Early biomarkers for post-stroke cognitive impairment

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Abstract The aim of this study was to investigate whether some biomarkers could predict cognitive impairment after stroke. One hundred fifty-two first-ever stroke patients were recruited within 6–72 h after the onset of symptoms. Blood was drawn within 1 h after admission for determining biomarkers. Cognitive function was assayed 2 weeks after stroke. The patients were divided into four groups: stroke, vascular cognitive impairment with no dementia (VCIND), vascular dementia (VaD), and mixed dementia (MD). Forty healthy subjects were used as controls. The results indicated that lower soluble receptor levels for advanced glycation end products (sRAGE) and higher β-secretase enzyme (BACE1) and neprilysin (NEP) levels were found in the VCIND, VaD, and MD groups. In addition, the percentages of ε3/ε4 genotypes and ε4 alleles in the VCIND, VaD, and MD groups were higher than in the stroke group. Correlation analysis determined that sRAGE, BACE1, and NEP were significantly related to the results of neuropsychological assessments. Logistic regression analysis, however, suggested that only sRAGE and BACE1 changed ahead of cognitive impairment after stroke. In conclusion, only BACE1 and sRAGE, not NEP or APOE genotypes, may be biomarkers diagnosing post-stroke cognitive impairment.

Keywords Biomarker · sRAGE · BACE1 · NEP · APOE · Post-stroke cognitive impairment

Introduction

Stroke is currently the second most common cause of acquired cognitive decline and dementia [23]; more than 64% of patients after stroke exhibit varying degrees of cognitive impairment [11]. Moreover, about one-fourth of stroke survivors develop dementia within 12 months after stroke [3]. Longitudinal studies have shown that within 2 weeks, 3 and 6 months after stroke, the prevalence of dementia is 16.3, 32.0, and 13.6–31.8%, respectively [16].

Currently, the diagnosis of cognitive impairment after stroke is based on clinical manifestations, neuroimaging, and a battery of neuropsychological tests. However, these methods are not sensitive enough to identify post-stroke cognitive dysfunction early, and they depend on the
cooperation of the patients. Therefore, it is very useful to find early biomarkers in the clinic.

Increasing evidence shows that VaD and Alzheimer’s disease (AD) share several pathological features. At least 40% of patients meeting the pathological criteria for VaD have concurrent AD pathology [1]. Furthermore, 87% of VaD patients were found to have either AD alone (58%) or AD in combination with cerebrovascular disease (42%) at autopsy. In addition, Aβ deposits and neurofibrillary tangles were present in 43% of VaD patients [13]. Our previous study indicated that in an Aβ-induced AD mouse model, the alterations in the levels of BACE1, sRAGE, and NEP in the cortex and hippocampus occurred ahead of Aβ accumulation as well as cognitive performance decline [28]. In both experimental stroke models and brain tissue of VaD patients, there are increased levels of BACE1 and an accumulation of Aβ42 [2, 17]. NEP, the major Aβ-degrading enzyme that was found to be downregulated in AD [7], may be altered in VaD. sRAGE, a secreted isoform of the receptor for RAGE, may be a marker for VaD and AD [9, 19]. Furthermore, the ε4 allele of apolipoprotein E (APOE) is a well-known risk factor for late-onset AD [14], and has been recognized as a risk factor for VaD in multiple ethnic groups [5].

The goal of this study was to test our hypothesis that changes in BACE1, sRAGE, NEP, and APOE occurred before cognitive functions could be assessed after stroke. We measured serum concentrations of these factors in acute ischemic stroke patients, and explored the relationship between these factors and different degrees of cognitive deficit after stroke. It will be helpful for physicians to predict and intervene against cognitive deficits early after stroke occurs.

Methods

Recruitment

Overall, 152 inpatients were recruited from the Department of Neurology of the Affiliated Drum Tower Hospital of Nanjing University Medical School between April 2010 and December 2010. The subjects were admitted to the hospital within 6–72 h after the onset of symptoms.

The subjects were aged 40–88 years and experiencing their first-ever ischemic stroke, involving the carotid artery system or vertebrobasilar artery system, which met the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and World Health Organization Multinational Monitoring of Trends and Determinants in Cardiovascular Disease. Computed tomography (CT) or magnetic resonance imaging (MRI) confirmed the diagnosis. Exclusion criteria included dementia or significant cognitive impairment before stroke, hemorrhagic stroke, consciousness disorders, severe aphasia or dysarthria, and serious depression (HAMD score ≥ 17). All patients were able consented to participate.

The 40 healthy control subjects (NC) were unpaid volunteers, recruited from the Medical Examination Center of the Affiliated Drum Tower Hospital of Nanjing University Medical School; they matched the patients for age, and had no history of stroke or other neurological or psychiatric disorders. This study was approved by the ethics committee in our hospital.

Neurological assessment and sampling

On admission, detailed medical history and neurological examination results were recorded. In addition, neurological deficits were assessed by NIHSS and mRs. Within 1 h after admission, a fasting blood sample was collected in gel clot activator tubes, and the serum was stored at −80°C. Blood specimens for the measurement of APOE genotypes were collected in sodium citrate-containing tubes, and stored at −80°C.

Neuropsychological assessment

Cognition was assessed within 2 weeks after stroke, which included the Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MOCA), Activities of Daily Living (ADL), digit span, Neuropsychiatric Inventory (NPI), Hamilton Depression Rating Scale (HAMD), clock-drawing task (CDT), digit-symbol coding from the Wechsler Adult Intelligence Scale (WAIS), Executive Dysfunction Index (EDI) [21], Informant Questionnaire of Cognitive Decline (IQCODE), Clinical Dementia Rating (CDR), and Hachinski Ischemic Score (HIS). All patients were divided into the following four groups on the basis of the cognitive assays:

1. The stroke group (no cognitive deficit) had an MMSE score between 27 and 30 (the MMSE score of subjects with no education adds 2 points, age ≥80 adds 1 point, and age <50 subtracts 1 point.), a MOCA score between 26 and 30, and an EDI ≤ 0.2, which indicate that these subjects had no executive dysfunction.
2. VCIND stroke patients met the following criteria [32]: (a) mild cognitive impairment: MMSE = 25 or 26, MOCA = 19–25, CDR = 0.5, and HIS > 4, in order to exclude pure AD; and EDI > 0.2, which indicate that these subjects had executive dysfunction; (b) abrupt or insidious onset of cognitive impairment after stroke, followed by a fluctuating, stepwise deteriorative, or gradually progressive course.
3. VaD stroke patients met the following criteria: (a) satisfied the DSM-IV dementia criteria [25] and the
Biochemical analysis

The sRAGE, BACE1, and NEP in serum were measured by commercial double sandwich enzyme-linked immunosorbent assay (ELISA) kits (R&D, DY1145E; Cusabio, CSB-E09824h; Cusabio, CSB-E13347h) according to the manufacturer’s instructions. APOE genotype analysis was performed by polymerase chain reaction–restriction fragment length polymorphism [33]. DNA was purified from leukocytes by the Wizard Genomic DNA Purification kit. Genomic DNA was amplified by PCR with the primers (F, 5’-TCCAAGGAGCTGACGGCAGCGC-3’; R, 5’-GGCTAAGGTACACTGCA-3’) to yield a 218-bp DNA fragment that spans both APOE polymorphic sites. Amplified DNA (15 μl) was digested simultaneously with 2.5 U of AflIII and 5 U of HaeII (New England Biolabs) for 24 h at 37°C, analyzed on 4% agarose gel, and visualized by ethidium bromide staining. All of these blood samples were analyzed at the same time using one batch of reagents at the laboratory of the Pharmaceutical Biotechnology Department of Nanjing University.

Statistical analysis

All statistical calculations were performed using SPSS 17.0. Data were presented as mean ± SEM. Intergroup differences were analyzed by one-way analysis of variance (ANOVA), with post hoc Newman-Keuls testing for multiple groups. Frequency data were analyzed by Pearson’s correlation coefficient (r). The predictive value of variables was tested by means of logistic regression analysis. Statistical significance was set at 0.05.

Results

Clinical characteristics

Two weeks after stroke, the 152 inpatients were divided into the following groups: stroke (64), VCIND (37), VaD (36), and MD (15), according to cognitive assays. The demographic differences between the NC and stroke group were not significant (p > 0.05). The male-to-female ratios and diabetes mellitus incidences among these groups were also not significantly different (p > 0.05). The age, education, hypertension incidences, and NIHSS and mRs scores in the VaD group were significantly different (p < 0.05) from those of the stroke group (Table 1).

Neuropsychological assessment

As shown in Table 2, patients with cognitive impairment had higher scores in IQCODE, ADL, CDR, EDI, and NPI assessments compared with NC and stroke patients (p < 0.05). Patients with cognitive impairment had, however, lower scores in MMSE, MOCA, CDT, digit span (in order), digit span (backward), and digit-symbol coding tasks (p < 0.05).

MRI characteristics

A total of 123 subjects underwent MRI, including 58 in the stroke group, 28 with VCIND, 27 with VaD, and 10 with MD. Compared with the stroke group, the percentages of images revealing white matter lesions in the VCIND and VaD were significantly higher. A similar tendency

Table 1 Clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>NC (n = 40)</th>
<th>Stroke (n = 64)</th>
<th>VCIND (n = 37)</th>
<th>VaD (n = 36)</th>
<th>MD (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64.0 ± 12.6</td>
<td>62.1 ± 1.6</td>
<td>65.5 ± 1.7</td>
<td>73.8 ± 2.1*</td>
<td>74.6 ± 2.2*</td>
</tr>
<tr>
<td>Education (years)</td>
<td>8.6 ± 4.6</td>
<td>8.2 ± 0.5</td>
<td>6.6 ± 0.8</td>
<td>5.6 ± 0.7*</td>
<td>7.3 ± 1.3</td>
</tr>
<tr>
<td>Male</td>
<td>13 (32.5%)</td>
<td>44 (68.8%)</td>
<td>19 (59.4%)</td>
<td>18 (58.1%)</td>
<td>6 (40.0%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>21 (52.5%)</td>
<td>41 (64.1%)</td>
<td>19 (59.4%)</td>
<td>25 (80.7%)*</td>
<td>9 (60.0%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>14 (35.0%)</td>
<td>42 (31.3%)</td>
<td>11 (29.7%)</td>
<td>11 (36.1%)</td>
<td>5 (33.3%)</td>
</tr>
<tr>
<td>NIHSS</td>
<td>–</td>
<td>3.7 ± 0.5</td>
<td>2.9 ± 0.6</td>
<td>5.0 ± 0.9*</td>
<td>2.7 ± 0.9</td>
</tr>
<tr>
<td>mRs</td>
<td>–</td>
<td>1.9 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>2.5 ± 0.3*</td>
<td>1.4 ± 0.4</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM. Gender, hypertension, and diabetes mellitus are presented as no. (%)

*p < 0.05 as compared with the stroke group
was seen with respect to subcortical lesions in the VaD, and subcortical and white matter lesions in the VCIND (Table 3).

Serum concentrations of sRAGE, BACE1, and NEP

Serum sRAGE, BACE1, and NEP are presented in Fig. 1. No significance difference was found between the NC and stroke group. The sRAGE in the VCIND, VaD, and MD groups was significantly decreased to 63.60% (p < 0.05), 55.75% (p < 0.05), and 43.43% (p < 0.01), respectively, in the stroke group (Fig. 1a). The BACE1 in the VCIND and VaD groups increased by 1.28- (p < 0.05) and 1.36-fold (p < 0.05), respectively, compared to the stroke group (Fig. 1b). Similarly, NEP in the VaD was increased by 1.45-fold (p < 0.05) in the stroke (Fig. 1c).

APOE genotype

As Table 4 shows, the percentages of the ε3/ε4 genotype in the VCIND, VaD, and MD groups increased by 1.98- (p < 0.05), 2.29- (p < 0.05), and 3.05-fold (p < 0.05), respectively, compared to the stroke group. The percentage of ε4/ε4 genotypes in the MD group was also higher than that in the stroke group (4.23-fold higher, p < 0.05). The frequency of the ε4 allele in the VCIND, VaD, and MD rose by 1.73- (p < 0.05), 2.17- (p < 0.05), and 3.32-fold (p < 0.05), respectively, when compared to the stroke group.

Table 2 Neuropsychological assessment of subjects

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>Stroke</th>
<th>VCIND</th>
<th>VaD</th>
<th>MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>IQCODE</td>
<td>3.2 ± 0.1</td>
<td>3.3 ± 0.3</td>
<td>3.6 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.3 ± 0.3</td>
<td>27.6 ± 0.2</td>
<td>25.5 ± 0.5</td>
<td>15.2 ± 1.1</td>
<td>16.3 ± 1.5</td>
</tr>
<tr>
<td>MOCA</td>
<td>27.1 ± 0.3</td>
<td>26.2 ± 0.2</td>
<td>19.7 ± 0.6</td>
<td>11.8 ± 0.8</td>
<td>13.5 ± 1.1</td>
</tr>
<tr>
<td>CDT</td>
<td>3.5 ± 0.1</td>
<td>3.6 ± 0.1</td>
<td>2.3 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>ADL</td>
<td>22.8 ± 0.9</td>
<td>32.3 ± 1.4</td>
<td>32.0 ± 1.8</td>
<td>45.1 ± 3.1</td>
<td>38.7 ± 5.2</td>
</tr>
<tr>
<td>CDR</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>EDI</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>HIS</td>
<td>–</td>
<td>–</td>
<td>6.3 ± 0.2</td>
<td>8.1 ± 0.4</td>
<td>5.7 ± 0.3</td>
</tr>
<tr>
<td>Digit span (in order)</td>
<td>8.0 ± 0.2</td>
<td>8.3 ± 0.1</td>
<td>6.3 ± 0.2</td>
<td>4.9 ± 0.4</td>
<td>6.3 ± 0.5</td>
</tr>
<tr>
<td>Digit span (backward)</td>
<td>4.2 ± 0.3</td>
<td>4.7 ± 0.1</td>
<td>3.6 ± 0.2</td>
<td>2.3 ± 0.3</td>
<td>2.7 ± 0.5</td>
</tr>
<tr>
<td>Digit-symbol coding</td>
<td>34.6 ± 1.7</td>
<td>30.0 ± 0.5</td>
<td>13.9 ± 5.9</td>
<td>6.2 ± 1.0</td>
<td>5.3 ± 1.7</td>
</tr>
<tr>
<td>NPI</td>
<td>0</td>
<td>0</td>
<td>0.1 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td>HAMD</td>
<td>3.7 ± 0.6</td>
<td>3.0 ± 0.2</td>
<td>4.2 ± 0.3</td>
<td>4.7 ± 0.3</td>
<td>4.1 ± 0.7</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM

IQCODE Informant Questionnaire of Cognitive Decline, MMSE Mini-Mental State Examination, MoCA Montreal Cognitive Assessment, CDT clock-drawing task, ADL activities of daily living, CDR clinical dementia rating, EDI executive dysfunction index variable, HIS Hachinski ischemic score, NPI neuropsychiatric inventory, HAMD Hamilton depression rating scale

Table 3 MRI characteristics of the stroke subjects

<table>
<thead>
<tr>
<th></th>
<th>Stroke</th>
<th>VCIND</th>
<th>VaD</th>
<th>MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of infarct (mm³)</td>
<td>3,318.1 ± 1,078.0</td>
<td>2,943.1 ± 587.6</td>
<td>5,075.4 ± 1,042.0</td>
<td>1,882.3 ± 337.3</td>
</tr>
</tbody>
</table>

One hundred twenty-three subjects had an MRI scan, including stroke 58, VCIND 28, VaD 27, and MD 10. Infarct characteristics are presented as no. (%). Infarct volumes are expressed as mean ± SEM

* p < 0.05 as compared with the stroke group
Correlations between serum biomarkers and clinical rating scales

Figure 2 indicates that there was a negative correlation between the serum level of sRAGE and the results of the IQCODE, ADL, CDR, EDI, HAMD, and NPI assessments. However, the MMSE, MOCA, CDT, digit span (in order and backwards), and digit-symbol coding assessments were positively correlated with serum levels of sRAGE. In addition, there was a negative relationship between the serum levels of BACE1 and the results of the MMSE, MOCA, CDT, digit span (backwards), and digit-symbol coding assessments. The serum level of NEP was negatively correlated with MOCA, CDT, EDI, digit span (in order and backwards), and digit-symbol results, and positively correlated with CDR and HIS results as well as the serum level of BACE1. There was no correlation between the volume of infarcts and the serum concentrations of biomarkers ($p > 0.05$). The effects of age, education, and the serum concentrations of biomarkers and the APOE ε4 allele on cognitive impairment prediction were tested by means of logistic regression analysis in all subjects. As shown in Table 5, only age (OR 1.045, 95% CI 1.003–1.089), serum sRAGE (OR 0.992, 95% CI 0.990–0.995), and BACE1 (OR 1.007, 95% CI 1.002–1.013) could predict cognitive impairment significantly ($p < 0.05$).
Discussion

This study indicates for the first time that changes in the serum levels of BACE1, sRAGE, and NEP are correlated with clinical manifestation of cognitive impairment after stroke, which itself has an association with the extent of cognitive impairment among acute ischemic stroke patients. Furthermore, stroke severity and lesion volume did not significantly modify this relationship, suggesting that serum BACE1, sRAGE, and NEP may be suitable early biomarkers of cognitive impairment post-stroke.

Increasing evidence has shown that aggregated Aβ is a major constituent of senile plaques in the brain; these plaques play a principal role in the pathogenesis of AD and VaD [2, 13, 15]. Moreover, Aβ deposits occur in VaD, and there is an overlap of risk factors and pathology between AD and VaD [2].

The steady-state level of Aβ in the brain is determined by the balance between its production and removal. The former is mainly related to BACE1, which cleaves amyloid precursor protein (APP) to generate Aβ peptide [30]; the latter is through sRAGE binding to Aβ, preventing free circulating Aβ to be transported into the central nervous system [24], as well as NEP, one of the major Aβ-degrading enzymes, degrading the accumulation of Aβ in the brain [29].

Previous research indicated that elevated BACE1 protein levels and activity occurred in postmortem AD brains [4]. The origin of BACE1 in blood cells or other tissues (heart, liver, muscle, etc.) remains unclear. Platelets are reported to contain APP and BACE1. High platelet BACE1 activity is present in MCI and AD patients [6]. AD and MCI patients also have higher BACE1 activity in the CSF. There is also strong positive correlation between CSF BACE1 activity and total tau levels in all MCI subgroups [31]. Furthermore, the low level of sRAGE was associated with VaD, AD, and severe leukoaraiosis. More importantly, a highly significant reduction in sRAGE levels is observed in AD [8, 22]. In addition, lower CSF NEP activity levels have been reported in dementia with Lewy body disease patients, but serum NEP activity levels were not significantly different in this study between the dementia and normal groups [20]. However, there are few reports of BACE1, RAGE, and NEP available in the context of VaD.

This study found that serum BACE1 and NEP levels were higher, and the sRAGE level was lower, in cases of post-stroke cognitive dysfunction relative to the group with stroke alone. These levels were altered at stroke onset (on
admission), while the clinical diagnosis of cognitive dysfunction was possible at 2 weeks post-stroke, suggesting that BACE1, sRAGE, and NEP levels could help neurologists to predict post-stroke cognitive impairment and treat the patients early.

In this study, we did not find any significant correlation between the volume of the infarcts and post-stroke dementia, consistent with the report of Assia Jaillard [10]. We also found that the APOE $\varepsilon 4/4$ genotype and $\varepsilon 4$ allele frequencies were higher in groups of patients experiencing post-stroke cognitive impairment than in stroke patients without cognitive impairment. The evidence indicates that both stroke and ApoE $\varepsilon 4$ are risk factors for dementia or AD [18]. The risk of AD associated with stroke was increased five- to six-fold in individuals with the ApoE $\varepsilon 4$ allele, and the increased risk of any dementia associated with stroke was increased three fold in individuals without the ApoE $\varepsilon 4$ allele [26]. In contrast, one longitudinal cohort study and one cross-sectional study did not find that ApoE allele status influenced the risk of any dementia associated with stroke [12]. In this study, correlation analysis revealed that the serum levels of sRAGE, BACE1, and NEP had a significant relationship with the results of neuropsychological assessments, including the MMSE, MOCA, CDT, EDI, CDR, and other tests. Logistic regression analysis, however, suggested that only age and serum concentrations of sRAGE and BACE1, and not education, NEP levels, and APOE $\varepsilon 4$ alleles, were significant for the prediction of cognitive impairment after stroke.

This study has several limitations. First, this was a single-center study. Multicenter trials with larger cohorts are needed. Second, we measured the biomarkers and neuropsychological assessments at only a single time point, and did not follow up.

In conclusion, the findings here suggest that sRAGE and BACE1 may be potential biomarkers for early diagnosis of cognitive impairment post-stroke, although more evidence is needed to be conclusive.

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Conflicts of interest None.

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