n (%)</th>
<th>t/χ²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>181 (53.6)</td>
<td>115 (61.5)</td>
<td>3.09</td>
<td>0.08</td>
</tr>
<tr>
<td>Age</td>
<td>65.4±10.9</td>
<td>67.4±11.4</td>
<td>-2.00</td>
<td>0.05</td>
</tr>
<tr>
<td>&lt;9 years of education</td>
<td>130 (38.5)</td>
<td>67 (35.8)</td>
<td>0.36</td>
<td>0.57</td>
</tr>
<tr>
<td>Living alone</td>
<td>40 (11.8)</td>
<td>32 (16.0)</td>
<td>1.85</td>
<td>0.17</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>224 (66.3)</td>
<td>130 (69.5)</td>
<td>0.58</td>
<td>0.45</td>
</tr>
<tr>
<td>History of diabetes</td>
<td>114 (33.7)</td>
<td>67 (35.8)</td>
<td>0.24</td>
<td>0.63</td>
</tr>
<tr>
<td>Lesion location</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>14 (4.1)</td>
<td>14 (7.5)</td>
<td>2.67</td>
<td>0.10</td>
</tr>
<tr>
<td>Parietal lobe</td>
<td>40 (11.8)</td>
<td>18 (9.6)</td>
<td>0.60</td>
<td>0.44</td>
</tr>
<tr>
<td>Temporal lobe</td>
<td>18 (5.3)</td>
<td>11 (5.9)</td>
<td>0.07</td>
<td>0.79</td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>22 (6.5)</td>
<td>14 (7.5)</td>
<td>0.18</td>
<td>0.67</td>
</tr>
<tr>
<td>Basal ganglia</td>
<td>103 (30.5)</td>
<td>68 (36.4)</td>
<td>1.90</td>
<td>0.17</td>
</tr>
<tr>
<td>Thalamus</td>
<td>19 (5.6)</td>
<td>26 (13.9)</td>
<td>10.54</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Brainstem</td>
<td>34 (10.1)</td>
<td>18 (9.6)</td>
<td>0.03</td>
<td>0.87</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>22 (6.5)</td>
<td>9 (4.8)</td>
<td>0.62</td>
<td>0.43</td>
</tr>
<tr>
<td>Other location</td>
<td>18 (5.3)</td>
<td>10 (5.3)</td>
<td>0.00</td>
<td>0.99</td>
</tr>
<tr>
<td>≥ Two locations</td>
<td>31 (9.2)</td>
<td>11 (5.9)</td>
<td>1.78</td>
<td>0.18</td>
</tr>
<tr>
<td>BI</td>
<td>71.48±16.11</td>
<td>68.90±17.34</td>
<td>1.71</td>
<td>0.09</td>
</tr>
<tr>
<td>Nihss score</td>
<td>4.91±2.54</td>
<td>5.35±2.55</td>
<td>-1.91</td>
<td>0.06</td>
</tr>
<tr>
<td>BUN (mmol/L)</td>
<td>5.17±1.56</td>
<td>5.23±1.60</td>
<td>-0.43</td>
<td>0.67</td>
</tr>
<tr>
<td>Cr (μmol/L)</td>
<td>67.15±17.96</td>
<td>65.97±18.91</td>
<td>0.70</td>
<td>0.48</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>21.77±9.28</td>
<td>20.42±9.13</td>
<td>1.61</td>
<td>0.11</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>71.30±17.36</td>
<td>70.10±18.94</td>
<td>0.74</td>
<td>0.46</td>
</tr>
<tr>
<td>HaMD17</td>
<td>2.95±2.03</td>
<td>3.04±2.00</td>
<td>-0.45</td>
<td>0.66</td>
</tr>
<tr>
<td>Uric acid (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤245.0</td>
<td>108 (32.0)</td>
<td>69 (36.9)</td>
<td>1.32</td>
<td>0.25</td>
</tr>
<tr>
<td>245.0–330.0</td>
<td>117 (34.6)</td>
<td>57 (30.5)</td>
<td>0.93</td>
<td>0.34</td>
</tr>
<tr>
<td>≥330.0</td>
<td>113 (33.4)</td>
<td>61 (32.6)</td>
<td>0.04</td>
<td>0.85</td>
</tr>
<tr>
<td>Total bilirubin (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤8.0</td>
<td>119 (35.2)</td>
<td>62 (33.2)</td>
<td>0.22</td>
<td>0.64</td>
</tr>
<tr>
<td>8.0–12.8</td>
<td>124 (36.7)</td>
<td>48 (25.7)</td>
<td>6.64</td>
<td>0.01</td>
</tr>
<tr>
<td>≥12.8</td>
<td>95 (28.1)</td>
<td>77 (41.2)</td>
<td>9.34</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; BI, Barthel index; BUN, blood urea nitrogen; Cr, creatinine; HaMD17, 17-item Hamilton Depression Rating Scale; IPSD, postischemic stroke depression; Nihss, National Institute of Health Stroke Scale.

Table 2 Univariate and multiple logistic regression analyses with risk factors (P≤0.1) of postischemic stroke depression

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate correlation analysis</th>
<th>Multivariate correlation analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI) P-value</td>
<td>OR (95% CI) P-value</td>
</tr>
<tr>
<td>Female</td>
<td>1.39 (0.96–1.99) 0.08</td>
<td>1.37 (0.94–2.00) 0.10</td>
</tr>
<tr>
<td>Age</td>
<td>1.02 (1.00–1.03) 0.05</td>
<td>1.01 (1.00–1.03) 0.23</td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>1.87 (0.87–4.02) 0.11</td>
<td>2.08 (0.94–4.60) 0.07</td>
</tr>
<tr>
<td>Thalamus</td>
<td>2.71 (1.46–5.05) &lt;0.01</td>
<td>2.63 (1.93–4.98) &lt;0.01</td>
</tr>
<tr>
<td>Nihss</td>
<td>1.07 (1.00–1.15) 0.06</td>
<td>1.08 (1.00–1.16) 0.05</td>
</tr>
<tr>
<td>BI</td>
<td>0.99 (0.98–1.00) 0.09</td>
<td>0.99 (0.98–1.00) 0.19</td>
</tr>
<tr>
<td>Total bilirubin ≥12.8 μmol/L</td>
<td>1.79 (1.23–2.61) &lt;0.01</td>
<td>1.93 (1.31–2.85) &lt;0.01</td>
</tr>
</tbody>
</table>

Abbreviations: BI, Barthel index; Nihss, National Institute of Health Stroke Scale; OR, odds ratio.
Table 3  The relationship between serum uric acid and total bilirubin on admission and postischemic stroke depression (iPsD) in different time periods

<table>
<thead>
<tr>
<th>No IPSD</th>
<th>Patients, n (%)</th>
<th>IPSD</th>
<th>Patients, n (%)</th>
<th>$\chi^2$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uric acid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group a1 (n=420)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile 1</td>
<td>148 (35.2)</td>
<td>Tertile 1</td>
<td>29 (27.6)</td>
<td>2.18</td>
<td>0.14</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>147 (35.0)</td>
<td>Tertile 2</td>
<td>27 (25.7)</td>
<td>3.27</td>
<td>0.07</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>125 (29.8)</td>
<td>Tertile 3</td>
<td>49 (46.7)</td>
<td>10.83</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Group a2 (n=372)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Tertile 1</td>
<td>124 (33.3)</td>
<td>Tertile 1</td>
<td>24 (50)</td>
<td>5.18</td>
<td>0.02</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>128 (34.4)</td>
<td>Tertile 2</td>
<td>19 (39.6)</td>
<td>0.50</td>
<td>0.48</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>120 (32.3)</td>
<td>Tertile 3</td>
<td>5 (10.4)</td>
<td>9.70</td>
<td>&lt;0.01</td>
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<tr>
<td>Group a3 (n=338)</td>
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<tr>
<td>Tertile 1</td>
<td>108 (32.0)</td>
<td>Tertile 1</td>
<td>16 (47.1)</td>
<td>3.17</td>
<td>0.08</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>117 (34.6)</td>
<td>Tertile 2</td>
<td>11 (32.4)</td>
<td>0.07</td>
<td>0.79</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>113 (33.4)</td>
<td>Tertile 3</td>
<td>7 (20.6)</td>
<td>2.23</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>Total bilirubin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group a1 (n=420)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile 1</td>
<td>161 (38.3)</td>
<td>Tertile 1</td>
<td>20 (19.0)</td>
<td>13.83</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>138 (32.9)</td>
<td>Tertile 2</td>
<td>34 (32.4)</td>
<td>0.01</td>
<td>0.93</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>121 (28.8)</td>
<td>Tertile 3</td>
<td>51 (48.6)</td>
<td>14.89</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Group a2 (n=372)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile 1</td>
<td>136 (36.6)</td>
<td>Tertile 1</td>
<td>25 (52.1)</td>
<td>4.33</td>
<td>0.04</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>132 (35.5)</td>
<td>Tertile 2</td>
<td>6 (12.5)</td>
<td>10.18</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>104 (28.0)</td>
<td>Tertile 3</td>
<td>17 (35.4)</td>
<td>1.15</td>
<td>0.28</td>
</tr>
<tr>
<td>Group a3 (n=338)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile 1</td>
<td>119 (35.2)</td>
<td>Tertile 1</td>
<td>17 (50.0)</td>
<td>2.92</td>
<td>0.09</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>124 (36.7)</td>
<td>Tertile 2</td>
<td>8 (23.5)</td>
<td>2.34</td>
<td>0.13</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>95 (28.1)</td>
<td>Tertile 3</td>
<td>9 (26.5)</td>
<td>0.04</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Notes: Tertile 1 = 245.0 μmol/L, 245.0 < tertile 2 < 330.0 μmol/L, tertile 3 = ≥330.0 μmol/L; tertile 1 = 8.0 μmol/L, 8.0 < tertile 2 < 12.8 μmol/L, tertile 3 = ≥12.8 μmol/L.

Group a1, no IPSD at 3 months; Group b1, IPSD at 3 months; Group a2, no IPSD at 6 months; Group b2, IPSD at 6 months; Group a3, no IPSD at 9 months; Group b3, IPSD at 9 months.

Table 4  Univariate and multiple logistic regression analyses with risk factors (P≤0.1) of postischemic stroke depression (IPSd) in different time periods

<table>
<thead>
<tr>
<th>Occurring time of IPSD</th>
<th>Variables</th>
<th>Univariate correlation analysis</th>
<th>Multivariate correlation analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td><em><strong>Three months post-stroke</strong></em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.02 (1.00–1.04)</td>
<td>0.07</td>
<td>1.02 (0.99–1.04)</td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>2.35 (1.05–5.26)</td>
<td>0.04</td>
<td>2.07 (0.88–4.87)</td>
</tr>
<tr>
<td>Thalamus</td>
<td>2.17 (1.12–4.20)</td>
<td>0.02</td>
<td>2.20 (1.11–4.37)</td>
</tr>
<tr>
<td>NIHSS</td>
<td>1.09 (1.01–1.19)</td>
<td>0.04</td>
<td>1.07 (0.98–1.16)</td>
</tr>
<tr>
<td>Uric acid ≥330.0 μmol/L</td>
<td>2.07 (1.33–3.20)</td>
<td>&lt;0.01</td>
<td>2.08 (1.33–3.27)</td>
</tr>
<tr>
<td>Total bilirubin ≥12.8 μmol/L</td>
<td>2.33 (1.51–3.61)</td>
<td>&lt;0.01</td>
<td>2.31 (1.47–3.63)</td>
</tr>
<tr>
<td><em><strong>Six months post-stroke</strong></em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>2.20 (1.15–4.24)</td>
<td>0.02</td>
<td>1.83 (0.93–3.60)</td>
</tr>
<tr>
<td>Age</td>
<td>1.03 (1.00–1.06)</td>
<td>0.10</td>
<td>1.03 (0.99–1.07)</td>
</tr>
<tr>
<td>NIHSS</td>
<td>1.20 (1.05–1.38)</td>
<td>0.01</td>
<td>1.27 (1.10–1.46)</td>
</tr>
<tr>
<td>Uric acid ≥245.0 μmol/L</td>
<td>2.00 (1.09–3.66)</td>
<td>0.03</td>
<td>2.05 (1.09–3.86)</td>
</tr>
<tr>
<td>Total bilirubin ≥8.0 μmol/L</td>
<td>1.89 (1.03–3.45)</td>
<td>0.04</td>
<td>2.79 (1.44–5.40)</td>
</tr>
<tr>
<td><em><strong>Nine months post-stroke</strong></em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>2.26 (1.05–4.88)</td>
<td>0.04</td>
<td>2.04 (0.93–4.51)</td>
</tr>
<tr>
<td>Age</td>
<td>1.05 (1.01–1.09)</td>
<td>0.02</td>
<td>1.05 (1.01–1.09)</td>
</tr>
<tr>
<td>Living alone</td>
<td>2.76 (1.20–6.34)</td>
<td>0.02</td>
<td>3.01 (1.26–7.17)</td>
</tr>
<tr>
<td>Uric acid ≥245.0 μmol/L</td>
<td>1.89 (0.93–3.86)</td>
<td>0.08</td>
<td>2.06 (0.98–4.33)</td>
</tr>
<tr>
<td>Total bilirubin ≥12.8 μmol/L</td>
<td>1.84 (0.91–3.74)</td>
<td>0.09</td>
<td>1.72 (0.83–3.57)</td>
</tr>
</tbody>
</table>

Abbreviations: NIHSS, National Institute of Health Stroke Scale; OR, odds ratio.
that acute ischemic stroke could cause the generation of free radicals and strong oxidative stress, and that this antioxidant capacity may limit the progression of ischemic injury.\textsuperscript{34} As powerful antioxidants, high concentrations of SUA and Tbil could reflect the level of oxidative stress in the first 3 months after stroke. Meanwhile, two previous meta-analyses reported that depression was strongly correlated to high oxidative stress by demonstrating a clear imbalance between antioxidants and oxidant activity.\textsuperscript{35} Previous research also implied that the pathophysiology of IPSD is probably involved in increased levels of oxidative stress in cerebral tissues.\textsuperscript{30} Firstly, oxidative stress, which involves enzymatic and nonenzymatic antioxidant factors expressed in the brain and periphery, could cause damage to proteins, DNA molecules, and lipids.\textsuperscript{37,38} Both SUA and Tbil are nonenzymatic antioxidants, much like glutathione and vitamins A, C, and E.\textsuperscript{12,37} Secondly, oxidative stress has been implicated in IPSD via the regulation of inflammation.\textsuperscript{35} A series of studies also provided support for the role of inflammatory factors in the development of IPSD.\textsuperscript{39–41} Under the influence of repeated inflammatory stimuli, microglia in the central nervous system can evolve into a source of inflammatory mediators, which may then influence brain neurotransmitter systems and neuronal integrity.\textsuperscript{32} Moreover, elevated levels of inflammatory cytokines can also affect the activity of 2,3-dioxygenase, which degrades tryptophan via the kynurenine pathway and ultimately leads to a reduction in the synthesis and availability of 5-hydroxytryptamine.\textsuperscript{43,44} It has also been reported that interaction between oxidative stress pathways and inflammatory pathways may lead to an increased level of neuronal apoptosis and neurodegeneration and a decline in the neurogenesis and neuroplasticity of depression.\textsuperscript{45,46} Thus, we can conclude that close associations between major IPSD and higher levels of SUA and Tbil in the first 3 months after stroke may be associated with high levels of oxidative stress. It has been reported that 60% of patients with depression during this particular time period showed recovery by 12 months post-stroke.\textsuperscript{47} It is possible that the symptoms of depression disappear at the same time as the disappearance of oxidative stress response.

However, this does not explain why patients with lower levels of SUA and Tbil at admission are more vulnerable to depression between 3 and 6 months post-stroke. One previous meta-analysis suggested that high levels of SUA at the onset of ischemic stroke could reduce levels of neurological damage and herald a better prognosis.\textsuperscript{15} Similar results were also reported by Perlstein et al for higher levels of Tbil.\textsuperscript{48} Some studies have also reported reduced levels of antioxidants in some patients with acute ischemic stroke.\textsuperscript{49} Thus, we can infer that lower levels of SUA and Tbil are related to more serious neurological damage than higher levels in acute stroke. It has also been reported that the more significant the

### Table 5 Relationship between sex and serum uric acid and total bilirubin levels

<table>
<thead>
<tr>
<th>Items</th>
<th>Tertiles</th>
<th>Patients (n)</th>
<th>Female (n, %)</th>
<th>(\chi^2, P)</th>
<th>Relevance, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid ((\mu\text{mol/L}))</td>
<td>(\leq245.0)</td>
<td>177</td>
<td>131 (74.0)</td>
<td>(\chi^2=46.47, P&lt;0.01)</td>
<td>(r=-0.30, P&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>245.0–330.0</td>
<td>174</td>
<td>99 (56.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\geq330.0)</td>
<td>174</td>
<td>66 (37.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total bilirubin ((\mu\text{mol/L}))</td>
<td>(\leq8.0)</td>
<td>180</td>
<td>99 (55.0)</td>
<td>(\chi^2=0.70, P=0.71)</td>
<td>(r=0.03, P=0.46)</td>
</tr>
<tr>
<td></td>
<td>8.0–12.8</td>
<td>172</td>
<td>95 (55.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\geq12.8)</td>
<td>173</td>
<td>102 (59.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6 Diagnostic performance of serum uric acid (SUA) and total bilirubin (Tbil) for the assessment of depression occurring in first 6 months post-stroke

<table>
<thead>
<tr>
<th>Items</th>
<th>Cutoff</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>YI</th>
<th>AUC (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three months post-stroke</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUA</td>
<td>406.5</td>
<td>0.760</td>
<td>0.816</td>
<td>0.576</td>
<td>0.834 (0.765–0.902)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>409.5</td>
<td>0.765</td>
<td>1.000</td>
<td>0.765</td>
<td>0.920 (0.870–0.969)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>385.5</td>
<td>0.739</td>
<td>0.762</td>
<td>0.501</td>
<td>0.756 (0.619–0.893)</td>
<td>0.001</td>
</tr>
<tr>
<td>Tbil</td>
<td>23.65</td>
<td>0.926</td>
<td>0.569</td>
<td>0.495</td>
<td>0.782 (0.704–0.860)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Six months post-stroke</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUA</td>
<td>194.0</td>
<td>0.694</td>
<td>0.750</td>
<td>0.444</td>
<td>0.676 (0.579–0.773)</td>
<td>0.007</td>
</tr>
<tr>
<td>Male</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.580 (0.401–0.760)</td>
<td>0.465</td>
</tr>
<tr>
<td>Female</td>
<td>214.5</td>
<td>0.515</td>
<td>0.938</td>
<td>0.501</td>
<td>0.722 (0.606–0.837)</td>
<td>0.006</td>
</tr>
<tr>
<td>Tbil</td>
<td>6.75</td>
<td>0.331</td>
<td>1.000</td>
<td>0.331</td>
<td>0.681 (0.580–0.783)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Note: YI = specificity + sensitivity – 1.
Abbreviations: AUC, area under the curve; YI, Youden Index.
Figure 2 ROC curve analysis for SUA and Tbil used for the assessment of IPSD in the first 3 months post-stroke. (A) SUA levels, (B) Tbil levels, (C) SUA levels in males, and (D) SUA levels in females.

Abbreviations: IPSD, postischemic stroke depression; ROC, receiver operating characteristic; SUA, serum uric acid; Tbil, total bilirubin.

stroke severity and functional impairment are, the greater the probability of suffering from depression.50 This is consistent with the higher NIHSS score in major IPSD patients observed between 3 and 6 months post-stroke. However, it is still unclear as to why patients with low levels of SUA and Tbil in our study developed depression after a 3-month delay instead of acutely. There may be several possible explanations for this phenomenon. Firstly, once the acute phase is over, patients with more severe ischemic stroke may gradually realize the seriousness of problems related to stroke,51 such as the decline in self-care and work ability, which would make such patients feel incompetent. Secondly, in patients with significant functional impairment such as post-stroke paresthesia, early paresthesia may lead to sleep disorders, and long-term sleep disorders inevitably lead to the occurrence of depression.52

From 6 to 9 months, we did not observe any significant association between major IPSD and the levels of SUA and Tbil. As we described in our previous publication, since biological changes and reconstruction in the brain tissue cease during this time frame, social psychology factors, rather than stroke severity, begin to play a more important role.53

In summary, our current study provides evidence that different levels of SUA and Tbil in the blood may be helpful in the early prevention of IPSD. This information may also be useful in selecting treatments for depression considering the
different mechanisms of depression which occur over different time periods. In the first 3 months after stroke, depression may not need excessive intervention because acute oxidative stress could disappear by itself. However, from 3 to 6 months, early post-stroke rehabilitation treatment has a more significant effect upon the prevention of depression and recovery because of the relationship between depression and stroke severity. Six months after stroke, antidepressive therapies, such as antidepressant drugs or psychotherapy, may begin to exert effects upon the treatments used for major IPSD. Compared with other biomarkers, there are several superiorities of the two biomarkers (SUA and Tbil) for IPSD. First, SUA and Tbil represent affordable routine clinical tools which do not incur additional expense as compared with BDNF, which is limited to precise laboratory tests and not suitable for extensive clinical studies and follow-up. Second, as compared with CRP, SUA and Tbil are less affected by the following factors, such as fever, infection, immune system diseases, and so on. Third, as compared with triiodothyronine and other biomarkers, SUA and Tbil may be used to monitor the development of IPSD, since a previous report showed that the level of SUA changes as depression improves.11

Acknowledgment

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Figure 3 ROC curve analysis for SUA and Tbil used for the assessment of IPSD from 3 to 6 months post-stroke. (A) SUA levels, (B) Tbil levels, (C) SUA levels in males, and (D) SUA levels in females.

Abbreviations: IPSD, postischemic stroke depression; ROC, receiver operating characteristic; SUA, serum uric acid; Tbil, total bilirubin.
Disclosure
The authors report no conflicts of interest in this work.

References


