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Predictors of severity and outcome and roles of intravenous thrombolysis and biomarkers in first ischemic stroke

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ABSTRACT

Aim: Stroke is one of the leading causes of death and disability. The proportion of patients receiving recombinant tissue plasminogen activator is low in our country. Biomarkers to identify patients at risk of severe disease, and guide treatment and prognosis would be valuable. This article aims to identify the factors that can independently prognosticate the acute phase of ischemic stroke.

Methods: All patients with the first episode of ischemic stroke admitted to the Neurology Department between 1st December 2017 to 31st March 2018 were included in this pilot study. Stroke severity was evaluated using the National Institute of Health Stroke Scale (NIHSS). Patients being admitted within 4.5 h of onset of symptoms were thrombolysed with injection alteplase. For each patient, 4 serum biomarkers (D-dimer, fibrinogen, C-reactive protein and neuron specific enolase) were evaluated at admission and 24 h later. Discharged patients were assessed on an outpatient basis using the modified Rankin scale. The study primarily aimed to identify the factors predicting the severity and outcome of stroke, and to evaluate the effect of thrombolysis on the outcome. The secondary aim was to evaluate the role of biomarkers to predict the unfavorable outcome and the chance of post thrombolysis hemorrhage.

Results: Out of 30 patients included in the study, 10 had NIHSS 0-4, 12 had NIHSS 5-15 and 8 had NIHSS 16-42. Sixteen patients had unfavorable outcome (mRS score $\geq$ 2), and 5 patients expired. Old age, history of diabetes, CHADS2 score $\geq$ 2, and total anterior circulation stroke (TACS) independently affected stroke severity, whereas low

ejection fraction < 35%, and TACS, independently predicted unfavorable outcome and mortality. High mean arterial blood pressure (MABP) and capillary blood glucose (CBG) at admission were significant predictors of stroke severity, unfavorable outcome, and mortality. Out of 10 thrombolysed patients, two had mRS score ≥ 2 and 3 had the post-thrombolysis hemorrhage. Thrombolysis significantly reduced the incidence of the unfavorable outcome, but did not significantly affect death. All the biomarker levels at admission were significantly higher among patients with severe stroke and those who subsequently had an unfavorable outcome. D-dimer levels significantly increased and fibrinogen level significantly decreased following thrombolysis. Higher MABP, CBG, and fibrinogen levels at admission predicted significantly higher chance to develop hemorrhagic complications post thrombolysis.

Conclusion: Low ejection fraction, occurrence of TACS and the higher levels of the biomarkers under study predicted poor outcome. Higher mean CBG and MABP and raised fibrinogen levels predicted higher chance of post-thrombolysis hemorrhage.

Keywords: First-ever ischemic stroke, thrombolysis, biomarkers, total anterior circulation stroke, fibrinogen

INTRODUCTION

Stroke is one of the leading causes of death and disability in India, which is facing the double burden of communicable and non-communicable diseases. The estimated adjusted prevalence rate of stroke range from 84-262/100,000 in rural to 334-424/100,000 in urban areas. The incidence rate is 119-145/100,000 based on the recent population based studies. These values were higher than those of high-income countries[1]. Case fatality rates vary widely, the highest being 42% in Kolkata[2]. Among patients presenting with the first-ever stroke in the Mumbai registry, 80.2% were ischemic strokes and 17.7% were hemorrhagic strokes[3]. In the Trivandrum registry, 83.6% were ischemic strokes, 11.6% were intracerebral hemorrhages, and 4.8% were subarachnoid hemorrhages[4]. Thirty-two percent of the patients in the Kolkata registry had hemorrhagic stroke, the highest reported so far from India[5]. The proportion of patients receiving recombinant tissue plasminogen activator (rtPA) is low in our country, being 11% (104 out of 967 patients) in the on-going Indo USA National stroke registry, due to lack of trained personnel. Intraarterial and mechanical thrombolysis was given in 3.5% (34 out of 967 patients)[6].

Age, stroke severity, stroke mechanism, infarct location, comorbid conditions, clinical findings, and related complications influence stroke prognosis. Interventions such as thrombolysis, stroke unit care, and rehabilitation also influence the outcome of ischemic stroke. Knowledge of these prognostic factors enables the clinician to make a reasonable prediction for each patient, to offer a rational treatment to the patients, and to help the family members understand the disease course[7]. Though clinical examination can excellently assess the stroke patients and the disease progression, biomarkers would be valuable to identify the patients at risk of severe disease, to guide treatment and to reasonably predict the prognosis. Though many such proteins which are markers of brain tissue damage, inflammation, and coagulation/thrombosis are associated with ischemic stroke, their successful translation to a biomarker useful in clinical practice has proven difficult due to the heterogeneity of ischemic stroke[8]. Moreover, they are not specific to ischemic stroke, as many other disease processes can damage brain tissue. The blood- brain barrier restrains the release of these biomarkers into the systemic circulation; hence, their levels may not correlate with the infarct volume, given that the anatomic locations of stroke have different impacts on blood-brain barrier breakdown[9]. Markers of ischemic brain injury include S100 calcium binding protein B (S-100B), neuron-specific enolase (NSE), myelin basic protein, and glial fibrillary acidic protein. Several proteins involved in inflammation and immune response have also been identified as biomarkers of ischemic stroke, including C-reactive protein (CRP), interleukin-6, tissue necrosis factor-alpha, vascular cell adhesion protein 1, intercellular adhesion molecule 1, N-methyl-d-aspartate receptor antibodies and matrix metalloproteinases. Similarly, molecules involved in

acute thrombosis have also been associated with ischemic stroke, including fibrinogen, D-Dimer and von-Willebrand factor[10].

Few studies have systematically evaluated the multimodal factors (demographic, clinical, radiological and biological markers) in unselected consecutive first-ever ischemic stroke patients. This article aims to identify the factors that can independently prognosticate the acute phase of ischemic stroke.

METHODS

All patients with the first episode of ischemic stroke admitted to the Neurology Department between 1st December 2017 to 30th April 2018 were included in this pilot study and written informed consent was taken from the patients or their family members to participate in the study. Stroke was defined according to the World Health Organisation criteria[11]. Ischemic stroke was diagnosed with a combination of clinical criteria and non contrast computed tomography imaging of brain, which was done for all patients upon admission, to exclude intracerebral hemorrhage. We recorded the medical history prior to the stroke and the congestive heart failure, hypertension, age, diabetes mellitus, prior stroke or transient ischemic attack (TIA), or thromboembolism (CHADS2) scores were calculated for all patients. The following variables were analyzed: gender, age, domestic arrangements (lives with other family members or alone), clinical history, and medications, vascular risk factors including history of hypertension, diabetes mellitus, heart diseases [ischemic heart disease, low ejection fraction and atrial fibrillation (AF), as a history of AF and/or AF diagnosed during the index admission by electrocardiography], TIA, current or former smoking, and hypercholesterolemia. Stroke severity was evaluated in the acute phase of the initial stroke by a neurologist certified in the use of the National Institute of Health Stroke Scale (NIHSS). Stroke severity by NIHSS[12] was categorized as mild (0-4), moderate (5-15), or severe (16-42). Furthermore, strokes were classified according to the Bamford criteria[13] in total anterior circulation stroke (TACS), partial anterior circulation stroke (PACS), posterior circulation stroke (PCS), and lacunar stroke (LS). Patients being admitted within 4.5 h of onset of symptoms were thrombolysed with injection alteplase, provided they did not have the contraindications for thrombolysis. For each patient, 4 serum biomarkers [(D-dimer, fibrinogen, CRP and Neuron specific enolase (NSE)] were evaluated at admission and 24 h later. D-dimer was assessed using enzyme linked immunosorbant assay (ELISA) kits from GenWay Biotech, San Diego, California, USA, and a level of more than 4 μg/mL was considered high. NSE was assessed using human NSE ELISA kit (Elabscience Biotechnology Co. Ltd., Houston, USA) and the normal value at spectrophotometers in the 450 nm wavelength was 7.2-12 ng/mL. Serum concentrations of CRP were quantified using a commercially available turbidimetric immunoassay (Transasia Bio-Medicals Ltd., Erba Diagnostics, Mannheim, Germany) and value < 6 mg/L was considered normal. Fibrinogen was assayed by FibroTek fibrinogen kit (R2 Hemostasis Diagnostics India Private Limited, Indira Puram, Ghaziabad, Uttar Pradesh) and the value of 150-400 mg/dL was considered normal.

Follow-up magnetic resonance imaging examination (1.5 Tesla system providing axial T1, T2, and proton density weighted images) or brain CT scan was repeated 5 days after the index event. Metabolic profile (renal and liver function tests); and hematologic parameters (complete hemograms and coagulation profile) were recorded in the registry on arrival and again at 24-48 h after stroke onset. The cardiological profile (electrocardiogram and transthoracic echocardiography) and a search for vasculitis (antinuclear factor and antiphospholipid antibodies) were also done. Previous infections were excluded by medical history, chest radiograph, routine urinalysis, and complete physical examination. The vital parameters were recorded continuously using multi-parameter monitors. The length of hospital stay was defined from the day of admission to a hospital ward to the day of discharge. Acute stroke management and secondary prevention in these patient followed current European Stroke Organization guidelines[14]. Discharged patients were followed up on a monthly basis through neurological examination and review of records. Their clinical outcomes were assessed using the modified Rankin scale (mRS) and categorized as favorable (score 0-1) or unfavorable (score 2-6). Exclusion criteria included history of recent infection, obvious signs of acquired infection before stroke

onset, and an initial CRP level > 10 mg/dL due to presumed infection.

Outcome measures - the primarily aims of the study were the following: (1) to identify the factors independently predicting: i) the severity of stroke; ii) the unfavorable outcome at 30 days post discharge; iii) the mortality; and iv) the chance of post thrombolysis hemorrhage; and (2) to evaluate the effect of thrombolysis on the outcome of ischemic stroke patients. The secondary aim was to evaluate the role of biomarkers to predict the unfavorable outcome and the chance of post thrombolysis hemorrhage and to evaluate the change of biomarker levels post thrombolysis.

The patient characteristics, comorbid risk factors, and hospital investigations were assessed by Chi-square test for categorical variables and independent-samples *t* test and one way analysis of variance for continuous variables. The variables analyzed were age, gender, body mass index, life conditions, comorbidities, NIHSS at admission, vascular risk factors, therapy prior to stroke, addictions, pathophysiologic and metabolic factors. Multivariate logistic regression models estimated the impact of possible determinants of stroke severity at admission. Differences between groups and effect of patient characteristics on the clinical outcome were assessed using Chi-square test. Statistical tests were considered significant when the value was ≤ 0.05. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 20.0 software version.

RESULTS

Out of 30 patients admitted with first ischemic stroke, 15 patients arrived at the hospital within 4.5 h. Out of them, 2 patients had NIHSS score > 25, 2 patients had stroke involving > 1/3 cerebral hemisphere, and 1 patient was taking oral anticoagulants for dilated cardiomyopathy - hence they did not receive thrombolysis. The rest 10 patients were thrombolysed with injection alteplase. The mean time (standard deviation or SD) of presentation of patients who were thrombolysed was 3.8 (0.7) and 7.8 (2.4) h for the remaining patients. Twelve patients had a mild stroke, 10 had a moderate stroke and 8 had a severe stroke. Fourteen patients had mRS score < 2, and 16 had mRS score ≥ 2, among whom 5 patients expired. Three out of ten thrombolysed patients developed intracerebral hemorrhage, among whom 1 patient expired. During admission, all the cases of AF were already diagnosed and were on anticoagulants as per the current guidelines. The results have been described in Tables 1-6.

DISCUSSION

Discussion on determinants of stroke severity and outcome

In this study on first-ever ischemic stroke patients, we demonstrated that risk factors such as old age, history of diabetes, CHADS2 score ≥ 2, and TACS independently affected stroke severity, whereas low EF < 35%, and TACS, independently predicted the unfavorable outcome (mRS score ≥ 2) and mortality. High mean arterial blood pressure (MABP) and capillary blood glucose (CBG) at admission were significant predictors for stroke severity, mRS score ≥ 2 and mortality. Female patients had significantly higher incidence of unfavorable outcome, but female gender was not a significant predictor of stroke severity and mortality. CHADS2 score significantly predicted the unfavorable outcome, but it was not a significant predictor when mortality was considered alone. Hypertension, hypercholesterolemia, smoking, ischemic heart disease, and AF showed a non-significant trend to be more prevalent among patients with severe stroke, unfavorable outcome and mortality. History of TIA was significantly associated with higher incidence of severe stroke and mortality. Home medications, living conditions, PACS and PCS did not significantly contribute to the severity and outcome of the stroke. However, patients of LS had a significantly lesser risk of having an unfavorable outcome. In the previous population-based studies, old age was found to be a strong independent predictor of ischemic stroke severity, outcome and mortality[7,15]. In Corso's study[7], patients > 85 years of age had 2.9 times higher risk for having a severe stroke. In our study, out of 3 patients > 80 years of age, 2 had a severe stroke [odds ratio (OR) = 7 (0.53-91.11), $P = 0.16$]. Previous reviews reported that female gender

Table 1. Patient characteristics at time of initial stroke

Characteristics	Mild stroke (n = 12)	Moderate stroke (n = 10)	Severe stroke (n = 8)	P value
Female sex	5	6	3	1.0
BMI (kg/m^2)	25.3 (0.3)	26.5 (0.5)	26.9 (0.8)	0.045
Age	58.2 (6.2)	62.4 (10.8)	66.3 (6.3)	0.01
Living condition				
Lives alone	2	1	3	0.6
Lives with family	10	9	5	
Vascular risk factors				
Hypertension	8	9	8	0.11
Hypercholesterolemia	8	7	7	0.6
Diabetes	3	8	7	0.02
Previous TIA	0	2	3	0.04
Current smoking	5	4	3	1.0
Ischemic heart disease	1	1	2	0.53
AF	1	2	2	0.53
Low EF (< 35%)	1	2	3	0.25
CHADS2 score				
0-1	10	1	2	
≥ 2	2	9	6	0.01
Bamford classification				
TACS	0	3	4	0.014
PACS	3	2	1	0.6
PCS	2	3	3	0.6
LS	7	2	0	0.14
MABP at admission	105.4 (8.8)	112 (9.2)	116 (7.8)	0.013
CBG at admission	152.1 (8.2)	163.7 (11.8)	181.4 (10.1)	< 0.0001
Home medications				
Statins	5	5	2	0.64
Antihypertensives	6	5	3	0.4
Anticoagulants	1	2	2	0.5

P value-statistical analysis was performed on the NIHSS 0-4 and the NIHSS ≥ 16 groups. The univariate analysis in Table 1 identified that higher BMI and age, diabetes, previous TIA, CHADS2 score ≥ 2, occurrences of TACS, higher MABP and CBG at admission significantly contributed to the occurrence of severe stroke. Multiple logistic regression analyses identified that the presence of diabetes [adjusted OR, i.e., AOR (95% CI) = 16.20 (2.5-180.45), P = 0.025], CHADS2 ≥ 2 score [AOR = 14.8 (1.2-130.17), P = 0.02, and occurrence of TACS (AOR = infinity, P = 0.016) independently influenced the higher NIHSS scores (≥ 16). Six patients had cardioembolic stroke. EF: ejection fraction; MABP: mean arterial blood pressure; CBG: capillary blood glucose; BMI: body mass index; TIA: transient ischemic attack; TACS: total anterior circulation stroke; PCS: posterior circulation stroke; LS: lacunar stroke; AOR: adjusted odds ratio

Table 2. Results of multivariate logistic regression analysis model for probability of unfavorable outcome (mRS score ≥ 2)

Characteristics	mRS score ≥ 2 (n = 16)	OR (95% CI)	RR (95% CI)	P value
Female sex (n = 14)	11	0.1636 (0.03-0.83)	0.47 (0.23-0.95)	0.032
Hypertension	15	6 (0.58-61.8)	1.3 (0.92-1.8)	0.15
Hypercholesterolemia	12	1.2 (0.23-6.0)	1.05 (0.67-1.6)	1.0
Diabetes	10	1.25 (0.2-5.4)	1.09 (0.6-1.97)	1.0
Previous TIA	4	4.33 (0.4-44.4)	3.5 (0.44-27.7)	0.33
Current smoking	9	4.7 (0.9-23.6)	2.6 (0.88-7.8)	0.07
Ischemic heart disease		0.85 (0.1-7.4)	0.87 (0.14-5.4)	1.0
AF	4	4.33 (0.4-44.4)	3.5 (0.44-27.7	0.33
Low EF (< 35%)	6			0.018
CHADS2 score ≥ 2	13	10.83 (1.9-59.8)	2.84 (1.2-6.7)	0.008
TACS	7			0.007
PACS	3	0.84 (1.4-5.07)	0.87 (0.2-3.65)	1
PCS	6	3.6 (0.59-21.9)	2.6 (0.62-10.9)	0.22
LS	2	0.14 (0.02-0.8)	0.25 (0.06-1.01)	0.045

The mean (SD) age and BMI among patients with mRS score ≥ 2 and mRS score < 2 were 68.6 (7.4) years *vs.* 62.7 (8.2) years (P = 0.047) and 26.3 (0.7) kg/m^2 *vs.* 25.8 (0.5) kg/m^2 (P = 0.043). At admission, the mean (SD) MABPs and CBGs among patients with mRS score ≥ 2 and mRS score < 2 were 115.5 (5.9) mm of Hg *vs.* 110.4 (4.6) mm of Hg (P = 0.025) and 186.6 (29.8) mg/dL *vs.* 150.4 (14.6) mg/dL (P = 0.0003). Thus, the univariate analysis in Table 2 identified female sex, smoking, low EF, CHADS2 score ≥ 2, the occurrence of TACS, higher age and BMI and higher MABP and CBG at admission as predictors of mRS score ≥ 2. Patients with lacunar stroke had a significantly lower incidence of mRS score ≥ 2 (P = 0.045). Multivariate analysis identified low EF (AOR = infinity), CHADS2 score ≥ 2 [AOR = 11.1 (2.1-54.2), P = 0.009], and occurrence of TACS (AOR = infinity) to be independent predictors of mRS score ≥ 2. EF: ejection fraction; TACS: total anterior circulation stroke; PACS: partial anterior circulation stroke; PCS: posterior circulation stroke; LS: lacunar stroke

Table 3. Results of multivariate logistic regression analysis model for probability of mortality

Characteristics	Mortality (n = 5)	OR (95% CI)	RR (95% CI)	P value
Female sex (n = 14)	3	1.9 (0.27-13.49)	1.7 (0.33-8.83)	0.64
Hypertension	4	0.76 (0.06-8.7)	0.8 (0.11-5.7)	1.0
Hypercholesterolemia	4	1.55 (0.14-16.4)	1.45 (0.18-11.14)	1.0
Diabetes	3	1.55 (0.14-16.4)	1.45 (0.18-11.14)	1.0
Previous TIA	3	17.25 (1.72-172.02)	7.5 (1.65-33.94)	0.02
Current smoking	2	1 (0.14-7.69)	1 (0.19-5.12)	1
Ischemic heart disease	2	7.66 (0.76-76.45)	5 (0.9-27.06)	0.118
AF	2	4.88 (0.96-42.3)	3.33 (0.73-15.08)	0.18
Low EF (< 35%)	3	7.33 (1.27-95.18)	4.16 (1.1-15.7)	0.041
CHADS2 score ≥ 2	4	3.69 (0.36-37.85)	1.53 (0.86-2.74)	0.35
TACS	4	29.33 (2.4-357.86)	6.66 (2.11-21.02)	0.0057
PACS	1	1 (0.09-11.02)	1 (1.39-6.8)	1
PCS	3	6 (0.78-46.14)	3 (1.03-8.67)	0.1
LS	0			0.28

The mean (SD) age of expired patients was 66.5 (7.4) years compared to 60.7 (9.3) years for surviving patients (P = 0.201), and mean BMI of expired patients was 25.4 (0.8) kg/m^2, compared to 24.9 (0.7) kg/m^2 for surviving patients (P = 0.16). At admission, the mean (SD) MABP among survivors was 115.2 (5.9) mm of Hg *vs.* 110.3 (4.2) mm of Hg among expired patients (P = 0.033). The mean (SD) CBGs at admission were 179.9 (12.5) mg/dL among survivors vs. 152.6 (20.6) mg/dL among expired patients (P = 0.0001). The univariate analysis in Table 3 identified previous TIA, low EF, the occurrence of TACS, higher age and BMI and higher MABP and CBG at admission as predictors of mortality. Multivariate analysis identified low EF [AOR = 7.21 (1.5-90.21), P = 0.043], and the occurrence of TACS [AOR = 28.9 (3.5-320.47), P = 0.006] to be independent predictors of mortality. TIA: transient ischemic attack; EF: ejection fraction; TACS: total anterior circulation stroke; PACS: partial anterior circulation stroke; PCS: posterior circulation stroke; LS: lacunar stroke

Table 4. Mean (SD) levels of biomarkers at admission among different groups of stroke patients

Biomarker	Mild stroke (n = 12)	Moderate stroke (n = 10)	Severe stroke (n = 8)	P value	mRS score < 2 (n = 14)	mRS score ≥ 2 (n = 16)	P value
CRP (mg/L)	4.7 (1.4)	5.7 (2.3)	6.9 (3.1)	0.043	5.6 (0.9)	6.9 (2.1)	0.040
Fibrinogen (mg/dL)	390.67 (20.25)	456.4 (40.50)	500.75 (37.86)	< 0.0001	430 (29.75)	478 (37.65)	0.0007
D-dimer (μg/mL)	4.7 (0.5)	6.4 (2.2)	8.8 (2.5)	< 0.0001	5.6 (1.4)	7.2 (2.5)	0.043
NSE (ng/mL)	24.5 (5.4)	37 (11.9)	56 (20.5)	< 0.0001	30.6 (6.8)	47 (16.87)	0.0021

Table 4 demonstrated that all the biomarker levels at admission were significantly higher among patients with severe stroke and unfavorable outcome (statistical analysis was performed on the mild and severe stroke groups). CRP: C-reactive protein; NSE: neuron specific enolase

Table 5. Comparison of final outcome and mean (SD) levels of the biomarkers 24 h after admission among thrombolysed *vs.* non-thrombolysed patients

Characteristics	Thrombolysed (n = 10)	Non- thrombolysed (n = 20)	P value
mRS ≥ 2 (n = 16)	2 (20%)	14 (70%)	0.018
Death (n = 5)	1 (10%)	4 (20%)	0.64
CRP	5.32 (0.9)	5.87 (2.1)	0.43
Fibrinogen	420 (20.5)	479.2 (29.4)	< 0.0001
D-dimer	6.6 (3.0)	4.9 (2.0)	0.047
NSE	36.5 (14.6)	40.2 (16.8)	0.54

Table 5 showed that thrombolysis significantly reduced the incidence of the unfavorable outcome, but did not significantly affect death. Three patients with TACS, two with PACS, three with PCS and two with LS underwent thrombolysis. One each with TACS and PCS had mRS ≥ 2. Thus, there was no significant difference in the efficacy of thrombolysis on stroke in various arterial territories (P > 0.05). D-dimer levels significantly increased and fibrinogen level significantly decreased following thrombolysis. CRP: C-reactive protein; NSE: neuron specific enolase; TACS: total anterior circulation stroke; PACS: partial anterior circulation stroke; LS: lacunar stroke.

has more severe strokes than men, with a 1-month case fatality of 24.7% *vs.* 19.7% for males[16]. The case fatality rates were lower in our study - 21.4% among females and 12.5 % among males. In Corso's study, the female patients were older and suffered more frequently from AF[17]; hence, females had a more severe stroke. Previous studies have reported that stroke patients with AF mostly present with large cortical infarcts, and less frequently with lacunar infarcts compared with patients without AF due to the lack of collateral vessels,

Table 6. Comparison of the baseline characteristics and biomarker levels at admission for patients developing hemorrhage (hge.) post thrombolysis

Characteristics	Patients with hge. complications (n = 3)	Patients without hge. complications (n = 7)	P value
BMI (kg/m^2)	26.5 (1.1)	26.8 (0.9)	0.66
Mean age (SD)	64 (10.8)	63 (8.2)	1.0
Vascular risk factors			
Hypertension	3	4	0.475
Hypercholesterolemia	3	4	0.475
Diabetes	2	3	1.0
AF	1	2	1.0
CHADS2 score			
≥ 2	1	3	1.0
Bamford classification			
TACS	2	2	0.5
PACS	0	3	0.475
PCS	1	2	1.0
MABP at admission	110.5 (4.2)	118.8 (4.8)	0.032
CBG at admission	251.4 (109.4)	143.2 (45.7)	0.048
Mean NIHSS at admission (SD)	10.4 (5.8)	20.2 (4.0)	0.0137
CRP (mg/L)	5.88 (0.24)	5.64 (0.32)	0.28
Fibrinogen (mg/dL)	478.5 (20.85)	421.4 (26.4)	0.01
D-dimer (μg/mL)	6.88 (1.4)	6.38 (1.7)	0.5
NSE (ng/mL)	42.1 (11.2)	30.8 (4.8)	0.07

Table 6 demonstrated that higher MABP, CBG, and fibrinogen levels at admission predicted significantly higher chance to develop post-thrombolysis hemorrhagic complications. Other three biomarkers were not significant predictors of hemorrhagic complications. (N.B- in Tables 1, 2, 3, and 6 some patients had multiple comorbidities). BMI: body mass index; TACS: total anterior circulation stroke; PACS: partial anterior circulation stroke; PCS: posterior circulation stroke; MABP: mean arterial blood pressure; CBG: capillary blood glucose; CRP: C-reactive protein; NSE: neuron specific enolase.

which develop and compensate for acute arterial occlusion in patients with gradual occlusion of arteries, such as in atherosclerosis of cervical or cerebral arteries[18]. Steger *et al.*[19] reported that for AF patients with ischemic stroke, the in-hospital mortality was higher (25% *vs.* 14%, $P < 0.0004$) and neurological outcome was poorer (65 *vs.* 90 Barthel index, $P < 0.0004$). But in our study, multivariate analysis did not establish AF as an independent predictor of mortality. AF was non-significantly more frequent among patients with severe stroke, unfavorable outcome, and among those who expired. Ntaios *et al.*[20] reported that compared with CHADS2 score 0, patients with CHADS2 score 1 and CHADS2 score > 1 had higher risks of ischemic stroke [hazard ratio 2.38 (95% CI: 1.41-4.00) and 2.72 (95% CI: 1.68-4.40), respectively] and death [hazard ratio 3.58; (95% CI: 1.80-7.12), and 5.45 (95% CI: 2.86-10.40) respectively]. In our study, CHADS2 score ≥ 2 significantly predicted stroke severity but did not predict mortality. Di Tullio *et al.*[21] in their "Reduced Ejection Fraction Trial", demonstrated that baseline left ventricular EF < 15% was inversely and linearly associated with the primary outcome, and mortality. Even in warfarin-treated patients, each 5% EF decrement significantly increased the stroke risk [adjusted hazard ratio 2.125 (95% CI: 1.182-3.818)]. In our study, EF < 35% [mean (SD) EF = 26.8 (5.8)] significantly predicted unfavorable outcome and mortality. Although Nedeltchev *et al.*[22] and Musolino *et al.*[23] reported current smoking, followed by hypercholesterolemia, family history of cerebrovascular disease, and hypertension to be the most prevalent risk factors among young ischemic stroke patients, these factors were non-significant predictors of stroke severity and outcome in our study. Osmani *et al.*[24] reported that TACS had the worst outcome with the highest number of mortalities (72.2%). The LS had a better outcome, i.e. 65.7% of the patients were functionally independent by the end of 6 months compared to 15% of TACS patients[24]. In our study, patients with TACS had a significantly higher incidence of severe stroke (57.14%), unfavorable outcome (100%) and mortality (57.14%). Huang *et al.*[25] also described that TACS was associated with a poor functional outcome, but patients had a better outcome with LS. Medications use like statins, anticoagulants and antihypertensives did not affect stroke prognosis, similar to Corso's study[7]. Koton *et al.*[26] described that systolic blood pressure (SBP) at admission was associated with stroke severity and disability at discharge or in-hospital death with an adjusted OR of 1.06 (95% CI: 1.04-1.08)

per 10mmHg change in SBP. In our study too, the higher MABP at admission was significantly associated with the stroke severity, the unfavorable outcome, and mortality. Bruno *et al.*[27] described that in all strokes combined (P = 0.03) and in non-LSs (P = 0.02), higher admission blood glucose levels were associated with the worse outcomes at 3 months according to multivariate logistic regression analysis adjusted for stroke severity, diabetes mellitus, and other vascular risks, thereby, corroborating with our findings.

Discussion on the role of thrombolysis

Our study demonstrated that thrombolysis significantly reduced the incidence of the unfavorable outcome, which is evidenced by the fact that only 20% of thrombolysed patients and 70% of non-thrombolysed patients had the unfavorable outcome. Mehta *et al.*[28] reported 67.1% good outcome and 32.9% unfavorable outcome post thrombolysis; diabetes, dyslipidemia, NIHSS at admission > 15, blood sugar > 250 mg/dL, dense cerebral artery sign and occlusion of large artery being significant predictors of the poor outcomes on multivariate analysis. Liu *et al.*[29] reported that age ≥ 70 years, NIHSS score > 20, serum glucose on admission > 9.0 mmol/L and cardioembolism were independent predictors of hemorrhage after thrombolysis in Chinese patients with acute ischemic stroke. Thirty percent patients in our study had post-thrombolysis hemorrhage. Blood glucose, MABP, higher mean NIHSS score and serum fibrinogen level at admission correlated significantly with post-thrombolysis hemorrhage. Higher NIHSS score increases the risk of hemorrhages since severe ischemic stroke is reflected by large areas of injured brain tissue, including injured blood vessels, which are prone to bleeding after rtPA treatment[30].

Discussion on the role of biomarkers

Our study demonstrated that the mean values of all the 4 biomarkers-CRP, fibrinogen, D-dimer, and NSE were significantly higher among patients with severe stroke. Fibrinogen level decreased and D-dimer level increased significantly following thrombolysis. Fibrinogen was the only biomarker, whose elevated levels could significantly predict post-thrombolysis hemorrhage. Results of a meta-analysis[31] indicated a significant association between the elevated baseline CRP and unfavorable long-term functional outcome. Although our study is based on the CRP values at admission, interestingly, two studies of the meta-analysis[32,33] showed a stronger association of poor outcome with hs-CRP measurements at 24-48 h and 7 days reflecting impairment of the recovery process due to prolonged inflammation after ischemic stroke.

Rothwell *et al.*[34] described that fibrinogen predicted ischemic stroke, with the association tending to be stronger in patients with nonlacunar than lacunar syndromes. Moreover, fibrinogen levels were found to be an independent predictor of early neurological deterioration among diabetic patients[35]. The Alteplase-Tenecteplase Trial Evaluation for Stroke Thrombolysis study demonstrated that alteplase was associated with prolongation of prothrombin time, reduced fibrinogen and plasminogen, elevated fibrin degradation products and d-dimer level[36]. Following ischemic stroke, tissue responds with mitochondrial dysfunction and increased nitric oxide (NO) production which vasodilates and maintains blood perfusion. In turn this leads to a burst in free radical production and the generation of peroxynitrite, which irreversibly nitrates proteins. Fibrinogen's up-regulation as an acute-phase reactive protein and increased permeability of the blood-brain barrier during ischemic stroke allowing extravasation of different plasma proteins into the brain parenchyma further potentiates fibrinogen nitrotyrosination. At early stages nitro-fibrinogen delays clot formation, but in the long term, it becomes harmful due to the production of fibrinolysis resistant clots and the induction of neuronal damage[37]. Hence, possibly more elevation in the fibrinogen level produces more cellular and local vascular damage, resulting in a higher chance of post-thrombolysis hemorrhage.

Previous studies demonstrated that acute ischemic stroke patients had significantly higher plasma median D-dimer levels as compared to healthy controls[38]. D-dimer levels increased with increasing severity of stroke and infarct volume and the positive trends existed even after correcting for possible confounding factors[38]. Thus, D-dimer concentrations may be considered a direct consequence of marked cerebral infarc-

tion. Although D-dimer levels are significantly associated with cardioembolic stroke, the significance of D-dimer levels in relation to the severity and functional outcomes of other stroke subtypes was investigated by Kim *et al.*[39]. Patients with higher D-dimer levels had significantly worse initial functional outcomes, and these worse outcomes were maintained throughout the 9-month follow-up period compared with the low D-dimer group. However, regardless of stroke subtype, D-dimer levels did not influence long-term longitudinal temporal changes of functional outcomes over the 9-month follow-up period[39]. In our study too, patients with significantly higher D-dimer levels at admission, had an unfavorable outcome.

In 2005, Anand and Stead[40] described that serum NSE level was significantly higher in stroke patients than in controls, and correlated with infarct tissue volume, but did not correlate with the functional outcome. This was explained by the disparity in sampling time because the NSE level has been shown to peak after 4-8 h and hence, a better correlation was expected from delayed sampling. However, in 2013, Zaheer *et al.*[41] found that NSE level in day 1 positively correlated with infarct volume and functional neurological outcome at day 30 and negatively correlated with Glasgow Coma Score at presentation. In our study, the mean (SD) time of presentation of the patients who underwent thrombolysis was 3.8 (0.7) h, and they also had elevated serum NSE levels. This signifies the requirement of more multicentre research with a larger sample size to determine the optimum time needed for NSE level to elevate in serum following an acute ischemic stroke.

Limitations

Small sample size confounded the results of factors predicting the severity and outcome of stroke and resulted in wider CI. Even important risk factors like hypertension, hypercholesterolemia, smoking, and AF could not be established as significant predictors. The short follow up time of 1 month, hindered the evaluation of the long-term neuro-disabilities. The computed tomography machine at our institution does not have the software to determine the infarct volume and so the correlation of the levels of the biomarkers with the volume of infarcted tissue could not be done.

What this study adds: (1) EF between 15%-35% also independently predicts severity and the outcome of ischemic stroke; (2) MABP and CBG at admission significantly predict severity and outcome of ischemic stroke, and meticulous management of these factors may improve the outcome; (3) serum NSE level can rise earlier than 4 h - this fact needs to be validated by other larger studies; (4) fibrinogen level > 478.5 mg/dL at admission significantly predicts the higher chance of post-thrombolysis haemorrhage; and (5) there is no significant difference in the efficacy of intravenous thrombolysis on stroke in various arterial territories.

DECLARATIONS

Authors' contributions

Provided intellectual inputs: Ghosh KC, Bhattacharya R, Mondal GP
Collected data: Ghosh S, Das S, Mahata M
Prepared the manuscript and acted for correspondence: Das S

Availability of data and materials

The data and material could be available to readers upon request.

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None.

Conflicts of interest

All authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

The study was approved by Institutional Ethicals Committee, Calcutta National Medical College, Kolkata and consent was obtained from the patients.

Consent for publication

Not applicable.

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