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Antithrombin III associated with fibrinogen predicts the risk of cerebral ischemic stroke

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ABSTRACT

Background and purpose: The purpose of this study is to examine the feasibility of developing plasma predictive value biomarkers of cerebral ischemic stroke before imaging evidence is acquired.
Methods: Blood samples were obtained from 198 patients who attended our neurology department as emergencies – with symptoms of vertigo, numbness, limb weakness, etc. – within 4.5 h of symptom onset, and before imaging evidence was obtained and medical treatment. After the final diagnosis was made by MRI/DWI/MRA or CTA in the following 24–72 h, the above cases were divided into two groups: stroke group and non-stroke group according to the imaging results. The levels of baseline plasma antithrombin III (AT-III), thrombin–antithrombin III (TAT), fibrinogen, D-dimer and high-sensitivity C-reactive protein (hsCRP) in the two groups were assayed.
Results: The level of the baseline AT-III in the stroke group was $118.07 \pm 26.22\%$, which was lower than that of the non-stroke group ($283.83 \pm 38.39\%$). The levels of TAT, fibrinogen, hsCRP were 7.24 ± 2.28 μg/L, 5.49 ± 0.98 g/L, and 2.17 ± 1.07 mg/L, respectively, which were higher than those of the non-stroke group (2.53 ± 1.23 μg/L, 3.35 ± 0.50 g/L, 1.82 ± 0.67 mg/L). All the *P*-values were less than 0.001. The D-dimer level was 322.57 ± 60.34 μg/L, which was slightly higher than that of the non-stroke group (305.76 ± 49.52 μg/L), but the *P*-value was 0.667. The sensitivities of AT-III, TAT, fibrinogen, D-dimer and hsCRP for predicting ischemic stroke tendency were 97.37%, 96.05%, 3.29%, 7.89%, but the specificity was 93.62%, 82.61%, 100% and 100%, respectively, and all the *P*-values were less than 0.001. High levels of D-dimer and hsCRP were mainly seen in the few cases with severe large-vessel infarction.
Conclusions: Clinical manifestations of acute focal neurological deficits were associated with plasma AT-III and fibrinogen. These tests might help the risk assessment of acute cerebral ischemic stroke and/or TIA with infarction tendency in the superacute stage before positive imaging evidence is obtained.

1. Introduction

Acute cerebral stroke is one of the leading causes of mortality and the most common cause of complex chronic disability worldwide [1,2]. Stroke survivors often suffer from long-term neurological disabilities, significantly reducing their ability to integrate effectively in society, which results in a heavy economic burden both for the family and for society [2]. The narrow time-window for successful treatment is the reason for the need of rapid and well-organized out-of-hospital and in-hospital systems of care. From the establishment of the penumbra concept, ischemic stroke has been recognized as a dynamic process [3]. Reopening the occluded artery and preventing ischemia quickly, before clear CT/MRI imaging evidence of cerebral infarction, can lead to good outcomes. Up to now, the only registered treatment with proven efficacy is thrombolysis with intravenous administration of recombinant tissue plasminogen activator (rt-PA), which has a very limited time window for use [4], and it is usually less than 3.0–4.5 h after the onset of symptoms [4–6]. In the acquired time-window, the earlier the thrombolysis performed, the better is the clinical outcome. So, promptly and correctly diagnosing ischemic stroke in the superacute stage, before clear imaging evidence, is the key step to successfully treating patients within the thrombolysis acquired time-window. However, absence of a sensitive and widely avail-

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able biomarker which can be used to predict cerebral ischemic stroke before imaging evidence is still the major problem at present. According to the study in 1998 among 2947 patients who were hospitalized for acute cerebral infarction [7], less than 1% with ischemic stroke received rt-PA for thrombolysis. Up to now, the absence of widely available and sensitive diagnostic tests for cerebral ischemic stroke and/or transient ischemic attack (TIA) with thrombophilia in the pre-symptomatic period or in the super-acute stage after onset remains a significant limitation. The diagnosis of acute ischemic stroke (especially TIA with thrombophilia) at most hospitals is made solely on clinical grounds after intracranial hemorrhage or a mass lesion is ruled out by CT and clinical scores [8]. However, other potential causes for acute focal neurological deficits, including complex migraine, demyelinating disease, cervicogenic vertigo, vertebral artery-type cervical spondylosis or even metabolic disturbances such as hypoglycemia, may be difficult to differentiate from acute ischemic stroke [9]. At most institutions, CT of the brain is performed as part of the initial evaluation of a patient with suspected stroke. The main advantage of this imaging modality is its widespread availability and sensitivity for hemorrhage, but it is usually insensitive to ischemic changes in the first few hours of cerebral ischemic stroke onset and is usually of little value for establishing the diagnosis of cerebral infarction in the early period of stroke [10]. Several technologies based on MRI have shown its advantage for the early diagnosis of cerebral ischemic stroke. For example, diffusion-weighted imaging (DWI) can demonstrate parenchymal changes early in the presentation of cerebral ischemic stroke [11]. In theory, a full MRI study, including diffusion- and perfusion-weighted imaging (DWI/PWI), MR angiography, and standard T1- and T2-weighted images, could be performed within 30 min [12]. However, as a practical issue, most hospitals do not have these specialized MRI services available in the acute setting. Thus, without a practical and widely available radiological test, the diagnosis of super-acute cerebral ischemic stroke remains largely a clinical decision. Although several simplified clinical stroke scales have been developed for the early evaluation of patients with suspected cerebral ischemia, at present they have limited utility. Obviously, any clinical neurological screening test will be limited by the training and experience of the examiner. This suggests an adjunctive clinical test is needed which can provide diagnostic information above and beyond screening clinical examinations. At many community hospitals, a formal stroke team or vascular neurologist may not be available, and the lack of a confirmatory diagnostic test may contribute to a physician's reluctance to initiate thrombolytic therapy. So another approach to support the diagnosis of acute ischemic stroke is greatly needed, which is the evaluation of plasma biomarkers showing the imbalance between coagulation and fibrinolysis [13]. However, the laboratory tests may be supportive but cannot substitute the clinical judgments. It is advisable that thrombolysis be performed only in centers where expert neurologists can take the decision according to the evidences obtained from many aspects. The markers of impaired hemostasis and thrombosis change within minutes or hours before and/or after clinical ischemic symptom occur. In addition, a rapid diagnostic tool would also be valuable in clinical trials evaluating novel therapeutic interventions to improve functional outcome after ischemic stroke. In this study, we noticed that some biomarkers might be interesting tools in supporting the diagnosis of ischemic stroke, and examined the feasibility of developing a novel panel of biomarkers, taking into account the complexity of the ischemic cascade to diagnose acute ischemic stroke.

Antithrombin III (AT-III) is synthesized by the endothelium of blood vessels and in the liver, which have combining sites for heparin and thrombin respectively. Once thrombin is produced, which is linked with AT-III with a 1:1 ratio and forms thrombin–antithrombin III (TAT) compound, it inhibits the thrombosis [14]. So AT-III and TAT are the biomarker in the earlier stage of blood coagulation activation.

Plasma fibrinogen is a kind of acute-phase reactive protein, which is a kind of blood coagulation factor and inflammation marker. It is deposited on the vessel wall after being changed to fibrin by thrombin, and combined with the glycoprotein IIb/IIIa receptor on the surface of the platelet membrane to accelerate platelet aggregation [15].

Plasma D-dimer is a specific degradation product of cross-linked fibrin hydrolyzed by fibrinolysin. Generally, a hypercoagulable state and secondary fibrinolysis exists *in vivo* in acute cerebral ischemic stroke, with a high D-dimer level [13,16].

High-sensitivity C-reactive protein (hsCRP), an inflammatory marker, is also a kind of acute phase reactive protein. There is evidence to show that it is associated with the risk of myocardial infarction (MI) [17].

We sought to develop and evaluate the effectiveness of these plasma biomarkers because they might be useful tools to identify patients with ischemic stroke in the super-acute stage, in order to increase the proportion of such patients receiving appropriate treatment, such as acute rtPA thrombolysis.

2. Subjects and methods

2.1. Patient enrolment

2.1.1. Inclusion criteria

(1) Patients from 40 to 70 years of age were coming from two stroke services in adjacent geographical regions in Beijing, China. (2) First-ever or recurrent sudden onset within 4.5 h of the symptoms grouped as limb numbness or weakness, dyskinesia, dysphasia, balance disturbance, diplopia, dizziness/vertigo/coordination, speech/language confusion, decreased level of consciousness, headache, visual changes and other local neurological symptoms. (3) The symptoms and signs above lasted for more than one hour. NIHSS scores were from 7 to 22. (4) Cerebral hemorrhage was excluded by emergency brain CT scan. (5) TIA diagnosis was according to the ICD-10 (ICD 10: G45.901) criteria, and TIA with thrombophilia (the short-term stroke risk after TIA) was valued with $ABCD^2$ criteria [18,19] and MRA/CTA imaging (the evidence of the local narrowing and the filling defect in the culprit artery).

2.1.2. Exclusion criteria

(1) Patients were on an anticoagulant therapy. (2) Patients with a clear family history of cerebral thrombosis at age less than 40 were excluded. (3) Patients who were suffering from cancer, infection, bone fracture, and cardiac insufficiency were excluded. (4) Hepatic and renal inadequacy (including alanine aminotransferase >40 U/L, aspartate aminotransferase >35 U/L, alkaline phosphatase >190 U/L, γ-GT >200 U/L, lactate dehydrogenase >240 U/L, urea nitrogen >7.9 mmol/L, creatinine >133 μmmol/L, and glomerular filtration rate <90 mL/min/1.73 m^2, calculated with MDRD formula) were excluded. (5) Recent child birth and contra-conceptive medicine use in the last month. (6) Atrial fibrilation demonstrated by electrocardiogram was excluded. (7) Most severe as well as the mildest forms were excluded (NIHSS scores less than 7 and more than 22) to avoid the affects of other confounding factors in the biomarkers. (8) Myelodysplastic syndrome (MDS) was excluded [20].

2.1.3. Documented risk factors

Documented risk factors included age, sex, smoking status, hypertension, diabetes, and hypercholesterolemia. Smoking status was categorized as current (patient admission of ≥1 cigarette