Heliyon 10 (2024) e26199

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Beyond diagnosis: Leveraging routine blood and urine biomarkers to predict severity and functional outcome in acute ischemic stroke

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ARTICLE INFO

Keywords:
Biomarker
Ischemic stroke
Liquid biopsy
Machine learning
Outcome
Prognosis
Severity

ABSTRACT

Background: The initial severity of acute ischemic stroke (AIS) is a crucial predictor of the disease outcome. In this study, blood and urine biomarkers from patients with AIS were measured to estimate stroke severity and predict long-term stroke outcomes.

Methods: The medical records of patients with AIS between October 2016 and May 2020 were retrospectively analyzed. The relationships of blood and urine biomarkers with stroke severity at admission were evaluated in patients with AIS. Predictive models for initial stroke severity and long-term prognosis were then developed using a panel of identified biomarkers.

Results: A total of 2229 patients were enrolled. Univariate analysis revealed 12 biomarkers associated with the National Institutes of Health Stroke Scale scores at admission. The area under the curve values for predicting initial stroke severity and long-term prognosis on the basis of these biomarkers were 0.7465, 0.7470, and 0.8061, respectively. Among multiple tested machine-learning, eXtreme gradient boosting exhibited the highest effectiveness in predicting 90-day modified Rankin Scale scores. SHapley Additive exPlanations revealed fasting glucose, albumin, hemoglobin, prothrombin time, and urine-specific gravity to be the top five most crucial biomarkers.

Conclusion: These findings demonstrate that clinically available blood and urine biomarkers can effectively estimate initial stroke severity and predict long-term prognosis in patients with AIS. Our results provide a scientific basis for developing tailored clinical treatment and management strategies for AIS, through incorporating liquid biomarkers into stroke risk assessment and patient care protocols for patients with AIS.

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https://doi.org/10.1016/j.heliyon.2024.e26199
Received 28 July 2023; Received in revised form 1 February 2024; Accepted 8 February 2024
Available online 10 February 2024

1. Introduction

Acute ischemic stroke (AIS) is a leading cause of death and disability worldwide [1,2] and places a considerable health and economic burden on patients and healthcare systems. With rapid socioeconomic development, an increasing population, and aging, the incidence of stroke events and their long-term sequelae and associated financial costs are expected to increase considerably in the near future [1–3]. Thus, enhancing prognosis capability and identifying factors associated with AIS severity are crucial for targeting high-risk patients and implementing timely interventions to optimize clinical outcomes. Many studies have developed predictive models for AIS prognosis, with stroke severity serving as a widely used variable for predicting AIS outcomes [4–7]. The National Institutes of Health Stroke Scale (NIHSS) is a 15-item neurological examination scale commonly employed to assess stroke severity and is one of the common predictors of AIS outcomes in stroke prediction models [4,6,8–10]. However, the NIHSS requires trained stroke specialists or neurologists to operate the scoring process. Furthermore, severe stroke is often associated with a poorer prognosis, making higher NIHSS scores inevitably imply worse outcomes. Despite the high efficacy of simplified predictive models using the NIHSS score [7,11,12], the direct employment of NIHSS scores as a predictor of prognosis may underestimate the positive benefits of AIS treatment by potentially biasing higher NIHSS scores towards a worse prognosis.

In addition to stroke severity, biomarkers that reflect the body's response to the damage caused by stroke have been widely explored to assess the prognosis of AIS [13]. Numerous biomarkers have been proposed for various applications in AIS prognosis, including early disease diagnosis, prediction of disease progression, and long-term prognosis [14,15]. While no single biomarker accurately measures stroke severity, several studies have suggested that neural biomarkers associated with neuronal injury and inflammation, including S100 calcium-binding protein (S100B), neuron-specific enolase (NSE), matrix metalloproteinase-9 (MMP-9), intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and glial fibrillary acidic protein (GFAP), play a critical role in the stroke process and may be utilized for monitoring or assessing stroke severity [14,15]. However, despite their potential relevance, biomarkers are not yet extensively used in clinical practice for stroke. Furthermore, many proposed biomarker measurements are not part of routine clinical protocols, and specific tests may require specialized equipment or techniques that are beyond the capabilities of a typical medical facility. Moreover, extensive research and validation are necessary to establish the reliability, consistency, and predictive power of biomarkers [14,15]. Thus, further studies are required to identify reliable biomarkers that can effectively assess stroke severity and long-term prognosis.

This study aimed to identify clinically accessible and routinely tested blood and urine biomarkers within the stroke unit that are associated with stroke severity. Subsequently, these biomarkers were utilized to formulate a predictive model for the severity of AIS. The identified biomarkers were then employed as predictors to assess their ability to estimate the long-term prognosis of stroke independently. Machine-learning techniques were employed to evaluate these predictors' importance and clinical significance within the model.

Previous studies have explored various biomarkers for assessing the severity and prognosis of stroke. However, the novelty of our research lies in the utilization of routinely available clinical biomarkers that can be rapidly and easily obtained in real-world practice. In contrast to emerging experimental biomarkers requiring advanced techniques or specialized equipment, our research employs common biochemical tests, enhancing the clinical utility and feasibility of our models.

Despite the well-established nature of the investigated biomarkers, predicting the severity of AIS using these markers remains a pioneering endeavor. Previous research has predominantly focused on the diagnostic or prognostic applications of these biomarkers in stroke. In contrast, our study introduces a new perspective by applying them concertedly for the prediction of initial stroke severity, offering fresh insights for clinical practice. Simultaneously analyzing multiple biomarkers, as opposed to single biomarker examined in prior studies, allows for a more comprehensive assessment of the complex pathophysiological processes in stroke. The integrative use of routine blood and urine tests to predict both the initial severity and long-term prognosis of stroke is a distinctive approach.

We further applied machine learning methods to analyze multidimensional and complex factors. Utilizing these readily accessible biomarkers, our models independently predict stroke outcomes without relying on additional clinical information frequently incorporated in prognostic models, such as age, sex, or stroke severity scores. The application of interpretable machine learning methods is also innovative, providing model transparency, enabling the assessment of feature importance, and supporting clinical decision-making.

In summary, the practicality of incorporating widely available blood and urine biomarkers, along with the utilization of machine learning for transparent predictions, and the simultaneous analysis of multiple markers, distinguishes this study from earlier investigations.

2. Materials and methods

2.1. Data source

This study retrieved medical records of patients diagnosed with AIS and treated at Taipei Medical University Shuang Ho Hospital between October 2016 and May 2020. All data were anonymized to ensure the confidentiality of patient information. The research procedures were approved by the Taipei Medical University-Joint Institutional Review Board (TMU-JIRB Approval No. N202103006).

Study design: Retrospective cohort study.

2.2. Participants

The inclusion criteria for this study are as follows: (i) patients aged 20 years or older, (ii) patients with AIS who were hospitalized and received treatment at Taipei Medical University-Shuang Ho Hospital, and (iii) patients who presented to the hospital within 10 days of the onset of AIS symptoms. However, patients who presented with acute intracranial hemorrhage, did not have a recorded NIHSS score at the time of admission, or had incomplete registration information were excluded from this study. At admission, all patients underwent non-contrast computed tomography (CT) of the head or magnetic resonance imaging (MRI) of the brain. Two neurologists and one radiologist independently interpreted all the CT and MRI findings. Demographic data—including age, sex, and the presence of cerebrovascular risk factors—were collected on admission. The overall severity of AIS was assessed by certified stroke specialists on the basis of the NIHSS scores, and functional outcomes 90 days after AIS onset were determined using modified Rankin Scale (mRS) scores [6,16,17]. At admission, blood count, prothrombin time, activated partial thromboplastin time (APTT), aspartate aminotransferase (AST), creatinine, and urine specific gravity were measured for each patient. Subsequently, within 72 h of admission, albumin, fasting glucose, glycated hemoglobin (HbA1c), cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDLC) levels were measured.

An mRS score of ≥ 2 at day 90 after AIS onset was considered an unfavorable functional outcome, indicating functional disability to varying degrees ranging from mild impairment to complete dependence or death [17]. An mRS score of ≥ 4 at day 90 after AIS onset was defined as a catastrophic outcome, signifying severe impairment of patients' functional independence, necessitating substantial or complete assistance in daily activities, or resulting in death [11,18,19].

2.3. Outcomes

In this study, selected biomarkers were applied to assess three outcomes.

i) Initial stroke severity, categorized as NIHSS ≥ 5 (no stroke symptom or mild stroke) and NIHSS < 5 (moderate to severe stroke) [8,20].
ii) Long-term AIS functional outcomes, categorized as favorable outcomes (mRS $= 0$ or 1) or unfavorable outcomes (mRS ≥ 2) 90 days after an AIS event.
iii) Long-term catastrophic outcomes, characterized as mRS scores of 4 or higher at 90 days after an AIS event [18,19].
iv) The mRS score at 90 days (measured on a scale of 0–6).

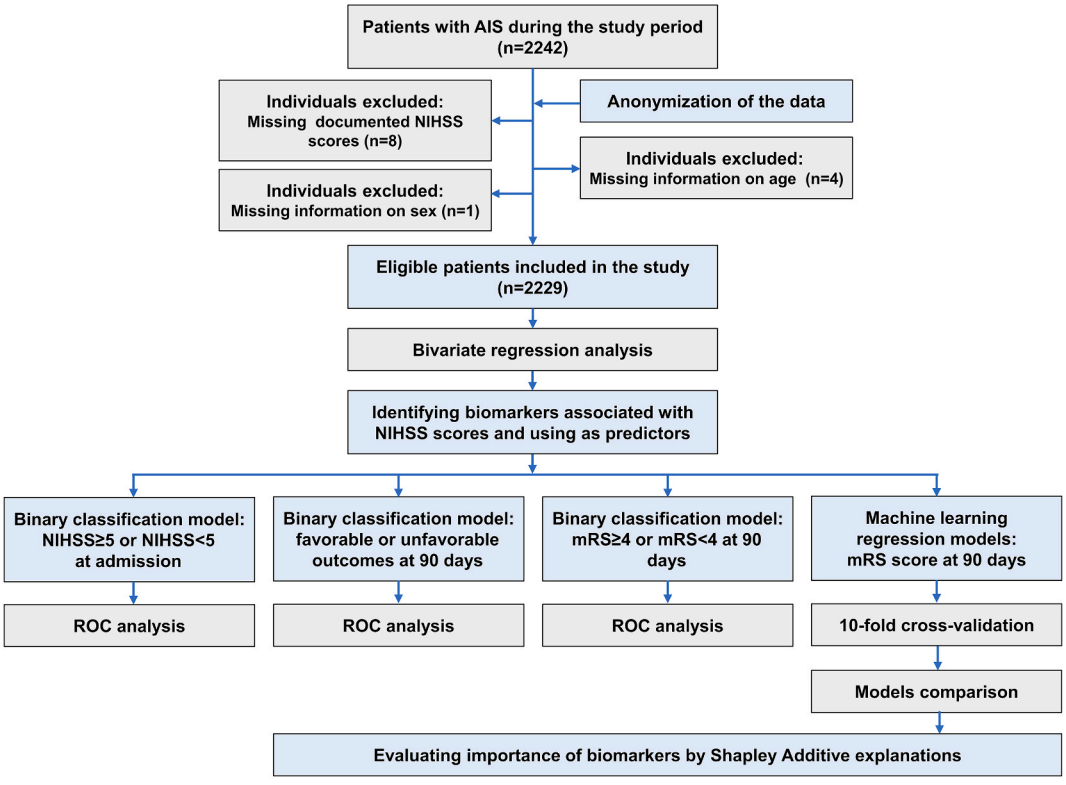

Fig. 1. Flowchart illustrating the process of developing predictive models for acute ischemic stroke (AIS) severity and long-term prognosis. AIS, acute ischemic stroke; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale.

2.4. Statistical analysis

In this study, continuous variables are expressed as mean ± standard deviation, and categorical variables are expressed as counts and proportions (%). Student's *t*-test was used to compare the means between the two groups of continuous variables. Pearson's chi-squared (X^2) test was employed to determine nonrandom associations in pairs of categorical variables in the groups with NIHSS ≥ 5 and NIHSS < 5. A one-way analysis of variance with Tukey–Kramer post hoc analysis was conducted to compare the means of NIHSS scores at admission among patients who received different thrombolytic treatments for AIS and with varying mRS scores at day 90. We used a univariable linear regression model to investigate the association between the level of biochemical tests and NIHSS scores at admission. Variables with a *p*-value of < 0.05 were considered potential biomarkers and thus were included in the multivariate logistic regression model to determine independent predictors of stroke severity and outcomes at 90 days. All hypothesis tests were two-sided, and $p < 0.05$ was considered statistically significant. Statistical analyses of the demographic and clinical characteristics of all the analyzed included patients were performed in STATISTICA version 14.0 (TIBCO Software Inc., Tulsa, OK, USA). Python version 3.10.9 (Python Software Foundation, Wilmington, DE, USA) was implemented to develop machine-learning models and to calculate predictor importance rankings.

2.5. Development of prediction models

This study investigated the association between biochemical test levels and NIHSS scores upon hospital admission for AIS and identified potential biomarkers. An analysis flowchart is presented in Fig. 1. First, bivariate regression analysis was conducted to determine the association between blood or urine biomarkers and NIHSS scores. The entire cohort was then divided into two groups based on their initial stroke severity: NIHSS ≥ 5 and NIHSS < 5. Subsequently, the ability of the identified biomarkers to estimate stroke severity was assessed. Next, the patients were divided into favorable outcome (mRS = 0 or 1) and unfavorable outcome (mRS$\geq$ 2) groups according to their 90-day mRS scores, and the previously identified blood and urine biomarkers were assessed for predicting stroke outcomes. We also used the same routine blood and urine biomarkers to predict catastrophic 90-day outcomes in patients with an mRS score of 4 or higher. To estimate the predictive ability of each model, a receiver operating characteristic (ROC) analysis was conducted, and the area under the ROC curve (AUC) was calculated.

Furthermore, we developed five machine-learning models—namely logistic regression (LR), random forest (RF), support vector machine (SVM), extra-trees, and eXtreme gradient boosting (XGBoost) models—and used the identified biomarkers to predict 90-day

Table 1
Baseline characteristics of patients in the current cohort (n = 2229).

Variables	Whole cohort	AIS severity at admission		
		NIHSS$\geq$ 5	NIHSS <5	*p*-value
Number of Patients, n (%)	2229 (100)	1091 (48.9)	1138 (51.1)	
Age (years)	68.5 ± 13.7	71.3 ± 13.9	65.8 ± 13.0	<0.0001**
Female, n (%)	839 (37.6)	470 (56.0)	369 (44.0)	<0.0001**
NIHSS at admission	7.5 ± 7.9	13.1 ± 8.0	2.2 ± 1.3	<0.0001**
Biochemical Data				
Albumin, g/dL	4.0 ± 0.5	3.86 ± 0.5	4.05 ± 0.4	<0.0001**
Fasting glucose, mg/dL	131.9 ± 50.1	140.1 ± 54.5	123.9 ± 43.9	<0.0001**
Random glucose, mg/dL	161.8 ± 76.4	161.5 ± 77.0	162.1 ± 75.7	0.8670
HbA1c, %	6.7 ± 1.9	6.7 ± 1.7	6.8 ± 2.0	0.2526
Hemoglobin, g/dL	13.9 ± 2.1	13.6 ± 2.2	14.2 ± 2.0	<0.0001**
WBC count, x10^3/uL	8.7 ± 3.2	9.1 ± 3.5	8.3 ± 2.8	<0.0001**
Platelet, x10^3/uL	223 ± 79.7	221.6 ± 80.8	226.1 ± 78.6	0.1913
Prothrombin time, sec	13.2 ± 1.6	13.4 ± 1.8	13.0 ± 1.3	<0.0001**
APTT, sec	37.3 ± 10.0	37.9 ± 12.6	36.8 ± 6.2	0.0216*
Cholesterol, mg/dL	187.9 ± 47.6	182.3 ± 46.3	193.3 ± 48.3	<0.0001**
Triglyceride, mg/dL	136.0 ± 105.7	122.1 ± 86.8	149.5 ± 119.9	<0.0001**
Low-density lipoprotein, mg/dL	115.8 ± 39.1	112.6 ± 40.0	118.9 ± 38.0	0.0002**
Creatinine, mg/dL	1.2 ± 1.1	1.3 ± 1.3	1.1 ± 1.0	0.0186*
AST, U/L	28.5 ± 23.0	29.4 ± 26.5	27.6 ± 18.9	0.0763
Urine specific gravity	1.019 ± 0.012	1.022 ± 0.015	1.016 ± 0.008	<0.0001**
Vascular risk factors, n (%)				
Hypertension	1565 (70.2)	753 (48.1)	812 (51.9)	0.2284
Diabetes mellitus	868 (38.9)	430 (49.5)	438 (50.5)	0.6544
Dyslipidemia	1446 (64.9)	672 (46.5)	774 (53.5)	0.0015**
Atrial fibrillation	396 (17.8)	287 (72.5)	109 ((27.5)	<0.0001**
Previous stroke or TIA	346 (15.5)	193 (55.8)	153 (44.2)	0.0057**
Ischemic heart disease	238 (10.7)	118 (49.6)	120 (50.4)	0.8360

Continuous variables were presented as mean ± standard deviation, and categorical variables were expressed as counts and proportions (%). AIS, acute ischemic stroke; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; HbA1c, Glycated Hemoglobin; NIHSS, National Institutes of Health Stroke Scale; TIA, transient ischemic attack; WBC, White blood cell. The *p*-value represents the comparison between the NIHSS$\geq$5 and NIHSS<5 groups. *p-value<0.05; **p-value<0.01.

mRS scores (from 0 to 6). The missing values of the biomarkers in the model were imputed through multivariate normal imputation [21].

2.6. Model evaluation

To avoid overfitting the machine-learning models, 10-fold cross-validation was performed to assess the generalizability of the analysis. Model performance was evaluated using the root mean squared error (RMSE). The performances of the five machine-learning models were compared on the basis of their respective RMSE values. During the cross-validation process, the model with the lowest mean RMSE was selected as the best-performing model. To analyze the contribution of predictors to the chosen model, SHapley Additive exPlanations (SHAP) was employed [22].

3. Results

3.1. Characteristics of the study population

Of the 2242 patients diagnosed with AIS and treated at our hospital during the study period, eight patients without documented NIHSS scores were excluded from the baseline analysis. Additionally, four patients with missing age information and one with missing sex information were excluded. Finally, 2229 patients (839 women and 1390 men) were included in the analysis (Fig. 1). The mean age of the entire cohort was 68.5 ± 13.7 years, and the mean baseline NIHSS score at admission was 7.5 ± 7.9 (Table 1). Among the cohort, 1091 patients (48.9%) had moderate to severe AIS, indicated by NIHSS ≥ 5 at admission. Age ($p < 0.0001$) and female sex ($p < 0.0001$) were significantly associated with more severe stroke at admission. The patients with a history of dyslipidemia tended to present with milder stroke; by contrast, those with atrial fibrillation (AF) and a history of stroke or transient ischemic attack tended to present with more severe stroke. A comparison of AIS patients with and without AF revealed that patients with AF had a longer prothrombin time at admission than did those without AF (14.0 ± 2.2 vs. 13.0 ± 1.4 s, respectively; $p < 0.0001$).

3.2. Correlation between NIHSS score at admission and 90-day mRS score

A significant correlation was observed between the mRS score 90 days after AIS onset and the NIHSS score at admission (Fig. 2A). Higher NIHSS scores at admission were associated with more severe stroke symptoms and poorer functional outcomes ($p < 0.0001$). A post hoc analysis further revealed that the NIHSS scores at admission differed significantly among patients with different mRS scores at 90 days after AIS onset (Fig. 2B).

3.3. The relationship between thrombolytic treatments, NIHSS score, and the outcomes

In our cohort, 192 patients (8.6%) received intravenous tissue plasminogen activator. Among them, 140 patients (6.3% of the entire cohort) exclusively received intravenous thrombolysis (IVT group), while 52 patients (2.3% of the whole cohort) underwent intra-

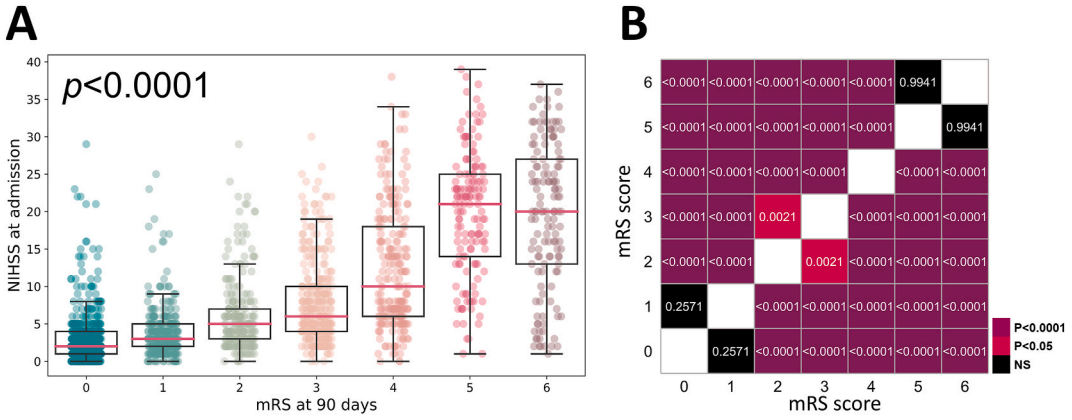

Fig. 2. Relationship between NIHSS score at admission and mRS score at 90 days after AIS onset. (A) The box plot shows the distribution of NIHSS scores at admission for multiple mRS groups 90 days after AIS. Each point represents an individual datum observation. The bottom and top of the box represent the lower quartile and upper quartile of the NIHSS score, respectively. The red line in the middle of the box indicates the median value. (B) Correlation matrix and heatmap of the NIHSS scores at admission for each group with multiple 90-day mRS scores. All pairs were compared using the Tukey–Kramer Honest Significance Test. The values in each cell indicate the *p*-values. Significant differences in NIHSS scores at admission were observed between the groups, except those with mRS scores of 0 and 1 and those with mRS scores of 5 and 6. AIS, acute ischemic stroke; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

arterial thrombolysis following IVT (IAT following IVT group). In addition, 81 patients (3.6% of the entire cohort) who received only intra-arterial thrombolysis were defined as the IAT group.

Patients' initial stroke severity and long-term prognosis showed distinct associations with various thrombolytic treatments. Patients who received non-thrombolytic therapy had notably lower NIHSS scores at the time of admission when compared to those in the IVT, IAT following IVT, and IAT groups. Compared to the IVT group, the IAT following IVT and IAT groups had relatively higher NIHSS scores upon admission (Fig. 3A). At 90 days after an AIS, patients who received non-thrombolytic therapy and IVT exhibited similar mRS scores ($p = 1.000$), whereas the IAT following IVT and IAT groups demonstrated higher mRS scores when compared to the former two groups (Fig. 3B).

3.4. Biochemical biomarkers associated with NIHSS scores at admission

Our bivariate analysis shows the association between each biochemical test result and the corresponding NIHSS score at admission with a p-value <0.05 (Fig. 4). Among the clinical biochemical test biomarkers listed in Table 1, 12 biomarkers, namely, albumin, fasting glucose, HbA1c, hemoglobin, white blood cell (WBC) count, prothrombin time, APTT, cholesterol, triglycerides, LDLC, AST, and urine specific gravity, demonstrated significant association with the NIHSS score at admission. These 12 biomarkers were explicitly selected as predictors in our prediction model.

A generalized linear model was employed to investigate the association between blood and urine biomarkers levels and NIHSS score at admission (Supplementary Table 1). Among the biomarkers, albumin, fasting glucose, HbA1c, hemoglobin, WBC count, prothrombin time, triglycerides, and urine specific gravity were associated with NIHSS score at admission.

3.5. Prediction of stroke severity at admission using biomarkers

The ROC curve analysis involving the aforementioned 12 biomarkers demonstrated an AUC value of 0.7465 for predicting moderate to severe AIS on the basis of NIHSS ≥ 5 at admission (Fig. 5A). Table 2 presents the crude and adjusted odds ratios (ORs) of the 12 biomarkers for NIHSS ≥ 5 at admission. Among the 12 biomarkers, albumin ($p < 0.0001$), fasting glucose ($p < 0.0001$), HbA1c ($p < 0.0001$), hemoglobin ($p < 0.0001$), WBC count ($p = 0.003$), prothrombin time ($p = 0.0344$), triglycerides ($p = 0.0182$), and urine-specific gravity ($p < 0.0001$) were significant predictors of moderate to severe stroke at admission. The notable AUC value suggested that these biomarkers could serve as surrogates of stroke severity and have the potential for predicting stroke severity upon hospitalization.

3.6. Association between biomarkers and 90-day mRS scores

In the long-term prognosis analysis of the entire cohort at 90 days of follow-up, 178 (8.0%) of the analyzed patients had missing mRS records. Overall, 2051 patients were included in the long-term prognosis analysis. Of these patients, 972 (47.4%) had favorable outcomes, and 1079 (52.6%) had unfavorable outcomes. The generalized linear model revealed that albumin, fasting glucose, HbA1c, hemoglobin, WBC count, prothrombin time, triglycerides, and urine-specific gravity were associated with 90-day mRS scores (Supplementary Table 2).

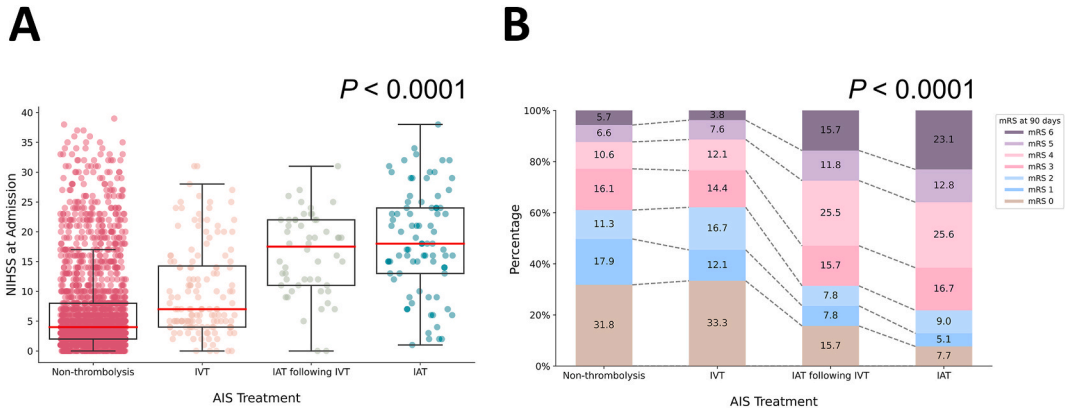

Fig. 3. Relationship between initial stroke severity and long-term prognosis across different thrombolytic treatments. (A) Box plots depict the distribution of NIHSS scores at admission among various treatment groups. Each data point corresponds to an individual observation. The lower and upper boundaries of the box signify the first and third quartiles of the NIHSS scores, while the red line within the box represents the median value. (B) Distribution of mRS scores at 90 days in different treatment groups for AIS. The number at the center of each colored bar represents the percentage of patients relative to the corresponding treatment group. AIS, acute ischemic stroke; IAT, intra-arterial thrombolysis; IVT, intravenous thrombolysis; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

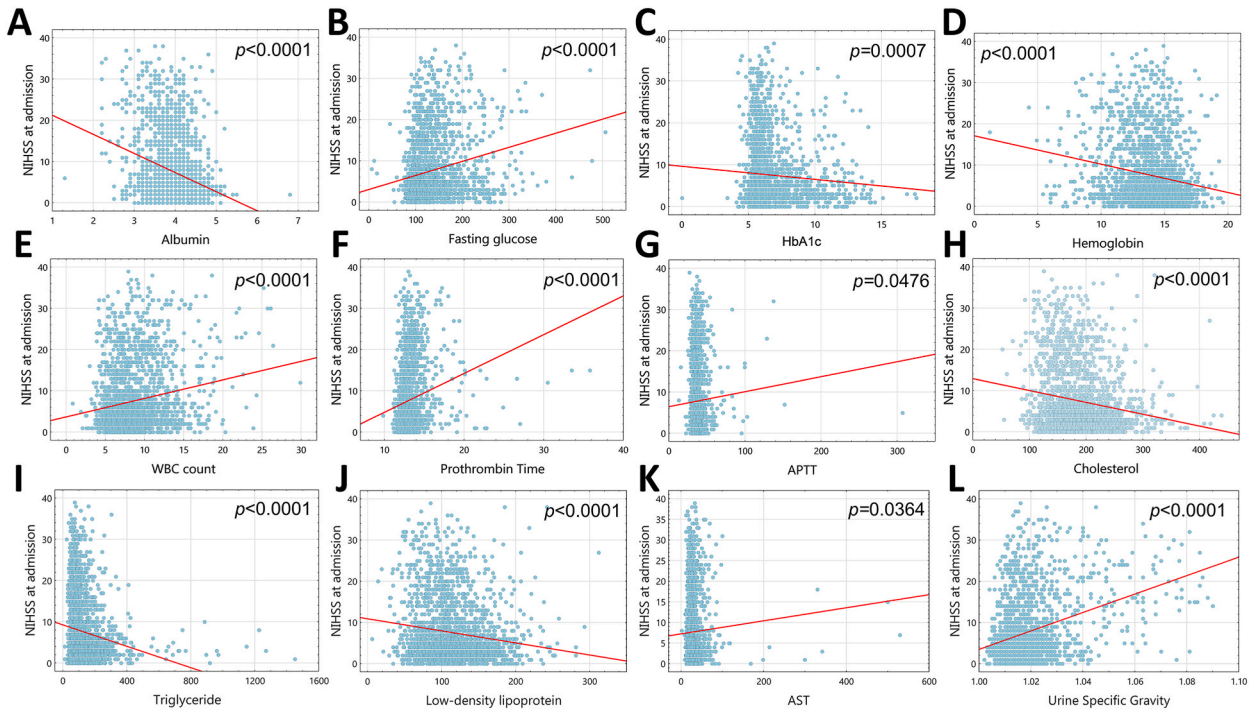

Fig. 4. Associations of blood and urine biomarkers with NIHSS score at admission. The x-axis indicates the value of each biomarker: (A) albumin, (B) fasting glucose, (C) HbA1c, (D) hemoglobin, (E) WBC count, (F) prothrombin time, (G) APTT, (H) cholesterol, (I) triglyceride, (J) low-density lipoprotein, (K) AST, (L) urine specific gravity. The y-axis corresponds to the NIHSS score at admission. APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; HbA1c, glycated hemoglobin; NIHSS, National Institutes of Health Stroke Scale; WBC, white blood cell.

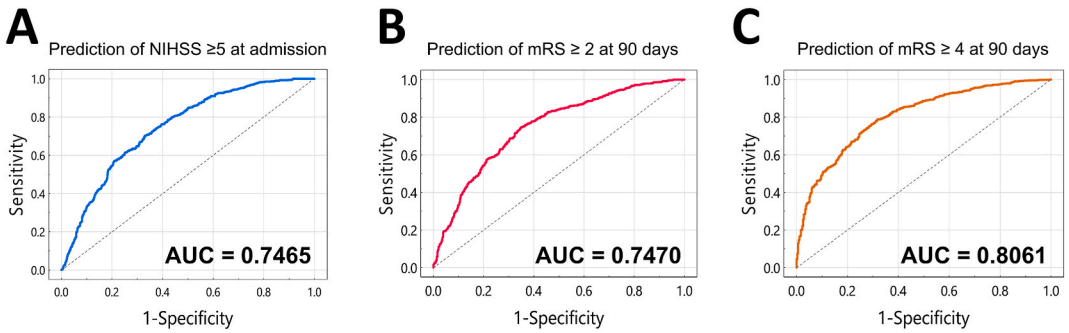

Fig. 5. AUC-defined performance of the prediction models based on blood and urine biomarkers. (A) ROC curves with corresponding AUC values of the model for predicting moderate to severe stroke based on an NIHSS score of ≥ 5 at admission and using the proposed 12 biomarkers. (B) ROC curves with corresponding AUC values of the model for predicting unfavorable outcomes at 90 days after AIS onset using the 12 biomarkers. (C) ROC curve and the associated AUC values for the model predicting catastrophic outcomes (mRS ≥ 4) at 90 days following AIS onset using the 12 biomarkers. AUC, area under the ROC curve; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; ROC, receiver operating characteristic.

3.7. Prediction of long-term prognosis using biomarkers

Table 3 presents the crude and adjusted ORs of the 12 biomarkers for an unfavorable outcome at 90 days. Albumin ($p < 0.0001$), fasting glucose ($p < 0.0001$), HbA1c ($p = 0.0001$), hemoglobin ($p < 0.0001$), WBC count ($p = 0.0007$), prothrombin time ($p = 0.0006$), triglycerides ($p = 0.0007$), and urine specific gravity ($p = 0.0008$) were significant predictors of unfavorable outcomes at 90 days following AIS onset.

Furthermore, in Table 3 we indicate the unadjusted and adjusted ORs for the 12 biomarkers regarding a catastrophic outcome (mRS ≥ 4) at 90 days. Notably, albumin ($p < 0.0001$), fasting glucose ($p < 0.0001$), HbA1c ($p < 0.0001$), hemoglobin ($p < 0.0001$), WBC count ($p = 0.01$), prothrombin time ($p < 0.0001$), triglycerides ($p < 0.0001$), and urine specific gravity ($p < 0.0001$) emerged as

Table 2
Multivariable analysis of factors associated with moderate to severe stroke (NIHSS ≥5) at admission.

	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Albumin	0.388 (0.315, 0.476)**	0.520 (0.388, 0.698)**
Fasting glucose	1.007 (1.005, 1.009)**	1.015 (1.011, 1.019)**
HbA1c	0.973 (0.929, 1.020)	0.763 (0.693, 0.839)**
Hemoglobin	0.863 (0.828, 0.900)**	0.875 (0.820, 0.934)**
WBC count	1.088 (1.058, 1.118)**	1.080 (1.036, 1.126)**
Prothrombin time	1.280 (1.182, 1.386)**	1.104 (1.007, 1.209)*
APTT	1.013 (1.001, 1.025)*	1.011 (0.995, 1.026)
Cholesterol	0.995 (0.993, 0.997)**	0.997 (0.990, 1.004)
Triglyceride	0.997 (0.996, 0.998)**	0.998 (0996, 1.000)*
Low-density lipoprotein	0.996 (0.994, 0.998)**	1.005 (0.996, 1.026)
AST	1.004 (0.999, 1.008)	1.004 (0.996, 1.011)
Urine specific gravity	1.75e+21 (1.27e+17, 2.40e+25)**	3.83e+18 (1.09e+13, 1.35e+24)**

APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; CI, confidence interval; HbA1c, Glycated Hemoglobin; WBC, white blood cell. The odds ratio represented per unit change in the regressor. *$p < 0.05$. **$p < 0.001$.

Table 3
Multivariable analysis of factors associated with AIS outcomes.

Associations between biomarkers and mRS ≥2 at 90 days		
	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Albumin	0.300 (0.239, 0.376)**	0.436 (0.319, 0.594)**
Fasting glucose	1.007 (1.005, 1.009)**	1.015 (1.010, 1.019)**
HbA1c	0.996 (0.949, 1.045)	0.827 (0.751, 0.912)**
Hemoglobin	0.796 (0.759, 0.834)**	0.820 (0.764, 0.880)**
WBC count	1.081 (1.050, 1.113)**	1.078 (1.032, 1.125)**
Prothrombin time	1.372 (1.253, 1.501)**	1.194 (1.079, 1.321)**
APTT	1.006 (0.996, 1.016)	0.997 (0.987, 1.008)
Cholesterol	0.995 (0.993, 0.997)**	1.003 (0.996, 1.010)
Triglyceride	0.997 (0.996, 0.998)**	0.997 (0995, 0.999)**
Low-density lipoprotein	0.994 (0.992, 0.997)**	0.999 (0.990, 1.007)
AST	1.004 (0.999, 1.010)	1.004 (0.995, 1.013)
Urine specific gravity	6.58e+13 (1.03e+10, 4.20e+17)**	1.89e+8 (2862.05, 1.25e+13)**
Associations between biomarkers and mRS ≥4 at 90 days		
	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Albumin	0.193 (0.149, 0.249)**	0.303 (0.213, 0.431)**
Fasting glucose	1.006 (1.004, 1.008)**	1.011 (1.006, 1.015)**
HbA1c	0.911 (0.856, 0.969)*	0.811 (0.727, 0.904)**
Hemoglobin	0.757 (0.720, 0.797)**	0.827 (0.766, 0.892)**
WBC count	1.085 (1.052, 1.118)**	1.061 (1.014, 1.110)*
Prothrombin time	1.406 (1.293, 1.530)**	1.223 (1.112, 1.346)**
APTT	1.005 (0.996, 1.015)	0.993 (0.979, 1.008)
Cholesterol	0.991 (0.989, 0.994)**	1.005 (0.997, 1.014)
Triglyceride	0.992 (0.991, 0.994)**	0.994 (0992, 0.997)**
Low-density lipoprotein	0.991 (0.988, 0.994)**	0.995 (0.985, 1.006)
AST	1.004 (1.000, 1.009)	1.002 (0.994, 1.010)
Urine specific gravity	4.37e+16 (1.22e+13, 1.56e+20)**	2.85e+13 (5.38e+8, 1.51e+18)**

AIS, acute ischemic stroke; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; CI, confidence interval; HbA1c, Glycated Hemoglobin; mRS, modified Rankin Scale; WBC, white blood cell. The odds ratio represented per unit change in the regressor. *$p < 0.05$. **$p < 0.001$.

significant predictors of mRS ≥ 4 at 90 days after AIS.

Using ROC curves for classifier performance efficiency, we demonstrated that the 12 biomarkers effectively predict unfavorable outcomes on the basis of mRS ≥ 2 at 90 days after AIS, as indicated by an AUC value of 0.7470 (Fig. 5B), indicating that the 12 biomarkers possess strong ability to predict the long-term prognosis of AIS. Similarly, the ROC curves of the 12 biomarkers in predicting mRS ≥ 4 after 90 days of AIS revealed an AUC value of 0.8061 (Fig. 5C), supporting the excellent predictive ability of these 12 biomarkers for AIS catastrophic outcomes. Supplementary Table 3 showed the optimal cut-off values for each biomarker in predicting NIHSS ≥ 5 at admission, unfavorable outcome, and catastrophic outcome at 90 days.

3.8. Machine learning for mRS prediction

The selected biomarkers were used as predictors, and five machine-learning models—namely LR, RF, SVM, extra-trees, and

XGBoost models—were applied to predict 90-day mRS scores ranging from 0 to 6. After 10-fold cross-validation, the mean and standard deviation of the RMSE were calculated as 2.381 ± 0.15, 1.629 ± 0.04, 1.783 ± 0.06, 1.618 ± 0.06, and 1.616 ± 0.03 for the LR, RF, SVM, extra-trees, and XGBoost models, respectively. Among these five models, the XGBoost approach achieved the lowest mean RMSE (Fig. 6).

3.9. Assessment of feature importance

The degree of contribution of each feature to the prediction model for an unfavorable prognosis in patients with AIS was calculated using SHAP. Fig. 7A presents the importance ranking of the 12 biomarkers in the XGBoost model, revealing the top five most crucial biomarkers to be fasting glucose, albumin, hemoglobin, prothrombin time, and urine-specific gravity in that order. Fig. 7B presents the distribution of each predictor's contribution to the model output. Each dot represents one case, with red and blue dots representing higher and lower biomarker values, respectively. The SHAP value on the horizontal axis indicates the degree of influence of the biomarker on the prediction result. Positive values signify a positive effect, and negative values indicate a negative impact.

4. Discussion

This research highlights the usefulness of clinically available biomarkers for developing models to predict both the severity and long-term prognosis of AIS. Additionally, machine-learning techniques were employed in this study to assess the importance of these biomarkers in their role as predictors in the models. The results revealed that initial stroke severity and long-term outcomes of AIS share common risk factors and that biomarkers that estimate stroke severity contribute to determining the long-term prognosis of AIS. This promising predictive performance suggests that models developed using these biomarkers have the potential to accurately predict stroke severity and long-term AIS prognosis. All the biomarkers used in the present models are readily and routinely accessible in clinical settings. Furthermore, the models developed using these biomarkers demonstrate the ability to make accurate predictions independently, eliminating the need for additional information commonly included in stroke prediction models, such as the patient's age, sex, and NIHSS score assessment. The application of machine-learning approaches to predict mRS scores of patients at 90 days after AIS onset and the analysis of the contribution of the aforementioned biomarkers to the models highlight the crucial roles of fasting glucose, albumin, hemoglobin, prothrombin time, and urine specific gravity in predicting the prognosis of AIS.

The National Institutes of Health (NIH) Biomarkers Definitions Working Group defined biomarkers as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention" [13,23,24]. Numerous studies have employed biomarkers in research related to AIS and have demonstrated the impact of these biomarkers in acute clinical settings. The use of biomarkers has been suggested for early screening and diagnosis of AIS, for predicting the risk of disease development or progression, for assessing the safety and efficacy of drugs, and for monitoring treatment effectiveness.

In addition, the use of many biomarkers in stroke diagnosis has been investigated. The levels of these biomarkers may reflect the body's pathophysiological processes in response to the damage caused by stroke, including neuronal or glial damage, platelet activation, thrombosis, endothelial cell activation, and microvascular circulation markers [14,15,25].

Elevated C-reactive protein (CRP) levels, observed after AIS onset, reflect stroke severity and are associated with stroke subtypes [26]. A study regarding patients undergoing intravenous thrombolysis for AIS demonstrated that higher initial serum lactate dehydrogenase (LDH) levels were associated with higher baseline NIHSS scores and larger final infarct size [27]. A meta-analysis of 136 biomarkers concluded that CRP, P-selectin, and homocysteine were the three biomarkers that significantly differentiated patients with

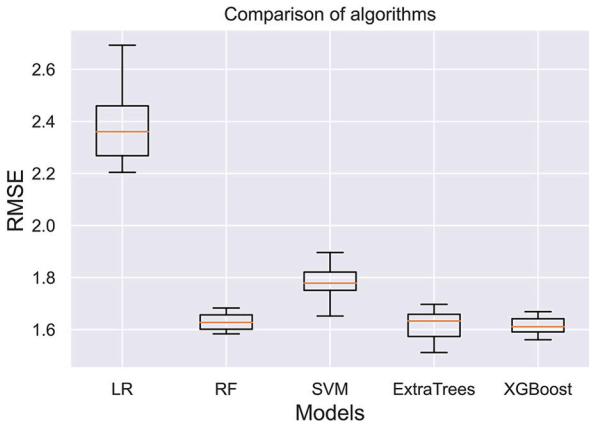

Fig. 6. Comparison of the performances of machine-learning models in predicting 90-day mRS scores. The box plot shows the distribution of the RMSE values for all the machine-learning models obtained after cross-validation. The orange line represents the median. LR, logistic regression; mRS, modified Rankin Scale; RF, random forest; RMSE, root-mean-square error; SVM, support vector machine; XGBoost, eXtreme Gradient Boosting. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

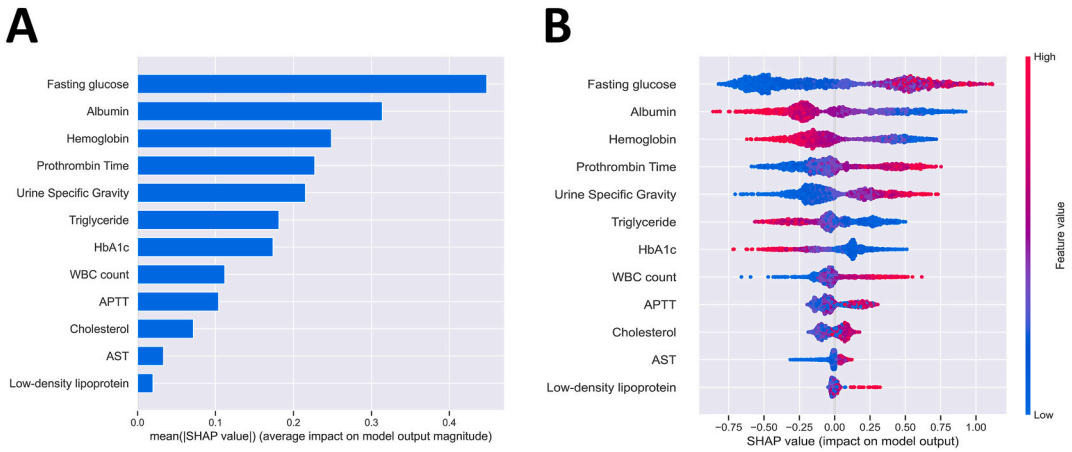

Fig. 7. Importance and ranking of biomarkers in the XGBoost model defined based on SHAP values. (A) Importance ranking of the 12 biomarkers. Longer bars indicate a more substantial influence on the prediction results. (B) Distribution of the impacts of each biomarker on the model output. The horizontal axis represents the SHAP value, which quantifies the degree of influence of each biomarker on the predicted outcome. Each dot represents one case in each row. The dots are colored from red to blue, representing the value of the biomarker itself from high to low, respectively. APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; HbA1c, glycated hemoglobin; WBC, white blood cell; XGBoost, eXtreme Gradient Boosting. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

ischemic stroke from healthy controls [25]. Elevated serum MMP-9 concentrations in the acute phase of AIS have been associated with larger infarct size, more severe stroke, and less favorable functional outcomes [14,28]. Levels of NSE, S100B protein, and neurofilament light chain may reflect the degree of neurological damage caused by stroke and have been demonstrated to correlate with stroke severity and infarct size [14,15,29,30]. Several proinflammatory and inflammatory cytokines—such as interleukin (IL)-6, IL-10, adhesion molecules, and circulating endothelial- or platelet-derived microparticles—have also been proposed as having associations with stroke severity and prognosis [15,31–33].

Although the aforementioned biomarkers exhibit potential relevance in AIS, their clinical use remains under investigation, and they are not yet widely used in clinical practice. Thus, further studies and validation are warranted to determine their reliability. To date, no biomarker has demonstrated sufficient power to diagnose or estimate stroke severity on its own.

The time-critical nature of AIS treatment emphasizes the need for prompt and reliable diagnostic and monitoring tools. Unlike tests that may require specialized equipment, techniques, or methods that may not be readily accessible in typical medical facilities, the current study used biochemical tests that are routinely used in clinical practice and that are accessible in routine clinical settings. This ensures the practical relevance of our findings, as these biomarkers have been extensively validated and hold clear value in guiding clinical decision-making. Moreover, using clinically accessible biomarkers allows for rapid access to results, enabling prompt and reliable diagnostic and monitoring outcomes to support clinical decision-making and treatment planning in AIS.

The current study developed models with 12 biomarkers to serve as predictors. Various pathophysiological processes underlie the occurrence and development of stroke. Consequently, a single biomarker may not fully reflect these complex processes, and thus, the simultaneous measurement of multiple markers may be required to provide sufficiently comprehensive information [14,15]. In addition, the simultaneous measurement of multiple biomarkers enables a more integrated analysis that considers the interactions and relationships between these biomarkers and provides insights into the underlying mechanisms of stroke development and progression. Furthermore, each patient with AIS has unique physiological conditions and pathological processes. Thus, by measuring multiple biomarkers simultaneously, these individual differences can be better accounted for, enabling more personalized prediction and treatment strategies.

The prediction model developed in this study identified fasting glucose, albumin, hemoglobin, prothrombin time, and urine-specific gravity as the top five most crucial biomarkers in the prognosis of AIS. Among these biomarkers, high fasting glucose levels were strongly associated with initial AIS severity and emerged as the most crucial factor in terms of prognostic relevance. The adverse effects of hyperglycemia on patients with AIS have been extensively validated. Specifically, hyperglycemia can have multiple detrimental mechanisms that affect the nervous system, including exacerbating brain tissue damage, increasing the infarct volume, reducing the efficacy of thrombolysis and thrombectomy, impeding the functional recovery of the brain, and contributing to increased disability and morbidity in patients with AIS [34–38]. Notably, in the present cohort, fasting glucose levels in the acute phase rather than HbA1c levels were associated with initial stroke severity. This finding is consistent with current knowledge, which suggests a critical role of stress-induced hyperglycemia, namely an elevation of blood glucose due to a sudden clinical event, in both the occurrence and progression of the disease and the development of treatment strategies for AIS [34–37].

Studies have demonstrated that lower plasma albumin levels are associated with larger infarct volumes, higher morbidity and mortality, and poorer functional recovery in patients with AIS [39–41]. These findings are consistent with the present results, which highlight the importance of albumin as an essential biomarker for assessing the severity and prognosis of AIS. One of the main

functions of albumin is to maintain sufficient colloid osmotic pressure in the blood [42]. Reduced plasma albumin levels may lead to increased blood viscosity and result in inadequate cerebral blood perfusion [41]. In addition, hypoalbuminemia may lead to abnormal blood coagulation and platelet aggregation activity and thus may increase the risks of atherosclerosis and thrombosis [41–43]. Furthermore, albumin is involved in the regulation of the inflammatory response; reduced plasma albumin may increase the degree of inflammatory response and worsen the severity and prognosis of stroke [41,44]. In patients with AIS, hypoalbuminemia may also reflect a systemic state of malnutrition, which in turn affects the severity and prognosis of stroke [39,44].

Hemoglobin, an essential component of red blood cells, plays a crucial role in transporting oxygen to various tissues and organs throughout the body. Ischemic stroke is characterized by the deprivation of oxygen to the brain as a result of a reduction or interruption in the cerebral blood supply. A decline in hemoglobin can exacerbate the insufficiency of oxygen delivery and thus can increase the severity of cerebral ischemia and AIS [45,46]. Furthermore, reduced hemoglobin leads to hyperdynamic circulation and upregulation of the expression of adhesion molecules on vascular endothelial cells, which in turn triggers an inflammatory response that induces thrombosis [45,46]. Consistent with existing knowledge in the field, the present study identified an association between hemoglobin levels and the severity of AIS and thus highlights the prognostic relevance of hemoglobin in stroke outcomes [46,47].

In our model, prothrombin time was considered a key feature. Prolonged prothrombin time indicates abnormalities in the coagulating function, which may be caused by interventions. In the present cohort, patients with AF had longer prothrombin times, which may be associated with the use of anticoagulant therapy. In line with the findings of previous studies, those of the present study revealed that patients with AF experienced more severe AIS and had worse prognoses compared with those without AF [48,49]. In addition, prolonged prothrombin time may indicate a specific hematological disorder or coagulation dysfunction, leading to a risk of more severe AIS and a less favorable prognosis [38,50].

Urine-specific gravity, a measure of the concentration of urine, serves as an essential indicator of the body's hydration status. Higher urine-specific gravity typically suggests an insufficient amount of body fluid or a state of dehydration. A dehydrated state may lead to increased blood viscosity, diminished circulation, and altered cerebral perfusion and thus may increase the risk of ischemic stroke. Studies have identified urine-specific gravity as a predictor of early neurological deterioration after ischemic stroke and a valuable indicator for guiding fluid therapy for patients with AIS [51,52]. Moreover, higher urine-specific gravity, which is indicative of dehydration, was identified as an independent risk factor for poor long-term prognosis in patients with AIS receiving thrombolytic therapy [53].

The high incidence of AIS has resulted in the accumulation of considerable amounts of clinical information, which in turn has provided researchers with opportunities to gain insights into stroke risk factors, prognosis, and treatment strategies. Regarding the analysis of these complex and large-scale data, the application of machine-learning methods in AIS research has gained considerable attention [5].

However, machine-learning algorithms often have many multidimensional, nonlinear parameters and complex internal structures, rendering the decision-making processes of these algorithms challenging to interpret and explain. To interpret the results of the present machine-learning model, we applied the SHAP method to describe the degree of contribution of each feature to the model prediction. By calculating SHAP values, the importance and influence of each feature in relation to the model's predictions were determined. Moreover, the SHAP values were used to determine the model's behavior, assess the significance of each feature, and create visualization tools to present the results of our prediction model. The incorporation of interpretability methods such as SHAP into machine-learning models for AIS prediction enables accurate assessment of the model's performance and, if necessary, helps optimize the model and enhance its performance, thereby facilitating more accurate predictions and contributing to clinical decision-making related to AIS.

In a more profound implication, the biomarkers used in our study, including fasting glucose, albumin, hemoglobin, prothrombin time, urine-specific gravity, triglyceride, HbA1c, APTT, and cholesterol, are all potentially modifiable factors. By identifying and managing these risk factors, healthcare professionals can establish personalized preventive measures with their patients to reduce the incidence and severity of stroke. Predictive models based on these modifiable markers can also support optimizing patient biomarkers in advance and play a key role in reducing the harm caused by AIS, facilitating clinical decision-making, and improving patient prognosis. Our findings demonstrate the potential of clinically available biomarkers to predict AIS severity and long-term prognosis. The models developed using these biomarkers provide independent predictions without relying on additional information commonly included in stroke prediction models. Fasting glucose, albumin, hemoglobin, prothrombin time, and urine-specific gravity emerged as significant predictors for AIS prognosis. These findings contribute to the understanding of stroke pathophysiology and facilitate the development of personalized prediction and treatment strategies. Further validation studies are warranted to confirm the utility of these biomarkers in clinical practice.

4.1. Study limitations

The study had the following limitations. First, this was a single-center study with a relatively moderate sample size. The study cohort represented a specific population at a single medical center and thus did not adequately represent the characteristics of the entire target population. This factor may have limited the external validity and generalizability of the study results. Second, the availability of routine medical tests is limited by the capacity and resources of individual medical institutions. Similarly, the availability of biomarkers may vary across healthcare settings and thus may hinder prediction accuracy. Third, stroke progression is a complex and dynamic process that may be influenced by multiple factors, such as a patient's physiological condition, pathological processes, coping ability, and therapeutic interventions. This study used only biomarkers measured at the time of hospitalization and did not capture potential changes in these biomarkers during different stages of stroke or in response to treatment; this factor also may

have limited the prediction accuracy. Fourth, our study did not exclude patients with a pre-morbid mRS score greater than 2. Including patients with higher pre-morbid mRS scores could introduce potential confounding and impact the generalizability of our findings. Lastly, although the models developed in this study exhibited promising results, room for improvement in terms of their accuracy remains. The field of stroke research is constantly evolving, with new biomarkers and applications repeatedly emerging. Future studies can continue to incorporate these advancements and refine the current prediction models to enhance their clinical reliability and feasibility.

5. Conclusion

The application of biomarkers in AIS research can facilitate the prevention, early diagnosis, and treatment of stroke and thus promote the development of individualized health management and precision medicine. Our study identified clinically available blood and urine biomarkers associated with initial stroke severity and long-term prognosis of AIS. The predictive models established in this study using these blood and urine biomarkers demonstrated notably high performance in predicting stroke severity and long-term outcomes. Furthermore, machine learning–based approaches were applied to develop and interpret the prediction models. Machine-learning methods enable the processing of large data sets and thus can reveal complex patterns and relationships that can optimize and enhance the prediction performance of models. By measuring an established set of multiple markers simultaneously, the occurrence and progression of AIS can be more comprehensively and more accurately assessed. Furthermore, the use of interpretable machine-learning methods can provide a more comprehensive understanding of the decision process and inference logic of machine-learning algorithms so that more accurate predictions can be made; such predictions can assist in clinical decision-making and provide a scientific basis for establishing more effective clinical treatment and management strategies to reduce the severity of AIS and improve its prognosis.

Funding

This research was funded by the National Science and Technology Council (grant number NSTC 111-2314-B-038-132-MY3) and Taipei Medical University Shuang Ho Hospital (grant number 112HHC-15 to Chen-Chih Chung).

Ethical approval

The present study, conducted by researchers affiliated with Taipei Medical University, was approved by that university's Joint Institutional Review Board (TMU-JIRB Approval No. N202103006). The board granted a waiver for informed consent owing to the retrospective nature of the study, which involved the secondary analysis of existing anonymized data. All procedures were performed in compliance with applicable guidelines and regulations.

CRediT authorship contribution statement

Oluwaseun Adebayo Bamodu: Writing – review & editing, Formal analysis. **Lung Chan:** Writing – review & editing, Investigation, Conceptualization. **Chia-Hui Wu:** Writing – original draft, Methodology, Formal analysis, Data curation. **Shun-Fan Yu:** Writing – original draft, Methodology, Formal analysis, Data curation. **Chen-Chih Chung:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors extend their appreciation to the reviewers for their insightful feedback.

The authors would like to thank the stroke case managers at the Shuang Ho Hospital, Ms. Le-Yan Lai and Ms. Ming-Hsiu Hsieh, for maintaining and managing the stroke registry data.

This manuscript was edited for English language use by Wallace Academic Editing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26199.

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