


Blood–Brain Barrier Integrity and Inflammatory Markers in Ischemic Stroke: A Clinical Study

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ABSTRACT

Background

Stroke is the second leading cause of death and the third leading cause of disability worldwide. Early damage of the blood-brain barrier after ischemic stroke leads to progressive neuronal death and brain edema. Tight junctions between endothelial cells, including occludins and claudins, are critical for BBB integrity, and their disruption contributes to stroke pathology.

Objective

To evaluate serum levels of occludin and claudin-5 in patients with acute ischemic stroke and their association with stroke severity, functional outcomes, and vascular risk factors.

Methods

This prospective study included 88 patients with acute ischemic stroke or transient ischemic attack. Baseline clinical characteristics, including NIHSS and modified Rankin Scale (mRS) scores, were assessed. Serum occludin and claudin-5 levels were measured on day one of admission. Patients were stratified by stroke severity (NIHSS) and outcomes (mRS), and subgroup analysis was conducted based on vascular risk factors. ROC curve analysis evaluated the predictive value of occludin and claudin-5 for mortality.

Results

The average NIHSS score was 10.2 ± 5.30 , and the mean mRS score was 3.72 ± 0.97 . Serum occludin and claudin-5 levels were 0.09 ± 0.34 ng/mL and 0.07 ± 0.44 ng/mL, respectively. No statistically significant differences were found in biomarker levels across NIHSS and mRS groups ($p > 0.05$), although claudin-5 showed a decreasing trend with worsening neurological and functional status. Patients with heart rhythm disturbances and diabetes mellitus exhibited slightly lower claudin-5 levels, though not significantly. ROC analysis revealed a limited discriminatory capacity of occludin (AUC = 0.525; $p = 0.6845$) and a moderate performance for claudin-5 (AUC = 0.614; $p = 0.0884$), with claudin-5 showing better sensitivity–specificity balance.

Conclusion

Serum occludin and claudin-5 levels did not correlate significantly with stroke severity or functional outcomes but displayed trends suggestive of their involvement in BBB disruption. Claudin-5 may hold moderate prognostic value in mortality prediction. Further large-scale studies are needed to clarify their diagnostic and prognostic potential in ischemic stroke.

Keywords

ischemic stroke; blood-brain barrier; occludin, claudin; biomarkers; prognosis.

INTRODUCTION

Stroke remains one of the most serious public health challenges worldwide. It is currently the second leading cause of death and the third leading cause of long-term disability globally ¹. The overall burden of stroke is substantial, with a reported global incidence of 185.01 cases per 100,000 person-years², reflecting its widespread and persistent impact on populations.

In Kazakhstan, stroke continues to pose a significant healthcare concern. According to Zhusupov et al., the incidence rate reaches 3.7 per 1,000 person-years, with acute stroke accounting for 52% of all reported cases. The mortality rate is estimated at 1.08 per 1,000 person-years ³. These figures highlight the considerable clinical and socioeconomic burden of stroke at the national level.

Among all stroke subtypes, ischemic stroke (IS) is the most common, representing approximately 65% of cases worldwide⁴. IS develops as a result of complex interactions between genetic predisposition and environmental exposures. A number of well-established and potentially modifiable risk factors contribute to its

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development, including arterial hypertension, diabetes mellitus, atrial fibrillation, hyperlipidemia, smoking, excessive alcohol consumption, high dietary salt intake, and physical inactivity⁵. Addressing these modifiable factors remains central to effective prevention strategies and reduction of stroke-related morbidity and mortality.

Recombinant tissue plasminogen activator (rtPA) stands as the sole pharmacological agent sanctioned by the US Food and Drug Administration for the management of acute IS. However, its utilization is restricted as per prevailing guidelines, mandating administration within 4.5 hours of stroke onset. Merely 5–7% of IS patients undergo intravenous tPA treatment, with an additional 1–2% receiving intra-arterial therapy, primarily owing to the risk of hemorrhagic transformation associated with tPA thrombolytic therapy⁶.

The compromise of the blood-brain barrier (BBB) emerges as an early occurrence subsequent to IS, inciting pathophysiological cerebral damage. Subsequent to BBB compromise, progressive neuronal demise and cerebral edema ensue, occasionally culminating in intracerebral hemorrhage post IS⁷. A pivotal constituent of the BBB is the intricate tight junction complex inherent in the vascular endothelial cells of the central nervous system⁸. Tight junction compromise contributes to the underlying pathology of numerous cerebral maladies, inclusive of IS; nevertheless, the precise pathological mechanisms precipitating tight junction compromise remain incompletely elucidated⁹.

Tight junctions interlinking endothelial cells consist of zonula occludens (ZO) and transmembrane proteins such as occludin, claudins, junctional adhesion molecules, and tricellulin, which are associated with cytoskeletal and cytoplasmic scaffold proteins. Their interconnections significantly influence BBB permeability¹⁰. Investigation in a rodent model of IS unveiled early-phase occludin degradation, concomitant with BBB breakdown, underscoring occludin's prospective utility as a hypertension risk predictor¹¹. Delving into occludin and claudin dynamics in IS patients holds paramount importance in dictating treatment modalities and prognosticating disease trajectory. These proteins, integral to vascular architecture, potentially wield pivotal influence in stroke pathogenesis. Enhanced comprehension of their roles fosters the formulation of more efficacious therapeutic interventions and the mitigation of recurrent stroke occurrences.

MATERIALS AND METHODS

Our prospective study, conducted from October 2022, included 88 patients with clinically manifested stroke identified according to WHO clinical criteria, confirmed by head computed tomography. Other main inclusion criteria were time from onset of stroke symptoms to hospitalization <24 hours and modified Rankin Scale (mRS) status >1 point.

All patients included in the study were analyzed according to the following criteria:

- Age of first stroke in life.

The presence of concomitant diseases, such as heart rhythm disturbance (HRD), arterial hypertension (AH), diabetes mellitus (DM), >50% atherosclerotic carotid artery stenosis (CAS, ipsilateral to acute ischemic brain injury).

- Type of treatment on the first day of hospitalization: rtPA, thrombectomy, stenting.

Their neurological status on the first day of stroke was assessed using the NIHSS (National Institute of Health Stroke Scale)¹².

Their functional state on the first day after the onset of stroke according to the mRS scale¹³.

Plasma concentrations of the following markers on the first day of stroke: claudin, occludin.

Markers assessment was performed by serum isolation. Serum was obtained by venipuncture. According to collection protocol, blood samples were left at room temperature to allow clotting for 60 min. Serum was isolated by centrifuging the blood sample at 1500×g for 15 min at room temperature, after which the supernatant was pipetted carefully into aliquots that were immediately stored at –70 °C. Hemolytic samples were excluded from the analysis.

Blood biomarker measurements were performed as such; target proteins were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocol (occludin, Cloud-Clone Corp., USA; claudin-5, Cloud-Clone Corp., USA); 500 µl for occludin, 500 µl for claudin-5. Mean serum concentrations of occludin, claudin-5 were assessed for all patients.

Analysis. All statistical analyses were weighted to ensure representativeness of the entire Aktobe city adult population over the age of 18 years, using individual

sampling weights to account for non-response. Normality of variables was assessed before proceeding with further analyses. All continuous variables were summarized as mean±SD, and categorical variables were summarized as count and percentage. The chi-square test was used for comparisons between groups for categorical variables. Correlation between variables was assessed using the Mann-Whitney U test and the Kruskal-Wallis test. Receiver operator characteristic (ROC) curve analysis was used to evaluate the predictive ability of occludin and claudin for poor outcome. All statistical analyzes were performed using SPSS 25 and MedCalc 22.0.

Ethical Clearance

The Research Ethics Board of the West Kazakhstan Marat Ospanov Medical University provided ethical approval for this study as part of a scientific and technical project (No. 9 of November 19, 2021). The study uses existing administrative healthcare databases and did not obtain individual patient consent

RESULTS

Table 1. Baseline Demographic, Clinical and Laboratory Characteristics of the Study Population.

No	Parameter	Means±SD n=88
1	Age,	64,2±11,5
2	Gender, n (%)	M=44 (50%)/W=44 (50%)
3	BMI	27,9±4,46
4	NIHSS	10,2±5,30
5	mRS	3,72±0,97
6	Occludin	0,09±0,34
7	Claudin	0,07±0,44
8	Rural, n (%)	23 (26,1%)
9	Died, n (%)	15 (17%)
10	Arterial hypertension, n (%)	87 (98,9%)
11	Heart rhythm disturbance, n (%)	19 (21,6%)
12	Diabetes mellitus, n (%)	24 (27,3%)
13	Cerebral stenosis >50%, n (%)	17 (19,3%)
14	Pneumonia, n (%)	6 (6,8%)
15	Cerebral angiography	22 (25%)
16	rtPA	13 (14,8%)
17	Thrombectomy	10 (11,4%)
18	Stenting	2 (2,3%)

The mean age of the study population was 64.2 ± 11.5 years. Gender distribution was balanced, with 44 men (50%) and 44 women (50%). The average body mass index (BMI) was 27.9 ± 4.46 kg/m², classifying the cohort as overweight according to WHO criteria. The mean National Institutes of Health Stroke Scale (NIHSS) score at admission was 10.2 ± 5.30 , indicating a moderate neurological deficit. The mean modified Rankin Scale (mRS) score was 3.72 ± 0.97 , corresponding to moderate to moderately severe disability.

Regarding biomarkers of blood-brain barrier integrity, the mean serum concentration of occludin was 0.09 ± 0.34 ng/mL, and that of claudin-5 was 0.07 ± 0.44 ng/mL. Both biomarkers demonstrated low mean values with high interindividual variability, suggesting heterogeneous disruption of the blood-brain barrier among patients.

During the observation period, 17% (n = 15) of patients died, and 26.1% (n = 23) resided in rural areas. Arterial hypertension was the most prevalent comorbidity, observed in 98.9% of cases. Heart rhythm disturbances were present in 21.6%, and diabetes mellitus in 27.3% of patients. Significant cerebral arterial stenosis (>50%) was identified in 19.3% of cases, and pneumonia was reported in 6.8% of patients.

In terms of treatment, 25% of patients underwent cerebral angiography, 14.8% received intravenous thrombolysis (rtPA), 11.4% underwent mechanical thrombectomy, and 2.3% had stenting procedures (Table 1).

Table 2. Serum Occludin and Claudin-5 Levels According to Stroke Severity Based on NIHSS Scores.

	NIHSS 0-8 n=45	NIHSS 9-15 n=22	NIHSS >16 n=21	p=0,05
Occludin	0,05±0,19	0,15±0,58	0,12±0,26	0,207
Claudin	0,13±0,61	0,08±0,02	0,02±0,04	0,076

Table 3. Serum Occludin and Claudin-5 According to Stroke Severity Based on Modified Rankin Scale (mRS).

	mRS 0-2 n=6	mRS 3-4 n=60	mRS >5 n=22	p=0,05
Occludin	0	0,11±0,55	0,05±0,13	0,851
Claudin	0	0,10±0,61	0,01±0,02	0,201

Occludin and claudin-5 levels were analyzed in relation to stroke severity and functional outcomes. Patients were stratified by NIHSS scores into three groups: mild (0–8, $n = 45$), moderate (9–15, $n = 22$), and severe (>16 , $n = 21$). The mean occludin concentrations in these groups were 0.05 ± 0.19 , 0.15 ± 0.58 , and 0.12 ± 0.26 ng/mL, respectively ($p = 0.207$). Claudin-5 levels were 0.13 ± 0.61 , 0.08 ± 0.02 , and 0.02 ± 0.04 ng/mL, respectively ($p = 0.076$), demonstrating a decreasing trend with increasing stroke severity, although the differences did not reach statistical significance.

Functional outcomes were assessed using the modified Rankin Scale (mRS), with patients categorized into favorable (0–2, $n = 6$), moderate (3–4, $n = 60$), and poor outcomes (>5 , $n = 22$). The corresponding occludin levels were 0, 0.11 ± 0.55 , and 0.05 ± 0.13 ng/mL ($p = 0.851$), while claudin-5 levels were 0, 0.10 ± 0.61 , and 0.01 ± 0.02 ng/mL ($p = 0.201$). Although not statistically significant, claudin-5 concentrations tended to decrease with worsening functional outcomes (Tables 2–3).

Table 4. Serum Occludin and Claudin-5 Levels in Patients With and Without Diabetes Mellitus and Heart Rhythm Disturbances.

	Occludin	Claudin
Heart rhythm disturbance, have not $n=69$	0,0807±0,351	0,0791±0,494
Heart rhythm disturbance, have $n=19$	0,1302±0,306	0,0432±0,122
p	0,521	0,171
Diabetes mellitus, have not $n=64$	0,1010±0,3809	0,0858±0,512
Diabetes mellitus, have $n=24$	0,658±0,205	0,0352±0,111
p	0,529	0,796

Subgroup analyses based on the presence of heart rhythm disturbances and diabetes mellitus did not reveal statistically significant differences in serum levels of occludin and claudin-5.

Among patients with heart rhythm disturbances ($n = 19$), mean occludin concentrations were slightly higher (0.1302 ± 0.306 ng/mL) compared to those without arrhythmias (0.0807 ± 0.351 ng/mL),

though the difference was not statistically significant ($p = 0.521$). Claudin-5 levels were lower in patients with arrhythmias (0.0432 ± 0.122 ng/mL) than in those without (0.0791 ± 0.494 ng/mL), showing a non-significant trend toward reduced levels in the presence of cardiac rhythm disturbances ($p = 0.171$). This may suggest a potential association between arrhythmias and greater blood–brain barrier (BBB) impairment, though confirmation in larger cohorts is needed.

In patients with diabetes mellitus ($n = 24$), mean claudin-5 levels were slightly lower (0.0352 ± 0.111 ng/mL) than in non-diabetic individuals (0.0858 ± 0.512 ng/mL). Occludin concentrations also showed a marginal reduction in diabetics (0.0658 ± 0.205 ng/mL) compared to non-diabetics (0.1010 ± 0.3809 ng/mL). However, these differences were not statistically significant ($p = 0.529$ for occludin; $p = 0.796$ for claudin-5), indicating no robust association between diabetes and these markers of BBB integrity in the present sample.

Although no statistically significant relationships were observed, the consistent trend toward lower claudin-5 levels in the presence of comorbidities may reflect subtle BBB alterations influenced by systemic vascular risk factors. These preliminary findings warrant further investigation in larger, stratified cohorts to better elucidate potential pathophysiological links (Table 4).

To assess the diagnostic precision of claudin and occludin concerning poor prognosis, ROC curve analyses were conducted (refer to Figure 1).

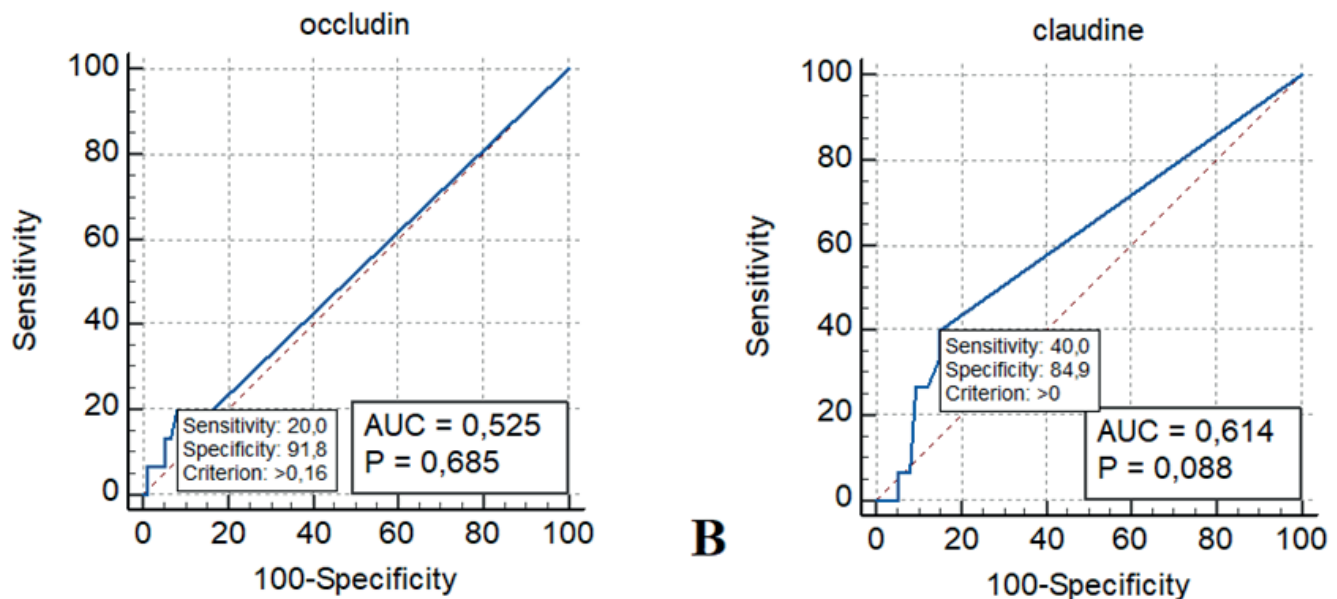
The resulting ROC curve for occludin yielded an Area Under the Curve (AUC) value of 0.525 ± 0.0607 (95% CI: 0.415–0.632). The model exhibited non-significance ($p = 0.6845$), indicating the limited discriminatory capacity of occludin within this cohort. The occludin threshold at 0.16 denoted a heightened risk of mortality, with values below it indicative of a diminished risk. The sensitivity and specificity of the model at this occludin threshold were 20.0% and 91.78%, respectively, illustrating high specificity but low sensitivity, thus constraining its clinical applicability.

Conversely, the resulting ROC curve for claudin yielded an AUC value of 0.614 ± 0.0670 (95% CI: 0.504–0.716). The model displayed borderline significance ($p = 0.0884$), suggesting a moderate discriminative capacity of claudin in distinguishing between surviving and deceased patients. The claudin threshold at

0.0 indicated an elevated mortality risk for values exceeding it, while lower levels signified a reduced risk. The sensitivity and specificity of the model at this claudin threshold were 40.0% and 84.93%, respectively, indicating a better balance between sensitivity and specificity compared to occludin.

ROC curve analysis delineated occludin's limited discriminatory capacity (AUC=0.525), rendering it less

adept in prognosticating patient outcomes. In contrast, claudin exhibited moderate discriminatory prowess (AUC=0.614), potentially rendering it a more valuable biomarker in clinical settings. However, neither biomarker demonstrated high accuracy, warranting further investigation and potential amalgamation with other diagnostic parameters to enhance predictive efficacy.



Picture 1. ROC Curves for Occludin and Claudin-5 in Predicting Mortality in Ischemic Stroke.

DISCUSSION

Age stands out as a potent risk factor for stroke morbidity and mortality. Our findings revealed a comparable incidence rate between genders, albeit men generally exhibit a higher lifetime risk of IS than women across most age cohorts¹⁴. Notably, in the United States, stroke ranked as the third leading cause of death among women in 2019, compared to the fifth leading cause among men. Women accounted for 57.1% of stroke-related deaths in 2019, with stroke contributing to 6.2% of all female deaths and 4.4% of all male deaths¹⁵.

Regarding risk factors, hypertension predominated among most patients in our study, while diabetes mellitus affected nearly one-third of the cohort. Heart rhythm disturbances ranked third in prevalence, and cerebral artery stenosis exceeding 50% was detected in almost 20% of cases. The GOAL study, encompassing 17,663 patients, identified smoking as the most prevalent risk factor (49.2%), followed by hypertension

(36.6%) and dyslipidemia (31.7%). 73.8% of patients presented with at least one vascular risk factor, with 40.9% exhibiting two or more risk factors. Among patients under 30 years old, 49.6% harbored at least one vascular risk factor. Traditional vascular risk factors, including hypertension, diabetes, dyslipidemia, and smoking, were more prevalent in men than women and displayed increased prevalence with advancing age¹⁶.

In the Young ESUS study, encompassing IS patients under 50 years old with an average age of 40.4 years, men constituted 56% of participants. Smoking emerged as the primary risk factor (45%), followed by hypertension (22%) and dyslipidemia (20%). Additionally, 6% of cases are presented with ipsilateral nonstenotic carotid plaques¹⁷.

Thirteen patients (14.8%) received thrombolytic therapy, while ten (11.3%) underwent thrombectomy. Previous animal investigations have indicated that rtPA induces vascular permeability, potentially

compromising BBB integrity [18]. The SITS-MOST study underscored that reperfusion therapies such as mechanical thrombectomy or intravenous thrombolysis elevate the risk of developing hypertension, a principal impediment to effective stroke therapy in most cases¹⁹.

Our study demonstrated elevated occludin concentrations in patients with diabetes mellitus and cardiac arrhythmias compared to those without these conditions. A study from Xuanwu Hospital of Capital Medical University (China, 2020) reported slightly elevated serum occludin levels in individuals over 80 years old and even higher levels in diabetic individuals compared to nondiabetic counterparts. Notably, hypertension and gender exerted no significant impact on baseline serum occludin levels. These data suggest that diabetes and advanced age may serve as weak risk factors for BBB damage. Additionally, the study revealed that heightened serum occludin levels, particularly evident in hypertensive patients, may effectively predict hypertension in individuals undergoing reperfusion therapy²⁰.

Lasek-Bal et al. reported that stroke localization in the anterior circulation was associated with elevated serum levels of occludin and claudin-5 during the acute phase, whereas occludin concentrations were significantly lower in cases of lacunar stroke. Similar to our findings, the authors did not observe a significant correlation between serum levels of claudin-5, occludin, or ZO-1 on the first day post-stroke and the neurological or functional status of patients during the acute phase of the disease²¹.

In a study conducted by Yuan et al. (2020), receiver operating characteristic (ROC) curve analysis demonstrated that combining serum occludin levels with NIHSS scores significantly enhanced the predictive value for hemorrhagic transformation (HT). An unbiased estimate of the area under the ROC curve (AUC) for the combined model was obtained using the leave-one-out cross-validation (LOOCV) method. According to DeLong's test, the AUC of the combined model (0.919) was significantly greater than that of either variable alone, suggesting superior diagnostic performance. These findings indicate that integrating serum occludin levels—reflecting blood–brain barrier (BBB) disruption—with NIHSS scores—reflecting stroke severity—substantially improves predictive accuracy for HT in patients undergoing reperfusion therapy²².

In conducting univariate analyses between occludin and deceased patients, we observed high specificity but low sensitivity, thereby limiting its practical utility. Conversely, claudin exhibited a more balanced performance between sensitivity and specificity compared to occludin.

Furthermore, despite the absence of significant differences, occludin and claudin levels manifested discernible trends that warrant further investigation. We recommend additional studies with larger sample sizes to comprehensively analyze these biomarkers and elucidate their roles in stroke pathogenesis.

CONCLUSION

Our study underscores the relevance of traditional vascular risk factors in stroke pathogenesis, with hypertension and diabetes mellitus being the most prevalent in our cohort. Elevated serum occludin levels were associated with diabetes, advanced age, and cardiac arrhythmias, suggesting their potential role as weak predictors of blood–brain barrier (BBB) disruption. Although neither occludin nor claudin levels showed strong correlations with neurological severity in the acute phase, the combined use of serum occludin levels and NIHSS scores significantly improved the predictive value for hemorrhagic transformation, as demonstrated by ROC analysis. Despite occludin's high specificity, its limited sensitivity may constrain clinical applicability, whereas claudin exhibited a more balanced diagnostic profile. Overall, our findings support the potential utility of BBB integrity markers in the prognostic assessment of ischemic stroke and highlight the need for larger, multicenter studies to validate these biomarkers and refine their clinical relevance.

Declarations

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical clearance: This study was conducted in accordance with ethical standards. Ethical approval was obtained from the appropriate institutional review board, and informed consent was secured from all participants prior to data collection.

Author Contributions: Conceptualization, Z.U.U.; Data curation, Z.U.U., A.A.K. and R.B.K.; Formal analysis, Z.U.U., A.A.K. and R.B.K.; Methodology, Z.U.U.,

A.A.K., G.B.K., and A.P.Y.; Project administration, A.A.K.; Resources, G.B.K., A.P.Y.; Software, Z.U.U., A.A.K., G.B.K., A.P.Y. and A.B.U; Supervision, Z.U.U. and A.A.K.; Validation, Z.U.U. and A.A.K.; Visualization, R.Y.N., G.B.K. and A.P.Y.; Writing, original draft, Z.U.U.; Writing, review and editing, Z.U.U., A.A.K., G.B.K., A.P.Y., A.B.U., R.B.K., N.A.S., O.M.A., R.Y.N., S.S.K., A.S.A. and S.Z.A. All authors have read and agreed to the published version of the manuscript.

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Limitations

This study has several limitations that should be acknowledged. First, the relatively small sample size (n = 88) may limit the statistical power to detect significant associations between serum biomarkers and clinical outcomes. The small number of patients with favorable or poor outcomes further constrained subgroup analyses and may have contributed to non-significant results despite observable trends.

Second, the study was conducted at a single center, which may limit the generalizability of the findings to

broader populations or healthcare settings with different demographics, stroke management protocols, or levels of care. The study cohort was also predominantly hypertensive, which could skew the results and reduce variability in vascular risk profiles.

Third, biomarker levels were assessed at a single time point—on admission—without longitudinal follow-up. This approach does not capture the dynamic changes in occludin and claudin-5 levels that may occur in the subacute or chronic phases of stroke and limits the ability to establish causal relationships.

Future studies should aim to include larger, multicenter cohorts, serial biomarker measurements, and multimodal imaging to better elucidate the role of occludin and claudin-5 as biomarkers of blood–brain barrier integrity and stroke prognosis.

Acknowledgments: Not applicable

Supplementary Material: Applicable

Appendices: Not applicable

Data Availability Statement: All data generated or analyzed in this study can be obtained from the corresponding author upon inquiry.

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