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ICAM-1 and CRP as biomarkers of 3-month outcome in acute ischaemic stroke

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ABSTRACT

Background It is clear that, inflammation deteriorates cerebral injury during the acute phase of stroke. While this process is going on, intercellular adhesion molecule-1 (ICAM-1) has a crucial role to play in mediating migration of immune cells into the damaged area. Furthermore, C reactive protein (CRP) is an essential inflammatory molecule in human organism. This research aims to investigate the association between ICAM-1, highly sensitive CRP(hs-CRP) and the prognosis of acute ischaemic stroke (AIS).

Methods 118 patients with AIS who were treated at Tashkent Medical Academy were participants in this research project. Blood samples were collected from patients on an empty stomach within 24 hours of admission. Modified Rankin Scale (mRS) was used in order to assess the functional prognosis in 3 months following the case of stroke in patients. The inadequate prognosis is described as mRS≥3. Each biomarker's potential to predict has also been evaluated with receiver operating characteristic analysis.

Results
ICAM-1 was identified to be an independent predictor of 3-month outcome (OR 1.05, 95 % CI 0.848 to 1.625; p=0.02) (area under the curve (AUC)=0.82 %). Independent associations with functional outcome were also found to be true for hs-CRP (OR 1.22, 95 % CI 0.78 to 1.86; p=0.03) (AUC=0.74 %).

Conclusions
The outcomes of a 3-month study carried out on patients with AIS showed ICAM-1 and hs-CRP to be independent predictors.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The higher tendency to suffer from hypertension and stroke has a direct link with highly sensitive C reactive protein (hs-CRP) and sICAM-1 among women. Furthermore, hs-CRP predicts further ischaemic events. Limited research has investigated the associations between ICAM-1 and stroke outcomes.

WHAT THIS STUDY ADDS

⇒ It has been the first research project to evaluate the prognostic capabilities of ICAM-1 and hs-CRP in 3 month period after stroke.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This research demonstrates potential role of ICAM-1 and hs-CRP as potential biomarkers to predict functional outcomes of acute ischaemic stroke and prognosis prediction helps to make decisions about therapies, monitoring and treatment at the different stages in stroke management.

INTRODUCTION

Stroke is considered to be one of the most frequent diseases on an international scale. The death and disability rate of stroke are significantly high, with 13.7 million new stroke cases in a year, and 5.8 million morbidity due to stroke.[1] For physicians, it is important to be able to predict outcome of acute ischaemic stroke (AIS) to come up with the most appropriate care. Prognosis prediction helps to make decisions about therapies, monitoring and treatment at different stages in stroke management. Primary clinical score, such as the National Institutes of Health Stroke Scale (NIHSS), is a reliable item to assess the effect of acute cerebral infarction. However, accurate outcome prediction methods have remained to be a challenge. Detecting and measuring blood biomarkers independent of other predictors of prognosis may allow for a more accurate prediction of outcome in AIS. The pathological processes that are underlying AIS are complicated.[2] Many recent study results demonstrate that inflammation plays an important role in the development and occurrence of AIS.[3] Cellular adhesion molecules and the cytokines development happen in the early stage of ischaemia and they play the part of the foundation for an ischaemic case evolving into an inflammatory one.[4–7] Several studies suggest that there is a correlation between short-term outcomes in AIS and C reactive protein (CRP).[8] Moreover, it was found that, there is a significant correlation between CRP levels with angina pectoris.[9] Cell adhesion molecules (CAMs) play an important role in the inflammation process after ischaemic injury[10] and provide

immune cells migration over the blood–brain barrier in cerebral parenchyma.[11] Intercellular adhesion molecule-1 (ICAM-1), also named CD54, contains five Ig domains.[12] According to the results of some studies, ICAM-1 concentrations are high in atherosclerosis, cardiovascular disorders and metabolic vascular neuropathy.[13–16] However, little is known about predictive ability of ICAM-1 and CRP in CVD. This study aims to examine the relationship between ICAM-1 and hs-CRP and outcome during a 3-month period.

SUBJECTS AND METHODS

Study population

This study was conducted from January 2022 to June 2022, all patients with AIS were consecutively included in the study and collected from the Department of Neurology of Tashkent Medical Academy. Patients were included if they met the following criteria: (1) aged 18 years or older and (2) admission for first-ever AIS within 24 hours; The exclusion criteria were as follows: (1) infection within 2 weeks before stroke; (2) patients with malignant tumour and autoimmune diseases; (3) history of transient ischaemic attack, cerebral infarction, intracranial haemorrhage, aneurysmal subarachnoid haemorrhage and venous sinus thrombosis; (4) severe hepatic and/or renal insufficiency. AIS sroke was defined by clinical presentation and proof of an ischaemic lesion or absence of a corresponding intracranial lesion other than infarction by using brain CT or MRI. Patients were not treated with thrombolysis as it is not available in our country. Among the enrolled patients, 149 were diagnosed with AIS, and there in 118 patients were involved in our analysis after excluding those with infection within 2 weeks before admission (n=3); malignant tumour and autoimmune diseases (n=2); history of stroke(n=9); severe hepatic and/or renal insufficiency (n=3) and patients without available data (n=14).[17] During the study, compliance with the Declaration of Helsinki was ensured and the local ethics committee confirmed that the researcher obeyed all the rules and regulations regarding moral standards. Respondents and their caretakers in the study voluntarily agreed to take part in the study and gave their full consent after finding out all the terms and conditions by signing the consent form.

Characteristics of patients with AIS

100% of the subjects who took part in the experiment went through a neurological check-up. Medical history (hypertension, diabetes, coronary heart disease and atrial fibrillation) and demographic data were gathered by asking questions. An electronic sphygmomanometer was employed to find out the blood pressure (BP) of patients. A neurologist identified the outcome following a 3-month period with the help of modified Ranking Scale (mRS) score.

Blood collection

For each included participant, blood samples were collected within 24 hours of admission. All blood samples centrifuged within 30 min at high speed and low temperature. Separated serum packed and stored in a refrigerator at −20°C until the measuring procedure started.

Biomarker measurements

The concentrations of serum hs-CRP and ICAM-1 were analysed by using the ELISA, which were produced by AO 'Вектор-Бест', Russia and BioVendor R&D, Czech Republic, respectively.

Functional outcome

The degree of disability or dependence on emergency department (ED) admission and 3-month period after AIS were assessed by using mRS. The score varied from 0 (no disability) to 6 (death), and each following grade showed progressive functional impairment. mRS<3 means a good prognosis, while a poor prognosis is determined as mRS≥3. Patients with AIS were divided into the good outcome group and poor outcome group according to the difference of mRS scores.

Statistical analyses

Continuous variables were reported as mean±SD. The receiver operating characteristic (ROC) curve is implemented to evaluate the cut point of ICAM-1 and hs-CRP to differentiate good from poor outcomes. Logistic regression analysis was used to determine the independent contribution of different variables to prognosis prediction. Statistical analysis was performed using SPSS V.29.0 software (SPSS), and a $p<0.05$ was set as statistically significant.

RESULTS

Characteristics of patients with AIS

Overall, 118 patients suffering from AIS whose mean age was 63.8 took part in the research. The mean systolic BP (SBP) was 154.4±28.6 mm Hg and diastolic BP was 91.8±7.9 mm Hg. Vascular risk factors included hypertension (n=111, 94%), diabetes mellitus (n=36, 30.5%), atrial fibrillation (n=17, 14.4%), coronary heart disease (n=11, 9.3%) and drinking (n=5%). According to the mRS score, they were divided into two groups, which are a good outcome group (n=72) and a poor outcome group (n=46). A good outcome was noticed in 61% of these patients. Clinical features and differences as well similarities between study subjects are represented on table 1 depending on outcome. The ages of patients with AIS in the good outcome group and poor outcome group were (62.97±7.07) and (64.71±12.28) years, respectively. First group included 62.5% of men with AIS, while second group consists of 45.6% men. Significant statistical difference was not determined between two groups in age and gender. In the following stage, we compared the anamnesis of both AIS patient groups and discovered that the occurrence of hypertension, diabetes, coronary heart disease, alcohol consumption and atrial fibrillation was not considerably different ($p>0.05$). As for clinical

indicators such as SBP, diastolic BP, there was also no significant statistical difference between the two groups (p>0.05). NIHSS score at admission was remarkably higher in the poor outcome group, in comparison to the good outcome group (p<0.001).

ICAM-1 and hs-CRP in relation to stroke outcome

The serum ICAM-1 and hs-CRP concentrations of patients with AIS in the poor outcome group were significantly higher compared with patients in the good outcome group (p<0.001). After logistic regression analysis, expected associations with a poor outcome arouse for the known predictors of poor outcome: sICAM-1 (OR 1.05, 95 % CI 0.848 to 1.625; p=0.024) (area under the curve (AUC=0.829) and hs-CRP (OR 1.22, 95 % CI 0.78 to 1.86; p=0.03) (AUC=0.748) (figures 1 and 2, table 2). As demonstrated in figures 1 and 2, in order to assess the diagnostic value of serum ICAM-1 and hs-CRP as a potential prognostic markers of AIS, we performd ROC curve analysis. For serum ICAM-1 and hs-CRP the AUC is 0.829 and 0.748, respectively. The best cut-off value for the ICAM-1 of the ROC curve for difference good outcomes from poor outcomes is 184.6 ng/mL, while this value for the hs-CRP equals to 2.93 mg/L. When the serum ICAM-1 and hs-CRP concentration is higher than 184.6 ng/mL and 2.93 mg/L, it means that the prognosis of patients with AIS is poor. Table 2 shows the predictive value of several clinical baseline characteristics which were incorporated into logistic regression analyses. The results of logistic regression demonstrated that after adjusting for baseline characteristics, serum ICAM-1 and hs-CRP levels are still the predictors of poor outcome in patients with AIS (p=0.024 and p=0.03, respectively).

Table 1 Characteristics of patients with AIS with poor outcome and good outcome

Characteristics	Good outcome	Poor outcome	P value
n	72	46	
Age, years	62.97±7.07	64.71±12.28	0.382
Male, n (%)	45 (62.5)	21 (45.6)	0.076
NIHSS on admission	5±4	14±5	<0.001
Systolic blood pressure, mm Hg	148.2±29.6	160±28.4	0.112
Diastolic blood pressure, mm Hg	91.7±8.1	92.4±7.8	0.846
Hypertension, n (%)	67 (93)	44 (95.6)	0.852
Diabetes mellitus, n (%)	15 (20.8)	21 (45.6)	0.212
Coronary heart disease, n (%)	5 (6.94)	6 (13)	0.345
Atrial fibrillation, n (%)	9 (12.5)	8 (17.4)	0.824
Alcohol n (%)	3 (4.16)	3 (6.5)	0.907
ICAM-1, ng/mL	161.7±64.9	225.39±173.73	<0.001
hs-CRP, mg/L	2.78±1.53	3.45±2.18	<0.001

AIS, acute ischaemic stroke; hs-CRP, highly-sensitive C reactive protein; ICAM-1, intercellular adhesion molecule-1; NIHSS, National Institutes of Health Stroke Scale.

DISCUSSION

It is well known that inflammation deteriorates damage to the brain at the stroke's acute phase. At the initial phase of the inflammation, proinflammatory cytokines release from necrotic cells such as interleukin-1β (IL-1β) and

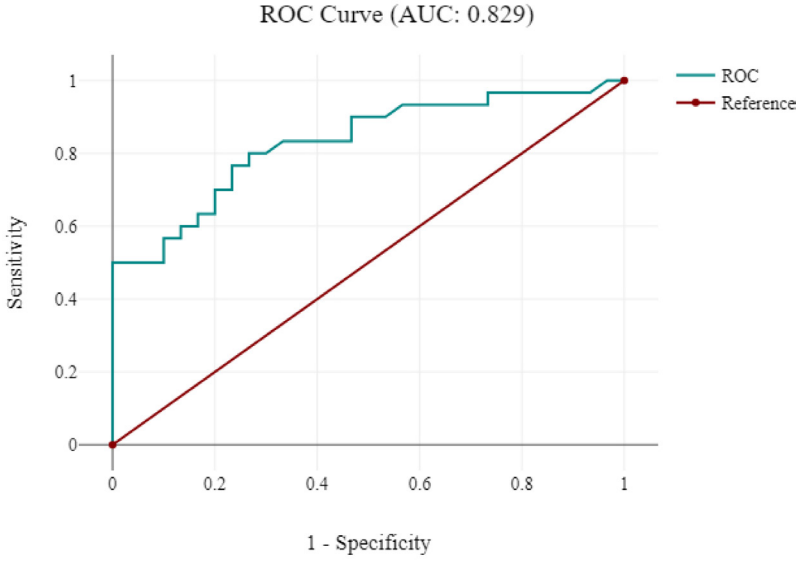

Figure 1 ROC analyses of ICAM-1. AUC, area under the curve; ICAM-1, intercellular adhesion molecule-1; ROC, receiver operating characteristic.

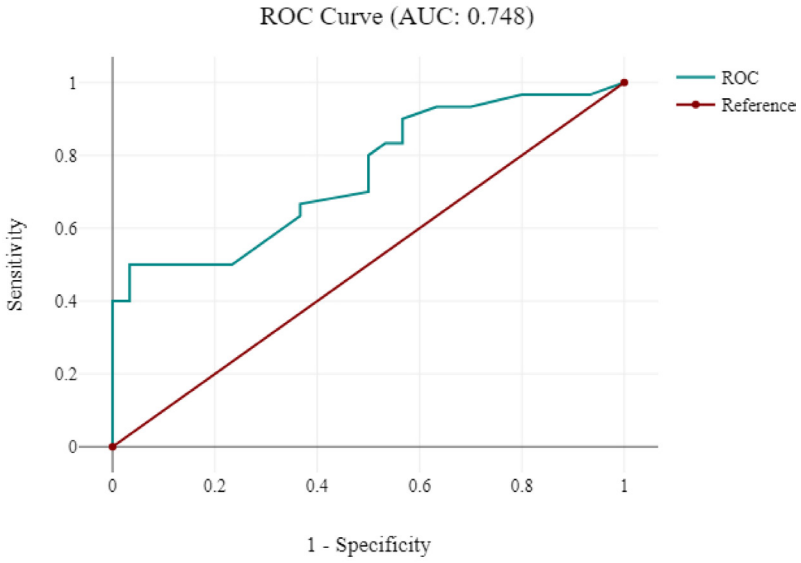

Figure 2 ROC analyses of hs-CRP. AUC, area under the curve; hs-CRP, highly sensitive C reactive protein; ROC, receiver operating characteristic.

tumour necrosis factor (TNF-α). The direct results are activation of the microglia and modification of permeability of the blood–brain barrier.[18] Endothelial cells of the arterial wall lose their tight junction and express CAMs.[19] These allow recruitment and migration of leucocytes into the brain parenchyma.[20] Infiltration of cerebral tissue with macrophages, neutrophil granulocytes and T-cells has been shown several times following experimental stroke.[21] First of all, attachment of leucocytes to the arterial wall would lead to microvessel obstruction.[4] More interestingly, immune cells release reactive oxygen species and MMP causing cell death and cytokines.[22] This leads to turning penumbra and brain swelling into expansion of infarction. In this study, predictive ability of ICAM-1 was noticed within the first day following the stroke. ICAM-1 mediate adhesion of the monocytes and granulocytes that get involved in the expansion of cerebral oedema,[11] whether the ischaemic or haemorrhagic one.[10 23] This could describe their association with prognosis of stroke's acute phase, early cytotoxic and vasogenic oedema being responsible for both the development of the ischaemic area and increasing intracranial hypertension.[10] The first phase of synthesis happens soon after stroke onset, following vascular destruction and activation of the arterial wall.[10 20] In vitro and in vivo researchs reveal ICAM-1 is the first to be released by activated endothelial cells during the first 24 hours with rapid decrease.[10 24] This allows the initial recruitment of granulocytes and monocytes by rolling on activated endothelium. High levels of ICAM-1 are found later in the blood and remain for several days.[5] This coincides with the migration of monocytes within brain parenchyma via ICAM-1. The extent to which the results of the current study are accurate is reinforced by the fact that this research project confirms the anticipatory potential of ICAM-1.

The concentration of CRP elevates in the first 48 hours after stroke, but it still increases at 7 days and stay high for 3–6 months after onset.[25 26] Hs-CRP plays a crucial role in the inflammation and may increase brain injury by encouraging atherosclerosis or lesion plaques.[27] While patients are going through the acute phase of ischaemic stroke, there is a rise in the hs-CRP in their blood owing to inflammation caused by cerebral ischaemic injury. In uninfected patients, hs-CRP concentration is rised may reflect the level of neuroinflammatory reaction after

Table 2 Risk variables for poor outcome at 3 months in patients with AIS

Characteristics	OR	95% CI	P value
Age, years	1.02	0.853 to 1.322	0.312
Male, n (%)	1	0.912 to 1.086	0.328
NIHSS on admission	1.27	0.83 to 1.93	0.05
Systolic blood pressure, mm Hg	1.03	0.94 to 1.079	0.245
Diastolic blood pressure, mm Hg	0.948	0.862 to 1.042	0.308
Hypertension, n (%)	1.32	0.887 to 1.379	0.267
Diabetes mellitus, n (%)	1.18	0.896 to 1.282	0.112
Coronary heart disease, n (%)	1.22	0.899 to 1.207	0.31
Atrial fibrillation, n (%)	1.31	0.970 to 1.361	0.496
Alcohol n (%)	1.09	0.317 to 2.102	0.508
ICAM-1, ng/mL	1.05	0.848 to 1.625	0.024
hs-CRP, mg/L	1.22	0.78 to 1.86	0.03

AIS, acute ischaemic stroke; hs-CRP, highly-sensitive C reactive protein; ICAM-1, intercellular adhesion molecule-1; NIHSS, National Institutes of Health Stroke Scale.

stroke, the higher the hs-CRP level, the more serious the inflammatory response.[28] Hs-CRP interacts with vascular endothelial cells and other cells, increases vascular inflammation and makes atherosclerotic plaques unstable, causing to a series of pathological and physiological processes like leucocyte adhesion, platelet activation and oxidation.[29] Excessive plasma hs-CRP levels may provide a useful biomarker to 3 months prognosis of ischaemic stroke.

There are limitations to this study. First, sample of patients have been chosen from one medical centre and the size of the sample is not large. Moreover, the level of biomarkers was not monitored consistently and rechecks on patients were not conducted in the long run. Hence, the relationship between biomarkers and prognosis in patients diagnosed with AIS following the treatment cannot be evaluated. Finally, we did not conduct subgroup analysis of patients with AIS with several causes of the disease.

CONCLUSIONS

Our research demonstrated that serum concentrations of ICAM-1 and hs-CRP at ED admission could be useful markers for predicting neurological recovery at 3 months after stroke, after adjusting for logistic regression analyses, they are still independent predictor of functional outcomes in patients with AIS.

Contributors GSR designed the study and drafted the Methods and Results sections. KBkA performed the statistical analyses and drafted the manuscript. KBkA is responsible for the overall content as guarantor. All authors revised the manuscript critically for important intellectual content and have given their final approval to the submitted form.

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Competing interests No, there are no competing interests.

Patient consent for publication Consent obtained from parent(s)/guardian(s).

Ethics approval All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Tashkent Medical Academy on 27 October 2021 with the statement No 3. Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available on reasonable request.

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