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Plasma GFAP, Co-peptin & MMP-9 concentrations among West African Stroke subjects compared with Stroke free controls

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Abstract

Background—Measurement of plasma molecular markers among stroke patients has been proposed as an avenue for improving the accuracy of stroke diagnosis. There is paucity of data on the potential role of these markers in resource-limited settings, where the burden of stroke is greatest.

Objective—To assess the potential diagnostic and prognostic performance of 3 proposed biomarkers for stroke in a resource-constrained setting.

Methods—Consecutive stroke subjects presenting at a tertiary medical center in Kumasi, Ghana, with radiologically confirmed diagnosis and etiologic subtype information available were recruited along with age- and sex-matched controls in a 2:1 ratio. Plasma concentrations of Glial Fibrillary Acidic Protein (GFAP), copeptin and Matrix Metalloproteinase-9 (MMP-9) among stroke cases and stroke-free controls were measured in duplicates using Enzyme Linked Immunoassays. Diagnostic and prognostic correlates were assessed using Area-under-the Curve measures of Receiver-Operator-Curves (ROCs) and Logistic regression analysis respectively.

Results—There were 156 stroke subjects with a mean age of 61.3 years of which 47.4% were females and 74 age- and sex-matched stroke-free controls. Median (IQR) time from symptom onset to hospital presentation for care was 7 days (5–11). Diagnostic accuracy of a single measurement of the three biomarkers for stroke using AUC (95%CI) plots were: 0.84 (0.77–0.91),

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$p < 0.0001$ for GFAP; 0.85 (0.79–0.92), $p < 0.0001$ for copeptin and for MMP-9 was 0.65 (0.56–0.73), $p = 0.0003$. None of the biomarkers was associated with stroke severity or mortality.

Conclusion—Plasma concentrations of GFAP and co-peptin demonstrated stronger associations with stroke occurrence in this West African cohort compared with controls.

Keywords

Plasma Biomarkers; GFAP; MMP-9; copeptin; Africa; stroke; diagnosis; prognosis

INTRODUCTION

Stroke is defined pathologically as an acute episode of focal cerebral, spinal or retinal dysfunction caused by infarction or spontaneous intraparenchymal or subarachnoid hemorrhage of central nervous system tissue of vascular origin.¹ The molecular perturbations immediately preceding or occurring after tissue infarction from stroke are accompanied by the elaboration of a diverse array of biomarkers of diagnostic, prognostic and predictive relevance.^{2,3} An elucidation of the bio-molecular profile of stroke subjects of African descent is a fundamental prerequisite for understanding its potential roles in settings where stroke patients usually present late for care and diagnostic neuro-imaging facilities are seldom available or accessible.

Glial fibrillary acidic protein (GFAP) is an intermediary filament expressed by astrocytes and ependymal cells and found in serum of patients due to necrotic brain cell destruction and Blood Brain Barrier (BBB) disruption, with a graded increase in circulating concentration from controls, ischemic stroke and hemorrhagic stroke subjects respectively.^{4–6} Copeptin is the C-terminal part of pro-vasopressin, a neuroendocrine stress marker associated with unfavorable outcomes among ischemic and hemorrhagic stroke subjects.^{7–10} Matrix Metalloproteinase-9 (MMP-9) is a protease induced by thrombin and blood, which increases capillary permeability, disrupts BBB, acts as a neurotoxic by degrading the endothelial basal lamina and extracellular matrix and is elevated after intracerebral hemorrhage where correlations between edema and neurological worsening have been identified.^{11–14} These biomarkers have been shown to be associated with stroke occurrence and stroke prognosis among predominantly Caucasian subjects but there is limited information on their potential utility among indigenous African stroke subjects.

Therefore the objective of this study is compare plasma concentrations of GFAP, copeptin and MMP-9 in a cohort of consecutive stroke patients matched with stroke-free control subjects encountered at a Ghanaian tertiary medical center. Our hypothesis is that, circulating plasma titers of GFAP, copeptin and MMP-9 may be significantly higher than among age- and sex-matched stroke free controls and that these biomarkers could have potential diagnostic and or prognostic relevance for stroke in African populations subject to validation in larger subsequent studies.

METHODS

Ethics

This study was approved by the Committee of Human Research Publications and Ethics of the Kwame Nkrumah University of Science and Technology and is part of the on-going Stroke Investigative Research and Educational Networks (SIREN) case-control study with protocol published elsewhere.¹⁵

Study subjects

Briefly, SIREN is a multicenter study involving 12 study sites in Ghana and Nigeria and has been running since August 2014. Stroke cases included consecutive consenting adults (aged 18 years or older) with first clinical stroke within 8 days of current symptom onset or 'last seen without deficit'. Cranial CT or MRI were performed within 10 days of symptom onset since beyond 10 days, ischemic and hemorrhagic strokes may be difficult to distinguish with great certainty. Stroke type classification was based on clinical evaluation and brain neuroimaging (CT or MRI of brain), ECG and/or transthoracic echocardiography, and carotid Doppler ultrasound performed by specially trained clinicians according to standardized protocols (SOP). Ischemic stroke was typed clinically using the presumed etiological sub-types as defined using the Trial of Org 10172 in Acute Stroke Treatment¹⁶ criteria. Intracerebral hemorrhage was classified etiologically into structural, medication-related, amyloid angiopathy, systemic/other disease, hypertension and undetermined causes (SMASH-U).¹⁷ Neuroradiological examination based on cranial CT imaging were performed independently by a radiologist who was blinded to all other data and reported on lesion topography, territories of vascular supply and volume of lesion which was calculated in all scans showing a clearly demarcated infarct area or hemorrhage. Measurement of Intracerebral hemorrhage volume (ml) was performed using the standard ellipsoid method.¹⁸ For ischemic stroke lesion size measurements, the area of abnormal low attenuation was traced on each CT slice and volume was derived from the area and the slice thickness. Adjudication of stroke subtypes were done by consensus between Neurologist (FSS), Cardiologist (LA) and Radiologist (MA) after reviewing clinical findings, CT scans and results of EKG, echocardiography and carotid Doppler. In-patient outcomes of stroke were classified according to the vital status at discharge namely dead or alive.

Controls were consenting stroke-free adults, mostly from the communities in the catchment areas of the SIREN hospitals where cases were recruited from.¹⁹ Stroke-free status was confirmed with the 8-item questionnaire for verifying stroke-free status (QVSFS) which has 98% negative predictive value.¹⁹ Controls were matched by age (± 5 years), sex and ethnicity.

Blood sampling and Biomarker measurements

At hospital admission, 5ml of blood was collected in a potassium EDTA Vacutainer tube (BD Company, UK) and rapidly transported to the laboratory of the Komfo Anokye Teaching Hospital and centrifuged at 1,500–2,000g for 10 minutes within 1 hour after blood draw. Plasma samples were immediately frozen and stored at -80°C until analysis. Plasma concentrations of copeptin, GFAP and MMP-9 were measured on stored samples using

Sandwich ELISA kits from Green Stone Swiss Ltd (China) according to manufacturer's instructions. The mean absorbance of duplicate standards, samples, and controls were calculated for each plate, and the mean zero standard was subtracted. A standard (best-fit) calibration curve was plotted for each analyte with lower limits of detection for GFAP, copeptin and MMP-9 of 30ng/L, 0.2/pmol/L, 45ng/L respectively.

Statistical analysis

Comparisons were performed using the Student's t-test or Analysis of Variance (ANOVA) for continuous variables with parametric distribution and the Mann-Whitney or Kruskal Wallis tests for continuous variables with nonparametric distributions respectively. Discrete variables were compared using Chi-square test and correlations between continuous variables were assessed using Pearson's correlation. Receiver Operator Characteristics (ROC) curves were used to calculate diagnostic accuracy for GFAP, copeptin and MMP-9 for distinguishing between strokes and controls, and for ischemic and hemorrhagic stroke subtypes using AUC of ROC curves with 95% CIs. Multiple logistic regression analysis was used to assess the predictors of stroke mortality with GFAP, copeptin and MMP-9 pre-specified as covariates in unadjusted models, however only variables attaining a p-value <0.10 were included in final adjusted model estimates. Statistical analyses were performed using the GraphPad Prism 7 software (GraphPad Software, Inc) and SPSS version 21. Missing data were excluded from the analysis, p-values <0.05 were considered significant with no adjustments for multiple comparisons.

RESULTS

Demographic & Clinical Characteristics of study populations

There were 156 stroke subjects and 74 stroke-free controls with no significant differences in mean age and gender distribution but significantly more controls resided in urban domicile compared with cases (Table 1). Furthermore, hypertension, diabetes mellitus and hyperlipidemia were significantly more frequent among stroke cases compared with controls as well as a marginal increase in heart diseases was noted. There were 99 (63.5%) ischemic strokes and 57 (36.5%) hemorrhagic strokes. Etiologically, ischemic stroke subtypes included lacunar strokes (50.5%), cardio-embolic (25.3%), large-vessel atheroembolic (19.2%), unknown (4.0%), and others (1.0%) whilst Hemorrhagic stroke subtypes include Hypertension (73.7%), Structural lesions such as aneurysms and AV-malformations (15.8%), unknown (7.0%), and Amyloid Angiopathy (3.5%).

Plasma levels of GFAP, copeptin & MMP-9

The mean $\pm$ SD plasma concentrations of GFAP of $1,657 \pm 604$ ng/L vs 676 ± 928 ng/L, $p < 0.0001$, copeptin of 21.2 ± 8.4 pmol/L vs 6.6 ± 10.7 pmol/L, $p < 0.0001$ and MMP-9 of 2524 ± 1726 ng/L vs 1876 ± 1929 ng/L, $p = 0.01$ were significantly higher among stroke cases compared with stroke-free controls respectively. Plasma concentrations of GFAP, copeptin and MMP-9 were significantly higher among ischemic and hemorrhagic stroke types compared with control subjects respectively as shown in Figure 1.

Overall, no significant differences in concentrations of the three biomarkers were noted when ischemic and hemorrhagic stroke subjects were compared. However, when analysis between the two primary stroke types were limited to subjects who presented within 3 days of symptoms onset, significant differences in serum concentrations were observed. The mean $\pm$ SD concentrations of GFAP among hemorrhagic stroke subjects presenting within 3 days of symptom onset (n=10) of 2106 ± 716 ng/L was significantly higher than 1498 ± 482 ng/L among ischemic stroke subjects (n=12), $p=0.03$. Copeptin was marginally higher among ischemic stroke subjects compared with hemorrhagic stroke with mean $\pm$ SD measurement of 26.3 ± 7.4 pmol/L vs 20.7 ± 6.8 pmol/L, $p=0.08$. However for MMP-9, there were no differences between the two stroke types amongst subjects presenting within 3 days of symptom onset.

Diagnostic correlates of GFAP, copeptin & MMP-9

The diagnostic accuracy of a single measurement of the three biomarkers for stroke using AUC (95%CI) plots were: 0.84 (0.77–0.91), $p<0.0001$ for GFAP; 0.85 (0.79–0.92), $p<0.0001$ for copeptin and for MMP-9 was 0.65 (0.56–0.73), $p=0.0003$. (Figure 2). At a serum GFAP cut-off value of 775.5ng/L, sensitivity and specificity for stroke diagnosis were 70% (95% CI: 59–82) and 95% (95% CI: 90–98) respectively. That of copeptin at cut-off value of 2.8 pmol/L were 80% (95% CI: 59–82) and 87% (95% CI: 80–91) respectively and MMP-9 had sensitivity and specificity of 61% (95% CI: 49–72) and 59% (95% CI: 51–67) respectively at a cut-off value of 331ng/L.

The diagnostic accuracy of GFAP for ischemic and hemorrhagic stroke compared with controls was 0.83 (0.77–0.90), $p<0.0001$ and 0.85 (0.78–0.92), $p<0.0001$ respectively; that for co-peptin was 0.86 (0.79–0.92), $p<0.0001$ and 0.85 (0.77–0.92), $p<0.0001$ respectively and for MMP-9 was 0.64 (0.55–0.73), $p=0.02$ and 0.66 (0.57–0.75), $p=0.001$ respectively.

The discriminatory properties of GFAP, copeptin and MMP-9 for ischemic stroke from hemorrhagic stroke were poor with AUC (95%CI) being 0.55 (0.45–0.63), 0.55 (0.45–0.64), and 0.54 (0.44–0.63) respectively. However, the ability of biomarkers to distinguish between ischemic and hemorrhagic stroke types may be time-dependent for specific biomarkers. For instance, the AUC for GFAP among ischemic compared hemorrhagic stroke subjects presenting within 48 hours was 0.83 (0.61–1.06), $p=0.04$, decreasing to 0.79 (0.59–0.99), $p=0.02$ among those presenting within 72 hours and then to 0.65 (0.47–0.84), $p=0.13$ for those presenting after 96 hours. That for copeptin appears to be better at day 3 with AUC of 0.73 (0.51–0.94) while for MMP-9 no such time-dependent relationships were observed.

Pathophysiologic correlates of stroke biomarkers

Among subjects with ischemic strokes, the median (IQR) concentrations of GFAP declined significantly among the three etiological subtypes being highest among large-vessel atheroembolic- 1745 ng/L (1617–2147), followed by cardio-embolic of 1569 ng/L (1304–1846) and then lacunar of 1569 ng/L (1207–1906), $p=0.03$ (Kruskal Wallis test). The mean $\pm$ SEM lesion volumes of large-vessel, cardio-embolic and lacunar strokes respectively were $85.8 \pm 36.6\text{cm}^3$, $82.1 \pm 22.0\text{cm}^3$ and $1.5 \pm 0.4\text{cm}^3$ respectively, $p<0.0001$. These differences

were not observed among ischemic stroke subtypes for co-peptin and MMP-9 and similarly for all three biomarkers among hemorrhagic stroke subtypes as shown in Figure 3.

The median duration of symptom onset and presentation to hospital after acute stroke was 6 (4–11) days among subjects with hemorrhagic strokes and 7 (5–11) days for those with ischemic strokes, $p=0.34$. Figure 4 depicts the concentrations of the three biomarkers by stroke types according to tertiles of time of presentation to hospital after stroke showing that there are wide variations in biomarker levels but some differences in biomarker trends were noted. For example whilst GFAP concentrations were highest within days 0–3 for hemorrhagic stroke subjects and steadily declined among those presenting at the second and third tertiles, that for those with ischemic strokes appeared to increase from days 0–3 to its highest at day 4–6 before declining among those presenting after day 7. (Figures 4A and 4B). Conversely, for copeptin, levels progressively diminished over duration of presentation among ischemic strokes while its levels tended to rise among those presenting between day 4–6 before declining although these changes were not statistically significant. (Figure 4C and 4D) Finally, MMP-9 seemed to show a steady rise with increasing time to presentation among hemorrhagic stroke subjects. (Figure 4E and 4F).

Prognostic correlates of plasma biomarkers

(a) Biomarkers and lesion volume—There were non-significant correlations between lesion volume and plasma biomarker concentrations measured with Pearson's $r = -0.08$ for GFAP with $p=0.30$, $r=-0.01$ for co-peptin with $p=0.87$ and $r=0.003$ for MMP-9, $p=0.97$.

(b) biomarkers and stroke severity—Among the three biomarkers, co-peptin was inversely correlated with stroke severity (measured using the NIHSS) with Pearson's correlation $r = -0.23$, $p=0.007$ with no significant correlations observed for MMP-9 and GFAP. A mild-to-moderate correlation between lesion volume and NIHSS score was noted with $r=0.28$, $p=0.0005$.

(c) biomarkers and stroke mortality—The concentrations of the three plasma biomarkers were not significantly different between those who survived and those who died from stroke. (Table 2). The median (IQR) lesion volume among stroke survivors of 5.1cm^3 (0.7–21.2) was significantly lower than 29.2cm^3 (5.5–63.8), $p=0.003$ among those who died. Similarly, the mean NIHSS score of 12.4 ± 7.4 among stroke survivors was lower compared with 21.5 ± 6.6 among those who succumbed, $p<0.0001$. Neither age at presentation, stroke type nor gender as associated with stroke mortality. A logistic regression model incorporating only copeptin, NIHSS score and lesion volume as covariates showed significant independent association between NIHSS score and mortality with an adjusted OR (95% CI) of 1.16 (1.08–1.26) but not copeptin nor lesion volume as shown in table 3.

DISCUSSION

We explored the profile of circulating levels of GFAP- an astroglial protein (GFAP), copeptin- a sensitive surrogate marker for arginine-vasopressin release indicative of stress response and MMP-9-a matrix degrading enzyme involved in remodeling of the extracellular matrix among West African stroke subjects compared with stroke-free control subjects.

Stroke subjects were predominantly septuagenarians, were more likely to have hypertension, diabetes and dyslipidemia compared with age- and sex- matched controls and tended to present after about a week of onset of stroke symptoms. This delay in seeking healthcare after such a devastating medical emergency would obviously impact on the prompt initiation of most guideline recommended evidence based interventions²⁰, offer partial explanations for the characteristically poor in-patient stroke outcomes in LMICs^{21–32} and also limited our ability to study the expression of the three biomarkers of interest at the very proximal phases of stroke in the majority of stroke subjects. These limited set of biomarkers were studied due to their known diagnostic and prognostic potentials and their pathophysiologic associations with stroke.

Potential diagnostic roles of selected biomarkers

We found that GFAP and copeptin were excellent biomarkers in discriminating stroke patients from stroke-free controls with accuracy of 0.84 and 0.85 respectively while MMP-9 had moderate-to-fair diagnostic capabilities in agreement with previous studies among Western and Asian populations.³³ Although GFAP and co-peptin maintained high diagnostic accuracies for both ischemic and hemorrhagic stroke types compared with stroke-free controls, none of the three biomarkers distinguished ischemic from hemorrhagic strokes over the broad range of time of presentation. There were hints from a limited sub-analysis that GFAP might distinguish between hemorrhagic and ischemic stroke cases within 3 days of stroke onset, which could be potentially useful in LMICs setting. It is well known that the release of tissue biomarkers after stroke follows distinct kinetic patterns. GFAP for instance is a highly cerebral-specific astroglial protein that is released rapidly into the blood stream after an intracerebral hemorrhage due to its instantaneous elaboration provoked by destruction of astrocytes and the BBB⁵. In contrast, due to preservation of structural integrity of brain cells and BBB in the setting of an ischemic stroke, GFAP release into the plasma follows a delayed kinetics with peak concentrations attained within 48–72 hours after stroke.⁶ Hence GFAP and S-100B, both astroglial proteins have been posited as promising biomarkers for distinguishing between hemorrhagic and ischemic strokes within hours of symptom onset and our study shows evidence in support of the notion that the window of utility for this purpose may fall within 72 hour window.³⁴ Future studies exploring the kinetics of GFAP among individual stroke subjects in LMICs would be required to provide corroborative evidence.

Prognostic correlates of selected biomarkers

Pathophysiologically, GFAP was associated with ischemic stroke subtypes being highest among large-vessel athero-embolic strokes compared with cardio-embolic and lacunar strokes which may be related to infarct volumes accompanying these stroke types. We observed as expected that large-vessel and cardio-embolic stroke had larger infarct sizes compared with lacunar strokes, hence the elaboration of GFAP may trend with lesion volumes among ischemic stroke subjects as previously reported³⁴ or could be related specifically to etiologic type of ischemic stroke via yet to be identified mechanisms. Overall however, formal analysis using correlation statistics did not yield any associations between individual GFAP measurements and lesion volumes. In studies where associations between lesion size and GFAP measurements were found, blood samples were drawn for analysis

nearly within 6 hours of symptom onset. However, in sub-Saharan Africa, stroke patients present rarely within 24 hours of symptoms onset.^{23,24} Furthermore, we did not observe associations between the three biomarkers and prognostic outcomes even when analysis were restricted to subjects who presented within 3 days of stroke onset. Rather, clinical indicators of severity measured using the NIHSS scale was potentially associated with stroke mortality and none of the biomarkers was independently predictive of mortality nor associated with stroke severity.

Study Implications, Strengths, Limitations and Future directions

The implications of our findings are that GFAP which is a glial tissue-specific biomarker may be explored further for stroke diagnosis in LMICs where neuroimaging is widely unavailable for evaluation of patients presenting with stroke symptoms. However due to late presentation of subjects in these milieus, the ability of GFAP to distinguish ischemic from hemorrhagic stroke may be limited to the first 72 hours after symptom onset. This will be an important limitation for the widespread use of biomarkers in LMICs, since specificity is challenged by time to presentation after stroke ictus in these settings as our study has shown. These are important considerations for stroke biomarkers which still remains as a biomedical research tool and yet to move into clinical utility.

The main strengths of this study are the rigorous measures instituted to ensure accurate phenotypic characterization of both ischemic and hemorrhagic stroke and the ascertainment of stroke-free status of community-based controls using a locally validated version of the Questionnaire for Verifying Stroke Free Status which has 98% negative predictive value.¹⁹ Also our ability to perform the molecular assays locally signals the coming of age of emerging LMIC centers with capabilities of conducting high-quality research and contributing to scientific knowledge in the field of biomarkers for stroke.

The sample size was relatively small in comparison with other published studies on stroke biomarkers. However the sample size was calculated to detect significant differences in biomarker concentrations between cases and controls. An important limitation was our inability to study the kinetics of biomarkers in individual subjects to gain a better appreciation of determinants of the wide fluctuations in inter-individual concentrations of analytes studied. These limitations would form the basis of subsequent studies. Indeed further studies assessing the discriminatory properties of GFAP in distinguishing stroke from stroke mimics relevant in LMICs such as HIV-related space occupying lesions, hypertensive encephalopathy, seizure disorders and so forth will be needed to assess the feasibility of GFAP as a bedside test. GFAP has also been shown to be elevated in glioblastoma^{35,36} and traumatic brain injury^{37,38}. Although MMP-9 and copeptin were both able to distinguish stroke cases from stroke-free controls, the diagnostic performance of MMP-9 for this purpose was unimpressive and copeptin is not necessarily specific for stroke diagnosis but is poor prognostic marker in diverse medical disorders such as stroke, traumatic brain injury³⁹, heart failure^{40,41}, sepsis⁴², an association that was not confirmed in the present study after adjusting for important demographic variables such as age, gender and stroke severity. Further validation studies evaluating a broader array of other promising biomarkers for stroke diagnosis and prognosis in multi-center collaborative studies in LMICs and other

international bodies such as the consortium on stroke biomarker research (<http://stroke-biomarkers.com/page.php?title=Resources>) are urgently needed to fill a knowledge gap in exploring their utility in routine stroke management in these settings.

Conclusion

In conclusion, among West Africans GFAP and copeptin appears to hold promise as potential marker for stroke diagnosis. However neither GFAP, copeptin nor MMP-9 were independently associated with poor stroke prognosis providing further impetus for further screening and validation of other biomarkers for routine utility in stroke management in LMICs.

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Figure 1. Comparison of mean $\pm$ SD serum concentrations of Glial Fibrillary Acid Protein (A), Co-peptin (B) and Matrix Metalloproteinase-9 (C) among stroke subtypes and controls.

Figure 2.
ROC curves for diagnosis of stroke with Area-under curve estimates for Glial Fibrillary Acid Protein (A), Co-peptin (B) and Matrix Metalloprotein-9 (C).

Figure 3. Profile of serum titers of Glial Fibrillary Acid Protein (A), Co-peptin (B) and Matrix Metalloproteinase-9 (C) according to pathophysiologic subtypes of stroke.

Figure 4.

Comparison of concentration of GFAP (panels A and B), copeptin (panels C and D) and MMP-9 (panels E and F) by stroke type according to tertiles of time to presentation after onset of stroke symptoms. Mean with standard deviations are presented at each tertile.

Table 1

Demographic characteristics of Stroke cases and Controls

Characteristic	Stroke Cases (n=156)	Controls (n=74)	P-value
Age, mean $\pm$ SD	61.3 $\pm$ 14.6	60.4 $\pm$ 14.7	0.66
Females, n (%)	74 (47.4%)	37 (50.0%)	0.78
Type of domicile			
Urban	82 (52.6)	63 (85.1)	<0.0001
Semi-urban	55 (35.3)	6 (8.1)	
Rural	19 (12.1)	5 (6.8)	
Formal education			0.55
None/Primary	68 (43.6)	37 (50.0)	
Secondary	62 (39.7)	24 (32.4)	
Tertiary or above	26 (16.7)	13 (17.6)	
Vascular risk factors			
Hypertension	97 (62.2)	24 (32.4)	<0.0001
Diabetes mellitus	29 (18.6)	5 (6.8)	0.018
Hyperlipidemia	10 (6.4)	0 (0.0)	0.026
Heart disease	6 (3.8)	0 (0.0)	0.09
Alcohol	20 (12.8)	10 (13.5)	0.88
Cigarette smoking	3 (1.9)	0 (0.0)	0.23
Stroke type		NA	
Ischemic	99 (63.5)	–	
Hemorrhagic	57 (36.5)	–	
Ischemic stroke subtype		NA	
Large-vessel	19 (19.2)	–	
Cardio-embolic	25 (25.3)	–	
Lacunar	50 (50.5)	–	
Other	1 (1.0)	–	
Unknown	4 (4.0)	–	
Hemorrhagic stroke		NA	
Structural	9 (15.8)	–	
Medication associated	0 (0.0)	–	
Amyloid angiopathy	2 (3.5)	–	
Systemic diseases	0 (0.0)	–	
Hypertension	42 (73.7)	–	
Unknown	4 (7.0)	–	

Table 2

Comparison of stroke subjects according to vital status at discharge

	Alive	Died	P-value
GFAP (ng/L), mean $\pm$ SD	1615 $\pm$ 545.3	1766 $\pm$ 762.2	0.34
Copeptin (pmol/L), mean $\pm$ SD	21.7 $\pm$ 8.6	19.0 $\pm$ 7.7	0.14
MMP-9 (ng/L), mean $\pm$ SD	2503 $\pm$ 1654	2546 $\pm$ 2065	0.91
NIHSS score, mean $\pm$ SD	12.4 $\pm$ 7.4	21.5 $\pm$ 6.6	<0.0001
Lesion volume (cm ³), median (IQR)	5.1 (0.7 - 21.2)	29.2 (5.5–63.8)	0.003
Stroke type			0.28
Ischemic stroke, n (%)	82	14	
Hemorrhagic stroke, n (%)	44	12	
Age (years), mean $\pm$ SD	61.4 $\pm$ 14.7	60.2 $\pm$ 13.9	0.71
Gender			0.83
Male, n (%)	65	14	
Female, n (%)	61	12	
Systolic B.P. at presentation			
mean $\pm$ SD	148.1 $\pm$ 25.9	145.4 $\pm$ 27.6	0.63

Table 3

Logistic regression modeling of predictors of in-patient mortality.

Covariate	Unadjusted OR		Adjusted OR	
	(95% CI)	p-value	(95 CI)	p-value
GFAP, each 1ug/L rise	10.9 (0.19–636.41)	0.25		
Co-peptin, each 1pmol rise	0.97 (0.92–1.02)	0.27		
MMP-9, each 1ug/L rise	1.52 (0.42–5.51)	0.53		
Age, each 10 year increase	0.99 (0.73–1.36)	0.97		
Male gender	0.65 (0.25–1.65)	0.36		
Hemorrhagic stroke	1.82 (0.71–4.63)	0.21		
NIHSS	1.17 (1.10–1.26)	<0.000 01	1.17 (1.08–1.26)	<0.000 01
Lesion volume >30cm ³	3.39 (1.41–8.15)	0.007	2.53 (0.94–6.76)	0.07