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Original Article

Serum mannose-binding lectin levels in patients with ischemic stroke

Ji Huang[1*], Xiao-Jun Ma[2*], Qiang Li[3], Guang-Jun Nie[3]

[1]*Department of Neurology, Tianmen First People's Hospital, Tianmen 431700, Hubei Province, P. R. China;* [2]*Department of Neurology, Traffic Hospital of Shandong Province, Jinan 250031, Shandong Province, P. R. China;* [3]*Department of Neurology, Central Hospital of Zibo, Zibo 255036, Shandong Province, P. R. China.* [*]*Equal contributors.*

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Abstract: Background: The purpose of this study was to investigate the serum levels of mannose-binding lectin (MBL) in the Chinese patients with acute ischemic stroke (AIS). Method: We conducted a case-control study at the emergency department of our hospital. Serum MBL levels and routine test were examined. Its value for biomarker diagnosis was appreciated through receiver operating curve (ROC). The National Institutes of Health Stroke Scale (NIHSS) score was assessed on admission blinded to MBL levels. Results: The results indicated that the serum MBL levels were significantly (P<0.0001) higher in AIS patients as compared to normal controls (1452; IQR, 1030-2232 ug/L *VS.* 903; IQR, 678-1193 ug/L, respectively). There was a modest correlation between levels of serum MBL and NIHSS score (r=0.704, P<0.0001). The median MBL levels in patients with artery arteriosclerosis were significantly higher than other stroke subtype (1689 [IQR, 1260-2785] *vs.* 1109 [IQR, 915-1614] ug/L, respectively; P<0.0001). Based on the ROC curve, the optimal serum concentration of MBL as a surrogate marker to support the diagnosis of ischemic cerebral injury was found to be 1080 ug/L, which yielded a sensitivity of 79.4% and a specificity of 71.3%, the area under the curve was 0.764 (95% CI, 0.707-0.826). Conclusion: The discovery study presented here show serum MBL at admission are related with stroke severity and subtype. MBL levels also could be considered as an independent stroke diagnosis marker in Chinese population.

Keywords: Mannose-binding lectin, acute ischemic stroke, risk, Chinese

Introduction

China has 2.5 million new stroke cases every year. In addition, approximately 1.6 million stroke patients will die and 7.5 million stroke survivors every year (15~30% of survivors will be permanently disabled), which has exceeded heart disease to become the second leading cause of death and adult disability [1]. Stroke places a tremendous burden on health resources in China. Early and accurate prediction of outcome in stroke is important and influences risk optimized therapeutic strategies [2].

Mannose-binding lectin (MBL) can activate the complement system independent of antibodies via MBL-associated serine proteases, thereby initiating the so-called MBL pathway of complement activation [3]. There is increasing evidence that the lectin pathway of complement activation plays an important role in the modulation of inflammation [4]. MBL deficiency has been shown to be associated with increased susceptibility to many diseases, for instance, myocardial ischemia [5] and atherosclerosis in different clinical situations [6]. In the cardiovascular system, there seems to be a fine balance as to when low MBL levels may be harmful [7] or beneficial [8] suggesting a role of MBL that is strongly dependent on the type of inflammation. In this study, we aimed to investigate the serum levels of MBL at admission in Chinese patients with AIS, and to assess the relation between MBL and stroke severity, subtype and risk.

Methods

Patients and study design

We conducted a case-control study at the emergency department of the Central Hospital of

Zibo, China. From November 2014 to October 2015, all patients with first-ever acute ischemic stroke were included. All patients were admitted within 24 hours of experiencing a new focal or global neurological event. Brain imaging (either CT or MRI) was performed routinely within 24 hours after admission. Exclusion criteria included: malignant tumor, intracerebral hemorrhage, precipitants of ischemic cerebral symptoms, renal insufficiency (serum creatinine >175 umol/L), severe edema, febrile disorders, acute and subclinical infections (A subclinical infection is one that is present in a host who shows no outward signs of symptoms and cannot be diagnosed without testing for a specific infectious agent) and severe liver dysfunction.

One hundred and seventy-five age- and sex-matched subjects (control group) were included as controls. The healthy subjects were enrolled from the Health Physical Examination Center of our hospital. They had no known diseases and were not using any medication. Records of potential controls were reviewed by a neurologist (not an author) to exclude the presence of stroke, other types of diseases. The Institutional Review Committee on Human Research of the Central Hospital of Zibo approved the study protocol. The patients or their relatives gave written informed consent prior to entering the study.

Clinical variables and neuroimaging

The following demographical and clinical data were taken: gender, age, body mass index (BMI) and history of conventional vascular risk factors (hypertension, coronary heart disease, family history for stroke, diabetes mellitus, atrial fibrillation, hyperlipoproteinemia, smoking habit, and alcohol abuse) were obtained. Stroke severity was assessed on admission using the National Institutes of Health Stroke Scale score by a neurologist [9]. The clinical stroke syndrome was determined by applying the criteria of the Oxfordshire Community Stroke Project [10] and Stroke subtype was classified according to TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria [11]. MRI with diffusion-weighted imaging (DWI) was available in some patients (N=155). In those patients, DWI lesion volumes were determined by one experienced neurologist unaware of the clinical and laboratory results. The infarct volume was calculated by using the formula $0.5\times a\times b\times c$ (where a is the maximal longitudinal diameter, b is the maximal transverse diameter perpendicular to a and c is the number of 10-mm slices containing infarct) [12].

Blood collection and quantification

Venous blood samples were taken in the morning's fasting state [within 0-3 (n=19), 3-12 (n=62), 12-24 (n=61) and 24-48 (n=33) hours from symptom onset]. The blood were taken into a serum tube and left to clot and serum was collected after centrifugation (After at least 30 min, but within 2 h, the tubes were centrifuged at 25°C for 10 min at 3,000 g, and the sera were stored frozen in plastic vials at -80°C until the time of consecutive analyses). Baseline serum samples were evaluated for stroke along with sex-age matched disease-free controls. MBL was measured by time-resolved immune-fluorometricassay on serum samples. Microwells coated with anti-MBL antibody were incubated with dilutions of patient serum, were developed with europium-labeled anti-MBL antibody, and europium was quantified with time-resolved fluorometric assay (Baoman Biological Technology Co., Ltd, Shanghai, China). The detection limit was 1.5 ug/L. The standard concentrations in these kits range from 1.5 to 100 ug/L, providing a range of 150-10000 ug/L at 1/100 dilution. The coefficients of variation (CV) for the intra- and inter-assay reproducibility are 5 and 9%, respectively.

Statistical analysis

Results are expressed as percentages for categorical variables and as medians (interquartile ranges, IQRs) for the continuous variables. The Mann-Whitney U test and Chi-square test were used to compare the two groups. Correlations among laboratory parameters were analyzed using Spearman's rank correlation test. Associations between severity of stroke evaluated by NHISS scores and serum protein markers were assessed using ordered logistic regression models in multivariate adjustment with possible confounders, namely, age, gender, conventional vascular risk factors, stroke syndrome and stroke subtype. A logistic regression analysis was used to evaluate the risk of stroke according to serum levels of protein markers. Results were expressed as adjusted

Table 1. Basal characteristic of patients with acute ischemic stroke

Baseline Characteristics	AIS patients (n=175)	Normal cases (n=175)	P[a]
Female sex, n (%)	77 (44.0)	77 (44.0)	NS
Median age (yr, IQR)	68 (57-75)	68 (57-75)	NS
BMI (kg/m^2, IQR)	25.8 (23.2-27.2)	25.5 (23.0-26.9)	NS
NIHSS Score (IQR)	7 (4-11)	-	
Systolic blood pressure (mmHg, IQR)	160 (145-170)	120 (105-130)	<0.001
Diastolic blood pressure (mmHg, IQR)	90 (85-100)	80 (70-85)	<0.001
Vascular risk factors			
Hypertention, n (%)	112 (64.0)	-	
Diabetes at baseline, n (%)	62 (35.4)	-	
Hypercholesterolemia, n (%)	71 (40.6)	-	
Atrial fibrillation, n (%)	31 (17.7)	-	
Coronary heart disease, n (%)	50 (28.6)	-	
Family history of stroke, n (%)	33 (18.9)	15 (8.5)	0.022
Cigarette smoking, n (%)	47 (26.9)	45 (25.7)	NS
Alcohol drinking, n (%)	41 (23.4)	42 (24.0)	NS
Time from onset to inclusion, (hr, IQR)	3.8 (2.0-8.5)	-	
Infarct volume (N=155; mL, IQR)	19 (10-42)	-	
TPA-T no. (%)	63 (36.0)	-	
Pre-stroke treatment, no. (%)	93 (53.1)	-	
TOAST classification			
a. Large artery, n (%)	29 (16.6)	-	
b. Small artery, n (%)	31 (17.7)	-	
c. Cardioembolism, n (%)	68 (38.9)	-	
d. Other cause, n (%)	16 (9.1)	-	
e. Unknown, n (%)	31 (17.7)	-	
Stroke syndrome			
a. TACS, n (%)	25 (14.3%)	-	
b. PACS, n (%)	71 (40.6%)	-	
c. LACS, n (%)	28 (16.0%)	-	
d. POCS, n (%)	51 (29.1%)	-	
Laboratory findings (Median, IQR)			
Total cholesterol (mmol L^{-1})	4.29 (3.44-5.18)	4.15 (3.40-5.12)	NS
Triglycerides (mmol L^{-1})	1.46 (1.20-1.75)	1.44 (1.22-1.75)	NS
High-density lipoproteins (mmol L^{-1})	1.40 (1.11-1.74)	1.52 (1.28-1.99)	0.036
Low-density lipoproteins (mmol L^{-1})	2.03 (1.21-2.59)	2.01 (1.22-2.64)	NS
FBG (mmol L^{-1}) 7.05 (6.18-8.55)	5.92 (4.73-7.12)	5.21 (4.51-5.70)	0.005
HCY, μmol/L	16.2 (12.0 -19.1)	13.8 (10.2-15.9)	<0.001
Hs-CRP, mg/dL	0.39 (0.23-0.59)	0.20 (0.12-0.33)	<0.001

[a]*p* value was assessed using Mann-Whitney U test or Chi-Square test. NS, not significant; IQR, interquartile range; Hs-CRP: high-sensitivity-C Respond Protein; HCY: homocysteine; FBG, fasting blood glucose; TACS, total anterior circulation syndrome; PACS, partial anterior circulation syndrome; LACS, lacunar syndrome; POCS, posterior circulation syndrome; PA-T: Tissue plasminogen activator-treated.

OR (odds ratios) with the corresponding 95% confidence interval (CI). Receiver operating characteristics (ROC) curves were constructed by calculating the sensitivities and specificities of a biomarker or the diagnostic score of a logistic regression model at different cutoff points for differentiating stroke cases from normal. All statistical analysis was performed with

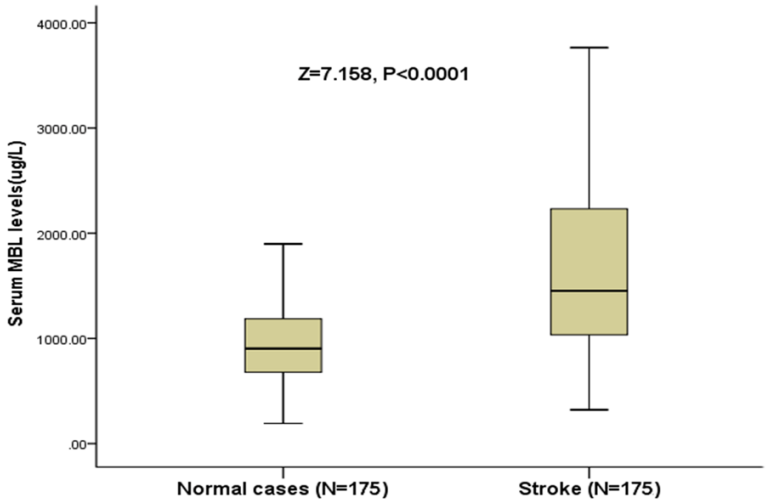

Figure 1. Serum MBL levels in acute ischemic stroke patients and control group. Mann-Whitney U-test. All data are medians and in-terquartile ranges (IQR). Significantly higher in stroke patients as compared to normal cases (P<0.0001). MBL = Mannose-binding lectin.

SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as P<0.05.

Results

In this study, 220 patients were screened and 175 patients with acute ischemic stroke were diagnosed and included in the analysis (18 with transient ischemic attack, 8 with hemorrhagic stroke, 6 with onset of symptoms >24 hours, 8 without informed consent, 3 with systemic infections and 2 with malignant tumor were not analyzed). The median age of patients was 68 (IQR, 57-75) years, while 44.0% were women. Thirty-three (18.9%) patients had a family history of stroke event. The median NIHSS score on admission was 7 points (IQR, 4-11). Six patients had NIHSS scores greater than 20 at the time of admission. The median time from stroke onset to inclusion in the study was 3.8 (IQR, 2.0-8.5) hours. Baseline characteristic in patients with acute ischemic stroke are provided in **Table 1**.

The results indicated that the serum MBL levels were significantly (P<0.0001) higher in AIS patients as compared to normal controls (1452; IQR, 1030-2232 ug/L *VS.* 903; IQR, 678-1193 ug/L, respectively; **Figure 1**). Serum MBL levels increased with increasing severity of stroke as defined by the NIHSS score. There was a modest correlation between levels of serum MBL and NIHSS score (r=0.704, P<0.0001; **Figure 2**). There was still a significant positive trend between serum MBL levels and NIHSS score (P= 0.005), using ordered logistic regression after multivariate adjustment for above possible confounders. There were no correlation between levels of serum MBL levels and sex (P=0.543), age (P=0.324).

Interesting, we found that serum MBL levels in patients with diabetes mellitus were higher than in others patients (1604; IQR, 1135-2308 ug/L vs. 1282; IQR, 899-1870 ug/L, respectively; P=0.002). There was a modest correlation between levels of serum MBL levels and Hs-CRP (r=0.225, P=0.008). In addition, serum MBL levels was correlated with the serum HDL levels (r=0.206, P=0.011). We also examined the relationship between serum MBL levels and stroke subtype. The median MBL levels in patients with artery arteriosclerosis were significantly higher than other stroke subtype (1689 [IQR, 1260-2785] *vs.* 1109 [IQR, 915-1614] ug/L, respectively; P<0.0001). In patients for whom MRI data were available (n=134), there was a positive correlation between levels of MBL and the infarct volume (r=0.489, P<0.001). There was still a significant positive trend between serum MBL levels and infarct volume (P=0.006), using ordered logistic regression after multivariate adjustment for above possible confounders.

Based on the ROC curve, the optimal serum concentration of MBL as a surrogate marker to support the diagnosis of ischemic cerebral injury was found to be 1080 ug/L, which yielded a sensitivity of 79.4% and a specificity of 71.3%, the area under the curve was 0.764 (95% CI, 0.707-0.826). The positive predictive value was 82.4%, and the negative predictive value was 87.9%. In the univariate model matching for gender and age, MBL as a continuous variable was associated with an increased risk of AIS, after adjustment for above possible confounders (OR 1.002, 95% CI: 1.001-1.003; P<0.0001). In multivariate analysis, there was an increased

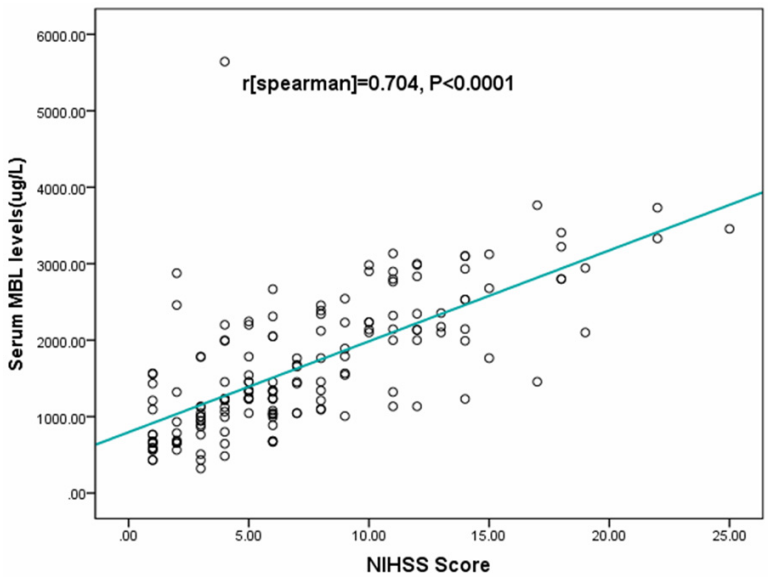

Figure 2. Correlation between the NIHSS score and serum MBL levels. MBL = Mannose-binding lectin; NIHSS = National Institutes of Health Stroke Scale.

risk of AIS associated with MBL levels ≥1080 ug/L (OR 4.03, 95% CI: 1.76-9.33) after adjusting for possible confounders.

Discussions

In this prospective study, we reported that serum MBL levels were significantly increased in cases of first AIS compared to age and gender matched normal controls. For the entire group, when adjusting for other possible risk factors, an elevated MBL level was an independent AIS risk factor, and serum MBL levels ≥1080 ug/L was associated with a 4.03-fold increase in AIS. In addition, we also found that that MBL levels increased with infarct volume, neurological deficit (assessed by the NIHSS) and stroke subtype. Recently, some clinical studies have provided evidence supporting activation of the complement system in patients with acute stroke [13]. Complement activation appears to contribute to the development of larger brain infarctions [14], hence depletion of complement results in beneficial effects [15], more readily observed in situations where the brain ischemia is followed by reperfusion [16]. MBL can activate the complement system independent of antibodies via MBL-associated serine proteases, thereby initiating the so-called MBL pathway of complement activation.

Wang et al. [17] found that elevated MBL levels could be considered as an independent stroke risk factor, suggesting a role of MBL and the lectin pathway of complement activation in the pathogenesis of stroke. Consistent with above conclusion, we also found that elevated MBL levels at admission was a stroke risk factor. Osthoff et al. [18] reported that MBL deficiency is associated with smaller cerebral infarcts and favorable outcome in ischemic stroke patients receiving conservative treatment, while Zhang et al. [19] reported that elevated serum MBL levels are a useful, complementary tool to predict functional outcome and mortality 90 days after stroke. In our study, the effects of circulating MBL on clinical outcome were not included in the study protocol. Further study should be considered.

The present study demonstrates that the MBL pathway serves as a critical mechanism of post-ischemic complement activation in the early post-stroke period. Recent work suggests that complement may promote both basal and ischemia-induced neurogenesis [20]. Multiple independent groups have examined the contribution of the classical pathway to stroke injury and have concluded that complement-mediated post-ischemic injury occurs independent of the classical pathway [21]. Two competing groups have recently published studies suggesting that MBL-mediated complement activation serves a deleterious role in stroke. The authors of these studies imply that MBL may serve as an effective therapeutic target in stroke [22, 23].

Some limitations of this observational study must be taken into account. First, MBL measurements were performed after the stroke and may not accurately reflect pre-stroke exposure. Second, some conditions which we did not realize could change serum levels of MBL. More work should be done to eliminate these effects in the future. Third, as the time of taking samples is not well defined (patients were

recruited within 24 hours after onset of symptoms) and serial samples were not taken, we cannot rule out whether increased MBL level is an atherosclerosis marker or rather reflects post-thrombotic state or related to initial stage of post-stroke infection. Lastly, further study might have needed a disease-positive control group as well, i.e. AMI, to demonstrate that the elevation of circulating MBL level indeed indicates something related more specifically to the ischemic brain damage.

Conclusions

In summary, the discovery study presented here show serum MBL at admission are related with stroke severity and subtype. MBL levels also could be considered as an independent stroke diagnosis marker in Chinese population. In the future, it is essential to evaluate the serum levels of MBL in patients with stroke and the relationship between MBL and clinical outcomes.

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Disclosure of conflict of interest

None.

Address correspondence to: Qiang Li, Department of Neurology, Central Hospital of Zibo, No 54, Gongqingtuan West Road, Zhangdian District, Zibo 255036, Shandong Province, P. R. China. Tel: +86-0533-2360441; E-mail: lwt9342@126.com

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