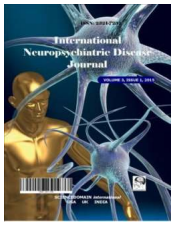

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Diagnostic and Prognostic Significance of Blood Biomarkers in Acute Ischemic Stroke

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Authors' contributions

This work was carried out in collaboration between three authors. Authors MES designed the study and wrote the protocol. Author AE preformed the statistical analysis, managed the literature search and wrote the first draft of the manuscript with assistance from author OAES. All authors read and approved the final manuscript.

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ABSTRACT

Background: The utilization of biomarker panels in acute ischemic cerebral stroke (AICS) could enhance the proper diagnosis that facilitate the identification of the cause of the cerebral stroke which is essential for rationally manage and avoid stroke recurrence.
Objectives: To inspect the vulnerable associations among a panel of blood biomarkers {D-dimer (DD), angiopoietin-1 (ANGPT1), S100 calcium-binding protein B (S-100b), and brain natriuretic peptide (BNP)} and AICS patients.
Patients and Methods: This is a prospective research performed on patients with AICS who admitted at Saudi German Hospital-KSA in corporation with the neurology department Mansoura faculty of medicine - Egypt during one and half years' duration. Demographics of the patients, fatality as well as the clinic and a panel of blood biomarkers serum levels were gathered. The clinical scales {National institutes of Health Stroke Scale (NIHSS) scoring for severity on admission, and Modified Rankin Scale (mRS) for outcome after 3 months were tested for all AICS patients.
Results: An overall of 150 patients with AICS was investigated, with a mean age of 62±14 years with males 52%. The AICS cases were set side by side to age and sex matched thirty healthy

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controls (HC) demonstrating that the patients were more likely to have significantly hypertension, and atrial fibrillation (71.3%, 20%, P< 0.05 respectively). The mortality after 3 months was 11% (15 cases). Regarding stroke severity NIHSS score mean was 11.6±6. The serum levels for a panel of blood biomarker (DD, S100b, and BNP) are significantly higher while for Angpt1 is significantly lower with AICS in comparison to HC. Multivariate predictors of patients with an unfavorable functional outcome, DD, S-100b, and BNP levels were significantly higher compared with the levels in patients with a favorable outcome. On the contrary, the level of Angpt1 is significantly decreased in patients with an unfavorable functional outcome. The stroke severity (NIHSS score) correlated significantly with the outcome (mRS) as less severe cases showed more favorable outcome. The clinical variables that showed significant correlation were age, diabetic, and atrial fibrillation.

Conclusion: Our findings highlighted that blood biomarkers can be accustomed as a valuable tool to investigate AICS and to anticipate initial neurological outcome that would assist in determining patients at risk of unfavorable outcome offering alert to launch therapies to avert aggravating of the patient's status.

Keywords: Acute ischemic cerebral stroke; biomarker; National Institutes of Health Stroke Scale; Modified Rankin Scale; D-dimer, angiopoietin-1; S100b, brain natriuretic peptide.

1. INTRODUCTION

Particular proteins are liberated subsequent to neuronal damage. Screening the degrees of fluctuation in the proteins would anticipate the level of the lesion. Distinct kinds of CNS diseases were the outcome of axonal degeneration and damage [1,2]. Therefore, a proper assay to identify the current brain injury would be beneficial for the detection of CNS damage. The ideal marker to discover brain injury needs to be precise can be easily measurable, and it needs to be refractory to destruction by proteases before or after its liberation. Several types of researches have attempted to identify markers for brain injury also they founded that the proteins are liberated inside the brain tissue and transferred to the cerebrospinal fluid (CSF) and the blood in patients of acute brain injury especially acute ischemic cerebral stroke (AICS). Although, nothing of these proteins are broadly utilized for the anticipation of clinical consequences and as diagnostic or predictive tool that assess for brain injury, due to the restrictions in specificity, sensitivity, and standardized measurability throughout many laboratories and researches [3].

D-dimer (DD) is a fibrin degradation product (FDP), a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. Newly, it is considered as an essential test conducted in patients with presumed thrombotic disorders. Its principal usage is to rule out thromboembolic disorder where the anticipation is low [4,5].

Angiopoietin 1 (ANGPT1) is a protein with significant functions in vascular angiogenesis and development improving healthy endothelial role in adults. As, it unites to an endothelial cell-specific tyrosine-protein kinase receptor. The protein encrypted by this gene is a secreted glycoprotein that activates the receptor by inducing its tyrosine phosphorylation. It performs an essential function in intermediation mutually-beneficial interact between the endothelium and neighboring matrix and mesenchyme. Also, the protein helps in blood vessel stabilization and maturation. Many previous types of researches stated that Angpt1 safeguards against cerebral ischemia [6-9].

S100 calcium-binding protein B (S100B) is a protein of the S-100 protein family. S100 proteins are situated in the cytoplasm and nucleus of a broad spectrum of cells, that implicated in the settlement of a number of cellular operations such as cell cycle differentiation and progression [10]. Also, it is released by astrocytes or could discharge from damaged cells to infiltrating the extracellular space or bloodstream. It's serum levels increase in cases while in the acute stage of brain injury [11]. Over the last decade, S100B has appeared as a peripheral biomarker of CNS damage and blood–brain barrier (BBB) permeability. High S100B levels specifically correspond the occurrence of neuropathological circumstances including neurodegenerative diseases or traumatic head injury. Ordinary S100B serum levels accurately rule out considerable CNS injury. So, the great benefit of using S100B is that elevations in serum or CSF levels give a sensitive indicator for identifying CNS damage at the molecular level prior the gross modifications elaborated, allowing appropriate delivery of essential medical

management prior irreversible brain injury appears [12].

Brain natriuretic peptide (BNP), is a counter-regulatory hormone liberated by the ventricles of the heart [13]. Its principal actions are vasodilation and natriuresis. Consequently, the final response of BNP is a reduction in blood volume, that decreases systemic blood pressure and afterload, leading to an increase in cardiac output, considerably as a result of a higher ejection fraction. Thus, the essential clinical role of BNP is that a normal level excludes acute heart failure in the emergency situations [14]. Increased serum BNP levels are accompanied with cardioembolic stroke chiefly due to atrial fibrillation which give rise to large brain infarct with unfavorable outcome [15].

So, this study was designed to investigate the susceptible associations between a panel of blood biomarkers DD, ANGPT1, S-100b, BNP and acute ischemic stroke patients.

2. SUBJECTS AND METHODS

One hundred fifty (150) cases with AICS admitted at Saudi German Hospital Kingdom of Saudi Arabia in corporation with the neurology department Mansoura faculty of medicine – Egypt. They were studied in the period between April 2014 and September 2015. Our patients group with AICS admitted within the first 24 hours after symptom onset. In addition, 30 healthy controls (HC) age and sex matched were included in this study as the control group.

2.1 On Admission

2.1.1 Clinical protocol

History of vascular risk factors was obtained for each patient. Complete general and neurological examinations were done. National Institutes of Health Stroke Scale (NIHSS) was done to assess the stroke severity and divided the patients into four subgroups that include minor (0–4), moderate (5–15), moderate to severe (16–20), and severe (21–42).

2.1.2 Neuroimaging protocol

All patients underwent computed tomographic brain scans. In some cases, MRI brain scans to confirm an ischemic stroke were done. CT and MRI brain were reviewed by a radiologist.

2.1.3 Blood samples and immunoassays tests

Samples were collected from stroke cases within 24 hours of stroke onset and before any treatment was administered to avoid drug-biomarker interference. Preparation of the plasma by centrifugation (15 minutes at 3000 rpm) and storage at −80℃ for analysis. Complete blood count, random and fasting blood sugar, rheumatoid factor, renal and liver functions. Peripheral blood samples were collected from stroke cases and controls subjects.

The biomarkers panel includes DD, ANGPT1, S-100b, and BNP. All biomarkers were assayed by ELISA. Immunoassays were forward immunometric (sandwich) assays performed in 384-well microtiter plates with a Tecan Genesis RSP 200/8 Workstation (Tecan). Each sample was tested twice with biotinylated antibodies (Biosite Inc, San Diego, Calif).

2.1.4 Other tests

ECG were performed in all patients while echocardiography and carotid duplex were done in some patients, if the patient was suspected to have a cardiac disease or in recurrent strokes or embolic strokes.

2.1.5 Exclusion criteria

Patients with chronic liver disease, high rheumatoid factor, history of malignancy, trauma, and recent surgery.

2.2 After 3 Months

2.2.1 Functional outcomes in stroke cases

Follow-up was conducted in stroke cases, after 3 months. Modified Rankin Scale (mRS) was used to assess disability. It denotes 7 outcomes: 0, no symptoms; 1, no significant disability despite symptoms; 2, slight disability; 3, moderate disability; 4, moderately severe disability; 5, severe disability; and 6, death [16,17].

2.2.2 Statistical analyses

Quantitative data were prescribed as interquartile and median range and comparison was done by Mann–Whitney *U* test. Nominal data were presented as number and percentages and compared by $\chi 2$ test. The association of plasma biomarker with stroke was assessed using

multiple regression analysis adjusting for age, male sex, previous myocardial infarction, hypertension, diabetes mellitus, smoking, and atrial fibrillation.

Stroke outcome was compared between patients with severe disability or death (mRS, 3–6) and those with minor or no disability (mRS, 0–2) using $\chi 2$ and Mann–Whitney U tests. To assess the association of blood biomarker with severe stroke disability, multivariate logistic regression was performed adjusting for age, atrial fibrillation, and stroke severity on admission (graded as minor, moderate, moderate to severe, or severe), which were noted to be associated with mRS score 3 to 6 on univariate analyzes.

3. RESULTS

AICS cases were compared to age and sex-matched HC showing that patients were more likely to have hypertension, diabetes mellitus, and atrial fibrillation. Mortality after 3 months was 11%. Regarding stroke severity NIHSS was 11.6±6 (Table 1).

Serum levels for a panel of blood biomarker (D-dimer, S-100b, and BNP) are significantly higher with AICS patients while the level of Angpt1 is significantly lower with AICS patients (Table 2) (Fig. 1).

Multivariate predictors of good functional outcome (modified Rankin Scale ≤2) and unfavorable outcome or death (modified Rankin Scale 3-6 or death of patient) in patients with acute ischemic stroke showing that patients with an unfavorable functional outcome, d-dimer levels, and S-100b levels, and BNP levels were significantly higher compared with the levels in patients with a favorable outcome. While in patients with an unfavorable functional outcome, levels were lower compared with the levels in patients with a favorable outcome. On the contrary, the level of Angpt1 is significantly decreased in patients with an unfavorable functional outcome. So a panel of blood biomarker (D-dimer, S-100b, BNP, and Angpt1) is an independent predictor of functional outcome and mortality in patients with acute ischemic stroke (Tables 3 and 4).

Table 1. Characteristics of patients and healthy controls (HC), vascular risk factors, stroke etiology and severity (NIHSS score on admission), and 3 months' outcomes

		Patients N (%)	Healthy controls N (%)	P value
No. of subjects		150	30	
Men		78(52%)	16(53.3%)	0.940
Mean age (SD), years		62.1±14.4	61.9±13.5	0.894
Risk factors	Hypertension	107(71.3%)	10(33.3%)	0.045*
	Smoker	60(40%)	8	0.339
	DM	38(25.3%)	5(16.6%)	0.414
	AF	30(20%)	0	0.015*
	Hyperlipidemia	23(15.3%)	1(3.3%)	0.109
	Previous stroke	9(6%)	0	0.181
	Coronary heart disease	9(6%)	1(3.3%)	0.578
NIHSS, mean (SD)		11.6 (6)	-	
Mortality		15 (11%)	-	

Table 2. Biomarker levels of patients and HC at the admission

Biomarker	Patients median (range)	HC median (range)	P value
Bnp (pg/ml)	191 (135–261)	31 (26.2- 63.7)	<0.001*
D dimer (ng/ml)	574.1(262-941)	150 (63.8-246.4)	<0.001*
S100b (pg/ml)	136 (88–297)	75 (0–138)	<0.001*
Angiopoietin-1 (ng/mL)	6.5 (5.21–10.22)	17.5 (14.45–23.12)	<0.001*

*P value by * Mann-Whitney U-Test*

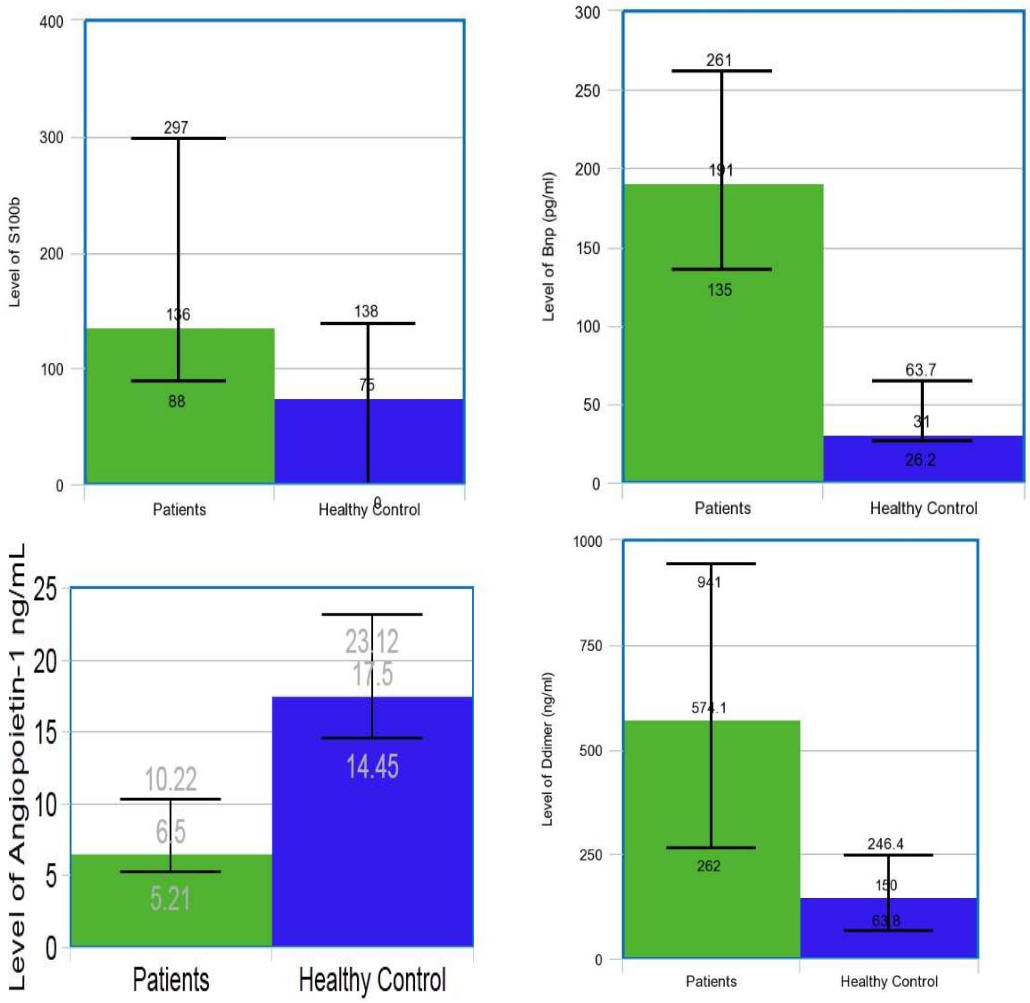

Fig. 1. Mean biomarkers levels in favorable and unfavorable ischemic stroke outcomes

The stroke severity (NIHSS score) correlated significantly with the outcome (mRS) as less severe cases [NIHSS mild (0-4) and moderate (5-15)] showed more favorable outcome. While, more severe cases [NIHSS moderate to severe (16-20) and severe (21-42)] showed more favorable outcome (Table 3).

The clinical variables that showed significant correlation were age (increase age associated with more chance for the unfavorable outcome), diabetic, and atrial fibrillation (Tables 3 and 4).

4. DISCUSSION

In spite of the majority of the diagnostic methods to the assessment of AICS depend on neuroimaging evaluation, an alternative approach would be the appraisal of blood-borne biomarkers of the tissue damage. This strategy has presented in the survey and initial investigations of other urgent medical situations. For instance, biochemical markers like BNP, CK-MB, troponin, and DD perform significant aspects in the assessment of congestive heart failure, pulmonary embolism, and myocardial infarction [18,19]. With a perfect clinical management, the rapid, and the noninvasive tool should assist in define the case in the inhabitants at high risk for brain infarction, that requires speedy opinion and prioritize. Additionally, it would contribute supplementary characteristic data for cases for whom practitioners are considering rapid interference.

Table 3. Correlation between risk factors, stroke severity (NIHSS score), panel of biomarker and stroke outcome (mRC) after 3 months

Variables		Outcome (mRC)		P value
		0–2 Favorable	3–6 Unfavorable	
Number (135)		95	40	
Age (years)		60.1 (13.26	67.9 (12.9)	<0.001*
Male		46(48.4%)	22(55%)	0.690
Hypertension		62(65.2%)	36(90%)	0.253
DM		18(18.9%)	16(40%)	0.05*
Smoking		35(36.8%)	21(52.5%)	0.288
CHD		2(2.1%)	3(7.5%)	0.619
AF		12(15%)	16(40%)	0.005*
Stroke severity on admission (NIHSS)	Mild (0–4)	59(62.1%)	8(20%)	
	Moderate (5–15)	33(34.7)%	19(47.5%)	< 0.001*
	Moderate to severe (16–20)	2(2.1%)	8(20%)	
	Severe (21–42)	1(1.1%)	5(12.5%)	
Bnp (pg/ml)		145 (135–212)	205 (156–261)	< 0.001**
Ddimer (ng/ml)		479 (262-621)	768.5(574.1-941)	< 0.001**
S 100b (pg/ml)		116 (88–261)	155 (102–297)	< 0.001**
Angiopoietin-1 ng/mL		8.12 (5.13–9.80)	5.26 (3.97–8.09)	< 0.001**

*P value calculation by * Fisher Exact Test ** Mann-Whitney U-Test*

Table 4. Predictors of outcome

Variables		Outcome (mRC)			
		Favorable		Unfavorable	
		OR	95% CI	OR	95% CI
Clinical	Age	0.97	0.4–0.99	1.0	0.97–1.04
	Severity (NIHSS)	0.86	0.8–0.96	1.1	1.01–1.19
	AF	0.81	0.42–1.6	3.6	1.2–13.2
Biomarkers	Angpt1	3.77	1.97–7.20	0.62	0.28–0.96
	D-Dimer	0.98	0.55-0.89	3.22	2.05-6.43
	S-100b	0.67	0.45-0.89	1.28	0.47–2.33
	BNP	0.64	0.41–0.98	1.75	1.36–2.24

Earlier researches had stated that Angpt1 could safeguard across cerebral infarction [20,21]. Localized brain ischemia has been demonstrated the downregulation of Angpt1 abrubtly [22]. Many earlier types of researches stated that Angpt1 hinders the capability of the vascular endothelial growth factor (VEGF) to trigger the blood–brain barrier (BBB) leakage and so decreases neuronal injury and brain infarction [20,23]. Unfavorable prognosis with middle cerebral artery obstruction was accompanied by the decline of Angpt1 [20], although the improvement of cerebral ischemia outcome following the exercise was related to the rise of Angpt1 [24]. So, the previous results indicated that Angpt1 levels could give significant predictive data on AICS prognosis. In the present research, the cases with AICS had median serum levels of Angpt1 that were statistically significant about 3-folds lower than HC [mean 6.5 ng/mL, 17.5 ng/mL, p <0.001, respectively]. Patients with the unfavorable outcomes at one month had lower plasma Angpt1 concentrations within 24 hours of their stroke admission when compared with favorable outcomes [mean 5.26 ng/mL, 8.12 ng/mL, p <0.001, respectively]. The present study concluded that Angpt1 is reduced after AICS and that the level of reduction is related to whole cerebral stroke prognosis. Also, AICS cases with fewer levels of Angpt1 would at higher susceptibility of the occurrence of cerebral ischemia. So, the estimation of the level of Angpt1 rapidly after AICS would give recent data for the outcome, of course, this needs more

investigation in larger AICS patients through prognostic studies. The present research indicates that the level of Angpt1 are downregulated in cases who have suffered a new AICS and so reduced levels of Angpt1 are linked to the unfavorable brain infarction prognosis. The plasma Angpt1 level is not an indicator risk of cerebral stroke incidence in the healthful elderly population. The results give a justification for additional investigations to consider if the upregulating Angpt1 enhances cerebral ischemia prognosis. These findings were in agreement with Golledge et al. [25] who stated that Plasma Angpt1 concentrations are low after ischemic stroke particularly in patients with poor stroke outcomes at 3 months and interventions effective at upregulating Angpt1 could potentially improve stroke outcomes.

Tomita et al. [26] tested the assumption that BNP concentrations are increased in cases with AICS independence of heart disease and correspond to AICS severity. The BNP concentration in AICS was positively linked to the NIHSS and infarct volume. Cerebral stroke volume and NIHSS were independently participants to the plasma BNP concentration in AICS. Furthermore, Kim et al. examined cases with AICS, and plasma BNP concentration was assessed in cases and HC. They concluded that the AICS cases had greater plasma BNP level in contrast to HC (mean 124.6 pg/mL versus mean 11.9 pg/mL, $p < 0.01$, respectively). The highest BNP level was correlated with increased initial NIHSS, advanced age, current non-smoker, increased white blood cell counts, atrial fibrillation, heart failure, cardioembolic cause, and increased infarction volume. The increased plasma BNP level associated with increased the volume of cerebral infarction [27]. These studies were in agreement with our research as BNP was significantly higher in AICs patients when compared with HC [mean 191 pg/mL, and mean 31 pg/mL, $p < 0.001$, respectively]. Also, it is significantly higher in AICS patients with unfavorable outcomes when compared with AICS patients with favorable outcomes [mean 205 pg/mL, and mean 145 pg/mL, $p < 0.001$, respectively].

The DD level could be increased in cases with myocardial infarction, deep venous thrombosis, pulmonary thromboembolism, trauma, disseminated intravascular coagulation, surgery or AICS [28-32]. DD, a biochemical marker of plasmin-mediated fibrin degradation, is related to fibrin degradation products (FDP) and denotes vessel obstruction. Plasmin separates the fibrin into FDP and DD when the coagulation and the fibrinolytic system is triggered. Many types of researches proved that DD, C-reactive protein, and other markers of hemostatic activation linked to diagnosis [33-41], progression, and death in AICS patients [33,42-44]. Laskowitz et al. [45] stated that a biochemical marker list could give time-sensitive and beneficial characteristic data to AICS appraisal and fast detection of cases with suspected AICS, that could widen the accessibility of time-limited therapeutic approaches. They furthermore stated that, for the assessment of earlier AICS, an approach assimilates the modern biochemical marker check in collaboration with non-contrast CT brain has significantly higher sensitivity than CT merely owns. They have evidenced the benefit of certain serologic markers, like DD, S100B, BNP, matrix metalloproteinase-9 (MMP-9), and, for identifying AICS. Skoloudík et al. [46] observed that the DD serum levels rise within 6 hours after AICS onset and it is higher in cases with large artery obstruction and in cases with cardioembolic AICS than it is in cases with lacunar cerebral stroke or in cases without arterial obstruction. Barber et al. [33] stated that the DD would assist practitioners aiming interventions for protecting against the beginning of neurological worsening after AICS. Though, previous researches concluded that DD measurement cannot be utilized as an AICS index, except for cardioembolic subtype [47,48]. These studies were in agreement with our research as DD was significantly higher in AICs patients when compared with HC [mean 574 ng/mL, and mean 150 ng/mL, $p < 0.001$, respectively].

The measurement of S-100B in acute neurological diseases like traumatic brain injury, ischemic or hemorrhagic stroke and global hypoxia illustrates the intensity of symptoms and prognosis. Conversely, the S-100B liberation relies on the pathophysiology of the damage, intensity, and topography (rapid appearance with traumatic brain injury follows the acute devastation of neuronal tissue or lagged liberation after AICS in which gradual breakdown of the BBB performs a critical function). In chronic brain disorders, information about the clinical importance of measurement of S-100B is little and more assessments are requested [49]. Nash et al. [50] investigated eighteen types of researches (1,643 patients). S100B peaks from symptom onset between 24 and 120 h with significantly elevated levels assayed from 0 to 120 h. Upper S100b levels stated significantly

greater infarction volumes, more severe cerebral strokes, and worse functional prognosis. There was a significant difference in S100 levels between AICS patients and controls. So, peak levels after stroke onset diversified. S100 was significantly raised after AICS onset, and corresponds with infarct volume, stroke severity, and functional outcome, and was a possible marker for current ischemia. Its serum level with AICS is a beneficial biochemical marker of infarct size and subsequently the clinical prognosis [50]. Weglewski et al. [51] investigated 67 patients, 53 with ischemic stroke and 14 with hemorrhagic stroke. Plasma levels of S100B was calculated on the 1st, 3rd, 7th and 14th day after stroke onset. The greatest levels of protein S100B were discovered in AICS mainly on the 3rd day and in hemorrhagic stroke on the 1st day. The levels of protein S100B were analogous in ischemic and hemorrhagic stroke on the 3rd, 7th and 14th day. Serum concentrations of protein S100B after stroke onset have shown a correspondence with infarct volume, particularly in cases with large or medium cerebral stroke. Ischemic and hemorrhagic cerebral strokes cause liberation of protein S100B into the blood. They found a good concordance between the liberation profile of S100B and volume of cerebral ischemia. S100B protein is the marker of brain injury during AICS. The S100B measurements could be used in the follow-up the stroke management [51]. These studies were in agreement with our research as S100B was significantly higher in AICs patients when compared with HC [mean 136 pg/mL, and mean 75 pg/mL, $p < 0.001$, respectively].

5. CONCLUSION

Blood biomarkers can be used as a useful tool to diagnose acute ischemic stroke and to predict the early neurological outcome. Also, they help to identify patients at risk of unfavorable outcome giving an alarm to initiate therapies to prevent worsening of the patient's condition.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Büki A, Povlishock JT. All roads lead to disconnection. Traumatic axonal injury revisited. Acta Neurochir (Wien). 2006; 148(2):181-93:Discussion 193-4.
2. Strong MJ, Strong WL, Jaffe H, Traggert B, Sopper MM, Pant HC. Phosphorylation state of the native high-molecular-weight neurofilament subunit protein from cervical spinal cord in sporadic amyotrophic lateral sclerosis. J Neurochem. 2001;76:1315–25.
3. Waheed M Radwan, Amany M Rabbah, Hamdy Saber, Mohamed O Elghonemi. A new marker for ischemic cerebrovascular stroke: Phosphorylated neurofilament H. The Egyptian Journal of Critical Care Medicine. 2013;1:105–8.
4. Suzuki T, Distante A, Eagle K. Biomarker-assisted diagnosis of acute aortic dissection: How far we have come and what to expect. Curr Opin Cardiol. 2010; 25(6):541-5.
5. Ranasinghe AM, Bonser RS. Biomarkers in Acute Aortic Dissection and Other Aortic Syndromes. J Am Coll Cardiol. 2010; 56(19):1535-41.
6. Fukuhara S, Sako K, Noda K, Zhang J, Minami M, Mochizuki N. Angiopoietin-1/Tie2 receptor signaling in vascular quiescence and angiogenesis. Histol Histopathol. 2010;25(3):387-96.
7. Hansen TM, Moss AJ, Brindle NP. Vascular endothelial growth factor and angiopoietins in neurovascular regeneration and protection following stroke. Curr Neurovasc Res. 2008; 5(4):236-45.
8. Shen F1, Walker EJ, Jiang L, Degos V, Li J, Sun B, Heriyanto F, Young WL, Su H. Coexpression of angiopoietin-1 with VEGF increases the structural integrity of the blood-brain barrier and reduces atrophy volume. J Cereb Blood Flow Metab. 2011;31(12):2343-51.
9. Zheng Q, Zhu D, Bai Y, Wu Y, Jia J, Hu Y. Exercise improves recovery after ischemic brain injury by inducing the expression of angiopoietin-1 and Tie-2 in rats. Tohoku J Exp Med. 2011;224(3):221-8.
10. Verma N, Karmakar M, Singh KP, Smita S. Structural and dynamic insights into S100B protein activity inhibition by melittin for the treatment of epilepsy. International journal of Computer Application National Seminar

on Application of Artificial Intelligence in Life Sciences, NSAAILS. 2013;1:55-60.

11. Zongo D, Ribéreau-Gayon R, Masson F, Laborey M, Contrand B, Salmi LR, Montaudon D, Beaudeux JL, Meurin A, Dousset V, Loiseau H, Lagarde E. S100-B protein as a screening tool for the early assessment of minor head injury. Ann Emerg Med. 2012;59(3):209-18.
12. Michetti F, Corvino V, Geloso MC, Lattanzi W, Bernardini C, Serpero L, Gazzolo D. The S100B protein in biological fluids: more than a lifelong biomarker of brain distress. J Neurochem. 2012;120(5):644-59.
13. Giannakoulas G, Hatzitolios A, Karvounis H, Koliakos G, Charitandi A, Dimitroulas T, Savopoulos C, Tsirogianni E, Louridas G. N-terminal pro-brain natriuretic peptide levels are elevated in patients with acute ischemic stroke. Angiology. 2005; 56(6):723-30.
14. Niederkofler EE, Kiernan UA, O'Rear J, Menon S, Saghir S, Protter AA, Nelson RW, Schellenberger U. Detection of endogenous B-type natriuretic peptide at very low concentrations in patients with heart failure. Circ Heart Fail. 2008; 1(4):258-64.
15. Maruyama K, Shiga T, Iijima M, Moriya S, Mizuno S, Toi S, Arai K, Ashihara K, Abe K, Uchiyama S. Brain natriuretic peptide in acute ischemic stroke. J Stroke Cerebrovasc Dis. 2014;23(5):967-72.
16. Banks JL, Marotta CA. Outcomes validity and reliability of the modified Rankin scale: Implications for stroke clinical trials: A literature review and synthesis. Stroke. 2007;38:1091–6.
17. Quinn TJ,Dawson J, Walters MR, Lees KR. Reliability of the modified Rankin Scale. Stroke. 2007;38:e144-e145.
18. Gibler WB, Blomkalns AL, Collins SP. Evaluation of chest pain and heart failure in the emergency department: Impact of multimarker strategies and B-type natriuretic peptide. Rev Cardiovasc Med. 2003;4(Suppl 4):S47-55.
19. Penttilä K, Koukkunen H, Halinen M, Rantanen T, Pyörälä K, Punnonen K, et al. Myoglobin, creatine kinase MB isoforms and creatine kinase MB mass in early diagnosis of myocardial infarction in patients with acute chest pain. Clin Biochem. 2002;35(8):647-53.
20. Shen F, Walker EJ, Jiang L, Degos V, Li J, Sun B, et al. Coexpression of angiopoietin-1 with VEGF increases the structural integrity of the blood-brain barrier and reduces atrophy volume. J Cereb Blood Flow Metab. 2011;31:2343–2351.
21. Cui X, Chopp M, Zacharek A, Ye X, Roberts C, Chen J. Angiopoietin/Tie2 pathway mediates type 2 diabetes induced vascular damage after cerebral stroke. Neurobiol Dis. 2011;43:285–292.
22. Zhang ZG, Zhang L, Tsang W, Soltanian-Zadeh H, Morris D, Zhang R, et al. Correlation of VEGF and angiopoietin expression with disruption of blood-brain barrier and angiogenesis after focal cerebral ischemia. J Cereb Blood Flow Metab. 2002;22:379–392.
23. Zhang ZG, Zhang L, Croll SD, Chopp M. Angiopoietin-1 reduces cerebral blood vessel leakage and ischemic lesion volume after focal cerebral embolic ischemia in mice. Neuroscience. 2002;113:683–7.
24. Zheng Q, Zhu D, Bai Y, Wu Y, Jia J, Hu Y. Exercise improves recovery after ischemic brain injury by inducing the expression of angiopoietin-1 and Tie-2 in rats. Tohoku J Exp Med. 2011;224:221–8.
25. Golledge J1, Clancy P, Maguire J, Lincz L, Koblar S, McEvoy M, Attia J, Levi C, Sturm J, Almeida OP, Yeap BB, Flicker L, Norman PE, Hankey GJ. Plasma angiopoietin-1 is lower after ischemic stroke and associated with major disability but not stroke incidence. Stroke. 2014;45(4):1064-8.
26. Tomita H, Metoki N, Saitoh G, Ashitate T, Echizen T, Katoh C, Fukuda M, Yasujima M, Osanai T, Okumura K. Elevated plasma brain natriuretic peptide levels independent of heart disease in acute ischemic stroke: correlation with stroke severity. Hypertens Res. 2008;31(9):1695-702.
27. Kim SH, Lee JY, Park SH, Jang HC, Lim EJ, Chang SJ, Lee SS. Plasma B-type natriuretic peptide level in patients with acute cerebral infarction according to infarction subtypeand infarction volume. Int J Med Sci. 2013;10(1):103-9.
28. Ageno W, Finazzi S, Steidl L, Biotti MG, Mera V, Melzi D'Eril G, et al. Plasma measurement of D-dimer levels for the early diagnosis of ischemic stroke subtypes. Arch Intern Med. 2002;162: 2589-93.

29. Dunn KL, Wolf JP, Dorfman DM, Fitzpatrick P, Baker JL, Goldhaber SZ: Normal D-dimer levels in emergency department patients suspected of acute pulmonary embolism. J Am Coll Cardiol. 2002;40:1475-8.

30. Hoffmeister HM, Szabo S, Kastner C, Beyer ME, Helber U, Kazmaier S, et al. Thrombolytic therapy in acute myocardial infarction: Comparison of procoagulant effects of streptokinase and alteplase regimens with focus on the kallikrein system and plasmin. Circulation. 1998;98: 2527-33.

31. Perrier A, Desmarais S, Miron MJ, de Moerloose P, Lepage R, Slosman D, et al. Non-invasive diagnosis of venous thromboembolism in outpatients. Lancet. 1999;353:190-5.

32. Skoloudík D, Bar M, Zapletalová O, Langová K, Herzig R, Kanovský P. D-dimer levels in acute stroke patients. Cesk Slov Neurol N. 2007;103:375-379.

33. Barber M, Langhorne P, Rumley A, Lowe GDO, Stott DJ. Hemostatic function and progressing ischemic stroke: D-dimer predicts early clinical progression. Stroke. 2004;35:1421-25.

34. Jauch EC, Lindsell C, Broderick J, Fagan SC, Tilley BC, Levine SR. Association of serial biochemical markers with acute ischemic stroke: The National Institute of Neurological Disorders and Stroke Recombinant Tissue Plasminogen Activator Stroke Study. Stroke. 2006;37: 2508-13.

35. Kelly PJ, Morrow JD, Ning M, Koroshetz W, Lo EH, Terry E, et al. Oxidative stress and matrix metalloproteinase-9 in acute ischemic stroke: The biomarker evaluation or antioxidant therapies in Stroke (BEATStroke) study. Stroke. 2008;39:100-4.

36. Koch HJ, Horn M, Bogdahn U, Ickenstein GW. The relationship between plasma D-dimer concentrations and acute ischemic stroke subtypes. J Stroke Cerebrovasc Dis. 2005;14:75-9.

37. Laskowitz DT, Blessing R, Floyd J, White WD, Lynch JR. Panel of biomarkers predicts stroke. Ann N Y Acad Sci. 2005; 1053:30.

38. Laskowitz DT, Kasner SE, Saver J, Remmel KS, Jauch EC. Clinical usefulness of a biomarker-based diagnostic test for acute stroke: The Biomarker Park SY, Kim MH, Kang SY, Suh JT, Lee WI. Inflammatory marker expression and its implication in Korean ischemic stroke patients. Korean. J Lab Med. 2007;27:197-204. Rapid Assessment in Ischemic Injury (BRAIN) study. Stroke. 2009;40:77-85.

39. Park SY, Kim MH, Kang SY, Suh JT, Lee WI. Inflammatory marker expression and its implication in Korean ischemic stroke patients. Korean J Lab Med. 2007;27:197-204.

40. Reynolds MA, Kirchick HJ, Dahlen JR, Anderberg JM, McPherson PH, Nakamura KK, et al. Early biomarkers of stroke. Clin Chem. 2003;49:1733-39.

41. Sibon I, Rouanet F, Meissner W, Orgogozo JM. Use of the triage stroke panel in a neurologic emergency service. Am J Emerg Med. 2009;27:558-62.

42. Feinberg WM, Bruck DC, Ring ME, Corrigan JJ Jr. Hemostatic markers in acute stroke. Stroke. 1989;20:592-97.

43. Muir KW, Weir CJ, Alwan W, Squire IB, Lees KR. C-reactive protein and outcome after ischemic stroke. Stroke. 1999;30: 981-85.

44. Tombul T, Atbas C, Anlar O. Hemostatic markers and platelet aggregation factors as predictive markers for type of stroke and neurological disability following cerebral infarction. J Clin Neurosci. 2005; 12:429-34.

45. Laskowitz DT, Blessing R, Floyd J, White WD, Lynch JR. Panel of biomarkers predicts stroke. Ann N Y Acad Sci. 2005; 1053:30.

46. Skoloudík D, Bar M, Sanák D, Bardon P, Roubec M, Langová K, et al. D-dimers increase in acute ischemic stroke patients with the large artery occlusion, but do not depend on the time of artery recanalization. J Thromb Thrombolysis. 2010;29:477-82.

47. Haapaniemi E, Tatlisumak T. Is D-dimer helpful in evaluating stroke patients? A systematic review. Acta Neurol Scand. 2009;119:141-50.

48. Skoloudík D, Bar M, Zapletalová O, Langová K, Herzig R, Kanovský P. D-dimer levels in acute stroke patients. Cesk Slov Neurol N. 2007;103:375-79.

49. Stroick M, Fatar M, Ragoschke-Schumm A, Fassbender K, Bertsch T, Hennerici MG. Protein S-100B - A Prognostic Marker

for cerebral damage. Curr Med Chem. 2006;13(25):3053-60.

50. Nash DL, Bellolio MF, Stead LG. S100 as a marker of acute brain ischemia: A systematic review. Neurocrit Care. 2008;8(2):301-7.

51. Weglewski A, Ryglewicz D, Mular A, Juryńczyk J. Changes of protein S100B serum concentration during ischemic and hemorrhagic stroke in relation to thevolume of stroke lesion. Neurol Neurochir Pol. 2005;39(4):310-7.

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