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ORIGINAL ARTICLE

Predictive Value of Combined Vitamin D, hs-CRP, and Cerebral Hemodynamic Parameters for Mid- to Long-Term Recurrence Risk of Ischemic Stroke

Nian-Ping Feng, Jia-Jia Sun, Wei-Wei Liang, Lin Cong, Si-Wen Ding, Xing-Jun Xiao

Department of Neurology, The Second Affiliated Hospital of Harbin Medical University, Harbin City, Heilongjiang Province, China

ABSTRACT

Background: **This study aimed to investigate the value of combined detection of vitamin D, high-sensitivity C-reactive protein (hs-CRP), and cerebral hemodynamic parameters in predicting the mid- to long-term recurrence risk of ischemic stroke.**

Methods: **A total of 409 patients with a first acute ischemic stroke were enrolled. Serum 25-hydroxyvitamin D [25(OH)D], hs-CRP levels, and transcranial Doppler examination were performed within 72 hours of symptom onset; the maximum pulsatility index (PImax) on the affected side was recorded. Recurrence events were recorded during a 12-month follow-up. Cox proportional hazards models were used to analyze the association of the three indicators with recurrence. A combined prediction model was constructed, and its discriminative ability (area under the curve [AUC], concordance index [C-index]), calibration (Brier score, calibration-in-the-large), and clinical net benefit (decision curve analysis) were evaluated.**

Results: **Within 12 months, 66 patients (16.1%) experienced recurrence. Multivariable Cox analysis, adjusted for confounders such as age and National Institutes of Health Stroke Scale score, showed that 1 standard deviation (SD) increase in PImax (hazard ratio [HR] = 2.53, 95% confidence interval [CI]: 1.92 - 3.34, p < 0.001), 1 SD increase in ln(hs-CRP) (HR = 2.38, 95% CI: 1.75 - 3.24, p < 0.001), and 1 SD decrease in 25(OH)D (HR = 2.21, 95% CI: 1.68 - 2.91, p < 0.001) were all independent risk factors for recurrence. The AUC for the three-indicator combined model was 0.869, while the AUC for the five-indicator model (including age and National Institutes of Health Stroke Scale score) was 0.875, with a C-index of 0.848. Both models demonstrated good calibration and showed significant net benefit within the 10% - 30% recurrence risk threshold range. Kaplan-Meier analysis revealed that the groups with high PImax, high hs-CRP, and low 25(OH)D levels had significantly higher 12-month cumulative recurrence rates (all Log-rank p < 0.001).**

Conclusions: **This study confirms that vitamin D deficiency, systemic inflammation activation, and elevated cerebrovascular resistance are independently associated with mid- to long-term ischemic stroke recurrence. The combined prediction model based on 25(OH)D, hs-CRP, and PImax demonstrates high differentiation ability for predicting recurrence risk. Integrating metabolic, inflammatory, and hemodynamic information can effectively identify high-risk populations for stroke recurrence, providing a basis for individualized risk stratification and precise secondary prevention.**

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Correspondence:
Xing-Jun Xiao
Department of Neurology
The Second Affiliated Hospital of Harbin Medical University
No. 246 Xuefu Road,
Nangang District
Harbin City
Heilongjiang Province, 150086
China
Email: XingJunxiao332@outlook.com

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INTRODUCTION

Ischemic stroke is one of the leading causes of disability and mortality worldwide, posing a significant public health burden [1,2]. Despite the widespread implementation of secondary prevention measures such as antiplatelet therapy, blood pressure, and lipid management, the risk of stroke recurrence remains high. Multiple systematic reviews and epidemiological studies indicate that stroke recurrence is common across different regions and populations, with reported rates ranging from approximately 6% to 50% [3]. The China National Stroke Registry also showed that even under standardized secondary prevention, the risk of recurrence within 12 months remains significant [4]. Recurrent events not only substantially increase the risk of disability and death but also impose a continuous burden on patients' long-term quality of life and healthcare resources [5]. However, existing clinical scoring systems have limited efficacy in identifying recurrence risk [6]. Therefore, developing a precise risk stratification tool that integrates multi-dimensional physiological and pathological information is crucial for the early identification of high-risk populations and for guiding individualized secondary prevention strategies.

Stroke recurrence is a pathological process involving the interaction of multiple factors, including systemic inflammation, endothelial dysfunction, cerebral hemodynamic abnormalities, and metabolic imbalances [7]. Chronic low-grade inflammation is considered one of the core drivers of stroke recurrence. Inflammatory markers such as high-sensitivity C-reactive protein (hs-CRP) have been confirmed as independent predictors of recurrence [8,9].

Inflammatory responses can disrupt the blood-brain barrier integrity and promote plaque instability and the progression of small vessel disease [8]. Endothelial dysfunction, by impairing vasomotor regulation, promoting local coagulation activation, and exacerbating inflammatory responses, serves as an important pathological basis for stroke recurrence [10]. Hemodynamically, prolonged perfusion time and elevated cerebrovascular resistance are closely associated with recurrence risk [11,12]. The pulsatility index (PI), measured by transcranial Doppler (TCD), reflects microcirculatory resistance and vascular compliance; its elevation is significantly correlated with cerebral small vessel disease and poor stroke outcomes [13]. This evidence suggests that the triad of inflammation activation, endothelial injury, and perfusion abnormalities may form a pathological loop driving stroke recurrence, necessitating a multi-dimensional comprehensive assessment strategy.

Beyond its role in calcium and phosphorus metabolism, vitamin D (25-hydroxyvitamin D [25(OH)D]) possesses immunomodulatory, anti-inflammatory, and endothelial protective functions [14]. Studies have shown that low 25(OH)D levels are significantly associated with the risk of ischemic stroke incidence and poor prognosis [15,16]. By modulating inflammatory pathways and improving endothelial function, vitamin D may influence vascular remodeling and recurrence risk after stroke [17]. Hs-CRP, a classic marker of systemic inflammation, is widely used to assess the activity of atherosclerosis and the degree of vascular injury [18,19]. Multiple studies have confirmed that elevated hs-CRP is independently associated with an increased risk of stroke recurrence and mortality [20,21]. The PI reflects changes in distal small vessel resistance and compliance; its elevation is associated with the burden of cerebral small vessel disease, white matter hyperintensities, and poor functional outcomes after stroke [22,23]. Although the individual predictive value of these indicators has been partially established, there is a lack of research systematically evaluating their combined predictive capability. Current research on predicting stroke recurrence often focuses on single-dimensional indicators, lacking comprehensive assessment models that integrate metabolic, inflammatory, and hemodynamic parameters. Based on the pathological framework of the inflammation-endothelium-hemodynamics axis, this study jointly measures 25(OH)D, hs-CRP, and the maximum pulsatility index (PImax) on the affected side. By using Cox regression analysis and model validation methods, it systematically evaluates the independent and combined predictive value of these three markers for mid- to long-term recurrence in ischemic stroke. The aim was to establish a quantifiable, easily generalizable risk stratification tool, providing a new strategy for precise secondary prevention and follow-up management.

MATERIALS AND METHODS

Study design and participants

This was a single-center cohort study. Patients with first-ever acute ischemic stroke (AIS) were enrolled. The inclusion criteria were as follows: 1) Patients were required to have all baseline tests (blood draw and TCD) completed after admission and within 72 hours of symptom onset. 2) Patients were required to be aged ≥ 18 years. 3) Patients were required to have an AIS confirmed by magnetic resonance imaging (MRI) and/or computed tomography (CT). All patients or their legal proxies/immediate family members provided written informed consent. The exclusion criteria were as follows: 1) Patients with hemorrhagic stroke or transient ischemic attack. 2) Patients with active severe infection, malignancy, severe hepatic/renal dysfunction, or autoimmune disease. 3) Patients who had received high-dose

vitamin D supplementation within the past 3 months. 4) Patients with pre-existing severe disability (modified Rankin Scale [mRS] score > 2) or a life expectancy of less than 1 year. 5) Patients who were pregnant or lactating. 6) Patients who were anticipated to be unable to complete the standard follow-up procedures at our institution. This study was approved by the Second Affiliated Hospital of Harbin Medical University´s Ethics Committee.

Baseline characteristics and variables

Demographic and clinical information were recorded upon admission, including age, gender, body mass index (BMI), current smoking status (yes/no), hypertension, diabetes mellitus, atrial fibrillation, hypercholesterolemia, and history of coronary heart disease. Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS; total score range 0 - 42) [24]. All patients underwent MRI and/or CT. Lesion laterality was independently determined by qualified stroke neurologists according to standardized imaging interpretation protocols, and etiological subtypes were classified based on the TOAST criteria [25]. The pre-stroke use of antiplatelet or anticoagulant medications (yes/no) was recorded, as well as acute phase treatment modalities, including intravenous thrombolysis (IVT) and mechanical thrombectomy (MT) (yes/no).

Laboratory measurements

Baseline blood sampling was performed after admission and within 72 hours of symptom onset, following the hospital's standard operating procedures. Blood samples were collected via peripheral venipuncture or an indwelling venous catheter. A 2 mL sample of dipotassium EDTA (K_2EDTA)-anticoagulated whole blood was used for complete blood count analysis, performed on a Beckman Coulter hematology analyzer (Beckman Coulter, USA). An additional 3 - 5 mL of blood was collected in a serum separator tube (SST) for biochemical and immunological testing. After complete clotting, the SST samples were centrifuged at 1,500 × g for 10 minutes at room temperature to separate the serum. Subsequent testing and quality control were performed on a Beckman Coulter fully automated immunoassay platform (Beckman Coulter, USA) according to the manufacturer's instructions. The laboratory tests covered routine hematological, biochemical, and immunological parameters, including hs-CRP, 25(OH)D, fasting plasma glucose (FPG), and low-density lipoprotein cholesterol (LDL-C). All results were included as baseline values for subsequent statistical analysis.

TCD measurements and PI

All patients underwent TCD ultrasonography within 72 hours of admission, performed by experienced operators under standardized conditions. A transcranial Doppler (TCD) ultrasound system (EMS-9A, Delica, China) was used to obtain spectral signals from the M1 segment of both middle cerebral arteries via the temporal window (standard depth 50 - 65 mm). With uniform gain, filter, and sample volume settings, at least three clear waveform segments (each lasting at least 4 - 6 seconds) were acquired from each side. Angle correction was applied when necessary, and all signals were visually reviewed to exclude artifacts and interference. The peak systolic velocity (PSV), end-diastolic velocity (EDV), and mean flow velocity [MFV = (PSV + 2 × EDV)/3] were recorded, and the PI was calculated as PI = (PSV - EDV)/MFV.

PImax was pre-defined as the maximum side-based PI, prioritizing the affected side, according to the following rules: 1) The maximum PI value from all valid waveforms on a given side was taken as the representative value for that side. 2) If the affected side could be determined, its representative value was used as the analysis variable PImax. 3) If the affected side could not be determined, the higher representative value from the left and right sides was used as PImax. 4) If signals were obtainable from only one side, the PI from that side was used as PImax. Cases where no valid spectra were obtained beyond 72 hours were recorded as missing and were excluded. Systolic blood pressure, diastolic blood pressure, heart rate, and body temperature were recorded simultaneously during the examination.

Outcomes and follow-up

The primary outcome was a new ischemic stroke event within 12 months, defined as a new vascular-related focal neurological deficit lasting > 24 hours and confirmed by clinical and imaging evidence. If a patient experienced multiple recurrent events, the first recurrence was defined as the event timepoint. If a patient died after a recurrence, the case was still classified as an event, with the event time being the date of the first recurrence. If a patient died without a prior recurrence, the data were censored at the last follow-up date in the primary outcome analysis. A uniform follow-up plan was established for all patients after admission. Outcome information was obtained through outpatient visits or telephone interviews at 90 days and 12 months post-discharge, with relevant medical evidence verified. If outcome information was unavailable, patients were censored at the date of their last follow-up.

Statistical analysis

All data analyses were performed using R software version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria). Continuous variables were presented as mean ± standard deviation or median (interquartile range) based on the normality test results, with group comparisons conducted using independent samples *t*-test or Mann-Whitney U test, as appropriate. Categorical variables were expressed as frequency (percentage) and were compared using the chi-squared test or Fisher's exact test.

Baseline variables of all enrolled patients were compared between groups to identify potential recurrence-related factors. Candidate variables showing statistically sig-

nificant differences ($p < 0.05$) were further incorporated into Cox proportional hazards models. Continuous variables were entered into the models after Z-score standardization. To ensure consistent directionality in the analysis, 25(OH)D values were inverted (-VitD) to represent the risk effect per 1 SD decrease. Univariate Cox models were used for initial screening of significant variables ($p < 0.05$), which were then included in multivariable models for adjusted analysis. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated. The proportional hazards assumption was verified using Schoenfeld residual tests.

Model differentiation ability was assessed using Harrell's C-index and the area under the receiver operating characteristic curve (AUC). Classification performance was described using sensitivity, specificity, false positive rate (FPR), false negative rate (FNR), and the Youden index. To further validate model stability, the calibration-in-the-large (CITL), calibration slope, and Brier score were calculated, and calibration curves were plotted. Internal validation was performed using bootstrap resampling with 500 iterations. Optimism-corrected calibration metrics (Brier score and calibration slope with 95% confidence intervals) were calculated to assess model stability. As a sensitivity analysis, a Fine–Gray subdistribution hazards model was additionally fitted, treating recurrent ischemic stroke as the event of interest and death without prior recurrence as the competing event. Clinical net benefit was evaluated using decision curve analysis (DCA). Kaplan-Meier survival analysis and Log-rank tests were performed for key continuous indicators. All tests were two-sided, and a p-value < 0.05 was considered statistically significant. This manuscript was written without any artificial Intelligence assistance.

RESULTS

Baseline characteristics

A total of 409 patients with first-ever AIS were enrolled, out of whom 66 experienced recurrence within 12 months. No patients were lost to follow-up. During the 12-month follow-up, 22 deaths occurred, including 20 deaths without prior recurrence and 2 deaths after recurrence. No outcome data were missing for recurrence status, death status, or event timing. Compared with the non-recurrence group, the recurrence group was older (62.99 ± 7.04 vs. 60.61 ± 6.46 years, $p = 0.013$), had higher admission NIHSS scores (7.50 ± 4.44 vs. 5.62 ± 3.65, $p = 0.002$), higher PImax (1.29 ± 0.21 vs. 1.11 ± 0.22, $p < 0.001$), lower 25(OH)D levels (33.63 ± 18.09 vs. 48.38 ± 19.20 nmol/L, $p < 0.001$), and significantly elevated hs-CRP levels (3.27 [2.07-4.36] vs. 1.85 [1.12-3.05], $p < 0.001$). No statistically significant differences were observed in other variables, including heart rate, SBP, DBP, BMI, FPG, and LDL-C (all $p > 0.05$). Medical histories including smoking, hypertension, diabetes, atrial fibrillation, dyslipidemia, and coronary heart disease, as well as pre-admission antithrombotic therapy and MT rates, also showed no significant differences (all $p > 0.05$). The IVT rate was lower in the recurrence group (4.5% vs. 13.4%), demonstrating a marginal difference ($p = 0.068$). The distribution of TOAST subtypes was similar between the two groups ($p = 0.807$). Regarding lesion laterality, the recurrence group had slightly higher proportions of bilateral or unidentifiable laterality (19.7% and 18.2% vs. 13.1% and 10.2%, respectively), with an overall marginal difference ($p = 0.093$). No significant difference was found in MFV (51.09 ± 11.31 vs. 49.26 ± 11.69 cm/s, $p = 0.235$).

Cox regression analysis

Based on the baseline differences between the recurrence and non-recurrence groups shown in Table 1, age, NIHSS score, ln(hs-CRP), 25(OH)D (per 1-SD decrease), and PImax were included in the Cox analysis. The univariate model showed that all five indicators were significantly associated with 12-month recurrence: a per 1-SD increase in age (HR = 1.393, 95% CI: 1.090 - 1.779, $p = 0.008$), a 1-SD increase in NIHSS score (HR = 1.599, 95% CI: 1.260 - 2.029, $p < 0.001$), a 1-SD increase in ln(hs-CRP) (HR = 2.104, 95% CI: 1.607 - 2.755, $p < 0.001$), and a 1-SD increase in PImax (HR = 2.317, 95% CI: 1.778 - 3.021, $p < 0.001$) all increased the recurrence risk; similarly, a 1-SD decrease in 25(OH)D also increased the recurrence risk (HR = 2.174, 95% CI: 1.648 - 2.867, $p < 0.001$) (Table 2).

In the multivariable model, these associations remained independent: PImax (HR = 2.53, 95% CI: 1.92 - 3.34, $p < 0.001$), ln(hs-CRP) (HR = 2.38, 95% CI 1.75 - 3.24, $p < 0.001$), and a 1-SD decrease in 25(OH)D (HR = 2.21, 95% CI: 1.68 - 2.91, $p < 0.001$) were strong independent factors for recurrence; age (HR = 1.30, 95% CI 1.01 - 1.67, $p = 0.039$) and NIHSS score (HR = 1.40, 95% CI: 1.11 - 1.76, $p = 0.004$) also remained significant (Figure 1). All HRs were based on standardized variables, representing per 1-SD increase for all except 25(OH)D, which represents per 1-SD decrease. The model demonstrated good differentiation ability (C-index = 0.848).

In a Fine-Gray competing-risk sensitivity analysis treating death without prior recurrence as the competing event, the main associations remained materially unchanged (Supplementary Table S1). In the 5-variable model, PImax remained independently associated with recurrence (SHR = 2.53, 95% CI 1.94 - 3.30, $p < 0.001$), as did ln(hs-CRP) (SHR = 2.37, 95% CI 1.78 - 3.16, $p < 0.001$) and lower 25(OH)D levels modeled per 1-SD decrease (SHR = 2.20, 95% CI 1.67 - 2.90, $p < 0.001$). Age (SHR = 1.30, 95% CI 1.02 - 1.64, $p = 0.032$) and NIHSS score (SHR = 1.40, 95% CI 1.11 - 1.76, $p = 0.005$) also showed directionally consistent effects.

Model differentiation ability and threshold performance

In predicting 12-month recurrence, the single-indicator models yielded the following AUC values: PImax 0.720, ln(hs-CRP) 0.714, and (-)VitD 0.703. The multi-

Table 1. Baseline characteristics by 12-month recurrence status.

Variable	Total (n = 409)	Non-recurrence (n = 343)	Recurrence (n = 66)	p-value
Age (years)	61.00 ± 6.61	60.61 ± 6.46	62.99 ± 7.04	0.013
Gender (male), n (%)	220 (53.8%)	178 (51.9%)	42 (63.6%)	0.106
Heart rate (bpm)	76.99 ± 12.21	77.25 ± 11.92	75.63 ± 13.65	0.370
SBP (mmHg)	149.72 ± 23.71	149.47 ± 23.84	151.03 ± 23.12	0.618
DBP (mmHg)	85.08 ± 14.29	84.99 ± 14.37	85.58 ± 13.96	0.756
BMI (kg/m²)	29.18 ± 5.03	29.17 ± 5.19	29.20 ± 4.14	0.957
NIHSS	5.92 ± 3.85	5.62 ± 3.65	7.50 ± 4.44	0.002
FPG (mmol/L)	7.25 ± 3.22	7.15 ± 3.26	7.75 ± 2.95	0.139
LDL-C (mmol/L)	2.96 ± 1.02	2.96 ± 1.01	2.95 ± 1.07	0.947
25(OH)D (nmol/L)	46.00 ± 19.77	48.38 ± 19.20	33.63 ± 18.09	< 0.001
PImax	1.13 ± 0.23	1.11 ± 0.22	1.29 ± 0.21	< 0.001
MFV	49.55 ± 11.64	49.26 ± 11.69	51.09 ± 11.31	0.235
hs-CRP (mg/L)	2.05 [1.22 - 3.25]	1.85 [1.12 - 3.05]	3.27 [2.07 - 4.36]	< 0.001
Current smoking (yes), n (%)	60 (14.7%)	48 (14.0%)	12 (18.2%)	0.490
Hypertension history (yes), n (%)	252 (61.6%)	212 (61.8%)	40 (60.6%)	0.964
Diabetes history (yes), n (%)	68 (16.6%)	52 (15.2%)	16 (24.2%)	0.102
Atrial fibrillation history (yes), n (%)	47 (11.5%)	40 (11.7%)	7 (10.6%)	0.972
Dyslipidemia history (yes), n (%)	175 (42.8%)	146 (42.6%)	29 (43.9%)	0.944
Coronary heart disease history (yes), n (%)	54 (13.2%)	47 (13.7%)	7 (10.6%)	0.630
Pre-stroke antiplatelet therapy (yes), n (%)	147 (35.9%)	126 (36.7%)	21 (31.8%)	0.534
Pre-anticoagulant therapy (yes), n (%)	26 (6.4%)	23 (6.7%)	3 (4.5%)	0.782
Intravenous thrombolysis (IVT, yes), n (%)	49 (12.0%)	46 (13.4%)	3 (4.5%)	0.068
Mechanical thrombectomy (MT, yes), n (%)	43 (10.5%)	37 (10.8%)	6 (9.1%)	0.847
TOAST classification, n (%)				
CE	135 (33.0%)	109 (31.8%)	26 (39.4%)	0.807
LAA	131 (32.0%)	113 (32.9%)	18 (27.3%)	
SVO	70 (17.1%)	59 (17.2%)	11 (16.7%)	
Other	32 (7.8%)	27 (7.9%)	5 (7.6%)	
Unknown	41 (10.0%)	35 (10.2%)	6 (9.1%)	
Lesion laterality, n (%)				
Left	155 (37.9%)	135 (39.4%)	20 (30.3%)	0.093
Right	149 (36.4%)	128 (37.3%)	21 (31.8%)	
Bilateral	58 (14.2%)	45 (13.1%)	13 (19.7%)	
Unclear	47 (11.5%)	35 (10.2%)	12 (18.2%)	

Continuous variables are expressed as mean ± SD or median [IQR], categorical variables as n (%). Between-group comparisons were performed using the independent-samples *t*-test for normally distributed data, the Mann-Whitney U test for non-normally distributed data, and the chi-squared or Fisher's exact test for categorical variables. A two-sided $p < 0.05$ was considered statistically significant.
SBP: Systolic blood pressure, DBP: Diastolic blood pressure, BMI: Body mass index, NIHSS: National institutes of health stroke scale, FPG: Fasting plasma glucose, LDL-C: Low-density lipoprotein cholesterol, 25(OH)D: 25-hydroxyvitamin D, PImax: pulsatility index with affected-side preference, MFV: mean flow velocity, hs-CRP: High-sensitivity C-reactive protein, IVT: Intravenous thrombolysis, MT: Mechanical thrombectomy, TOAST: Trial of org 10172 in acute stroke treatment, LAA: large-artery atherosclerosis, CE: cardioembolism, SVO: small-vessel occlusion.

Table 2. Univariate Cox regression for 12-month recurrence.

Variable	Unit	Scaling	HR	95% CI	p-value
Age	years	per SD increase	1.393	1.090 - 1.779	0.008
NIHSS score	—	per SD increase	1.599	1.260 - 2.029	< 0.001
25(OH)D	nmol/L	per SD decrease	2.174	1.648 - 2.867	< 0.001
hs-CRP (ln-transformed)	mg/L	per SD increase	2.104	1.607 - 2.755	< 0.001
PImax	—	per SD increase	2.317	1.778 - 3.021	< 0.001

All variables were standardized by SD units. The entry "25(OH)D per SD decrease" reflects the hazard associated with lower circulating vitamin D levels. Hazard ratios (HRs) are presented with 95% confidence intervals (CIs). A two-sided $p < 0.05$ was considered statistically significant.

Table 3. Differentiation and threshold-based performance of candidate models for 12-month recurrence.

Model	AUC	Cutoff	Sensitivity	Specificity	FPR	FNR	Youden
PImax	0.720	1.28	0.515	0.808	0.192	0.485	0.323
ln(hs-CRP)	0.714	2.51	0.682	0.650	0.350	0.318	0.332
(-)VitD	0.703	41.20	0.667	0.641	0.359	0.333	0.308
3-marker	0.869	0.78	0.833	0.787	0.213	0.167	0.621
5-marker	0.875	0.54	0.848	0.726	0.274	0.152	0.574

Optimal cutoffs were selected according to Youden's index from the ROC curve. Performance metrics comprise sensitivity, specificity, false-positive rate (FPR = 1 - specificity), false-negative rate (FNR = 1 - sensitivity), and Youden's index (sensitivity + specificity - 1). Model definetions correspond to those presented in Figure 2.
Cutoff interpretation: Single-marker cutoffs are reported in original clinical units for direct application: PImax ≥ 1.28 (dimensionless ratio), hs-CRP ≥ 2.5 mg/L, and 25(OH)D ≤ 41.2 nmol/L (approximately 16.5 ng/mL) indicate elevated recurrence risk. Multi-marker model cutoffs (0.78 for 3-marker, 0.54 for 5-marker) represent predicted 12-month recurrence risk probabilities (0 - 1 scale). Patients exceeding these thresholds should be considered for intensified secondary prevention.
AUC: Area under the curve, ROC: Receiver operating characteristic, PImax: Pulsatility index with affected-side preference, NIHSS: National institutes of health stroke scale.

indicator models achieved AUCs of 0.869 for the three-indicator model and 0.875 for the five-indicator model (Figure 2). By using the threshold determined by the Youden index, the three-indicator model demonstrated a sensitivity of 0.833 and a specificity of 0.787 (FPR 0.213, FNR 0.167, Youden index 0.621). The five-indicator model showed a sensitivity of 0.848 and a specificity of 0.726 (FPR 0.274, FNR 0.152, Youden index 0.574) (Table 3). Overall, both the three-indicator model combining vitamin D, hs-CRP, and cerebral hemodynamic parameters and the five-indicator model demonstrated high differentiation ability and good classification performance for predicting mid- to long-term ischemic stroke recurrence.

KM stratification analysis

To validate the risk stratification ability of key biomarkers, Kaplan-Meier analyses stratified by quartiles of (-)VitD, ln(hs-CRP), and PImax were performed (Figure 3). All three biomarkers showed consistent dose-response trends, with the recurrence-free survival curves for the higher quartile groups showing consistently lower survival (all Log-rank $p < 0.001$). Specifically, the highest quartile of (-)VitD (representing the lowest 25(OH)D levels) was associated with the highest 12-month recurrence rate, followed by the high quartile groups of hs-CRP and PImax. This suggests that vitamin D deficiency, inflammatory activation, and elevated cerebrovascular resistance are all associated with an increased recurrence risk. Regarding the combined modeling, grouping based on tertiles of the linear predictor (LP) from the five-indicator multivariable Cox model showed clear curve separation. The high-risk group exhibited event accumulation earlier in follow-up, with a significantly higher recurrence rate at 12 months compared to the low-risk group (Log-rank $p < 0.001$). This stratification performance is consistent with the direction of the multivariable regression and ROC results, supporting the robustness and clinical value of combined detection for assessing mid- to long-term recurrence risk.

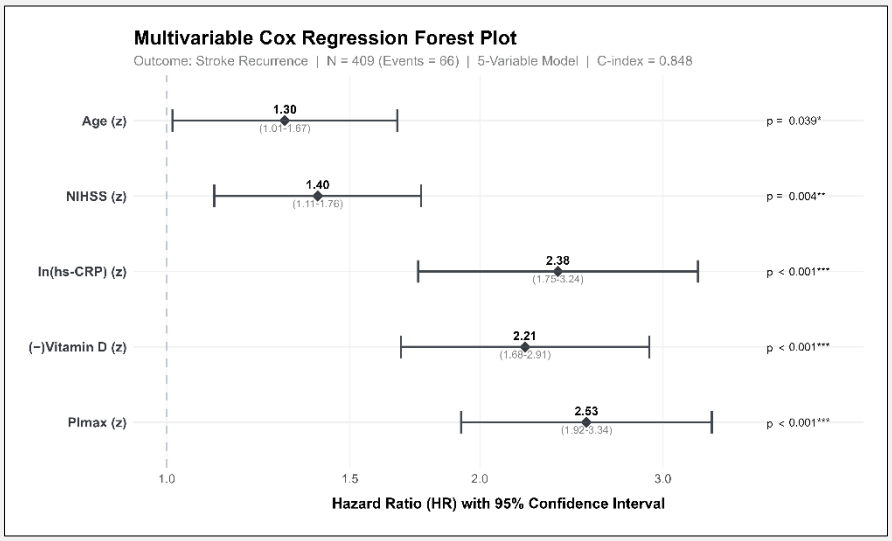


Figure 1. Multivariable Cox regression for 12-month recurrence.

HRs correspond to a 1-SD change for all covariates, (-)Vitamin D indicates a per-1-SD decrease in 25(OH)D. The multivariable model included age, NIHSS score, ln(hs-CRP), (-)Vitamin D, and PImax (n = 409; events = 66).

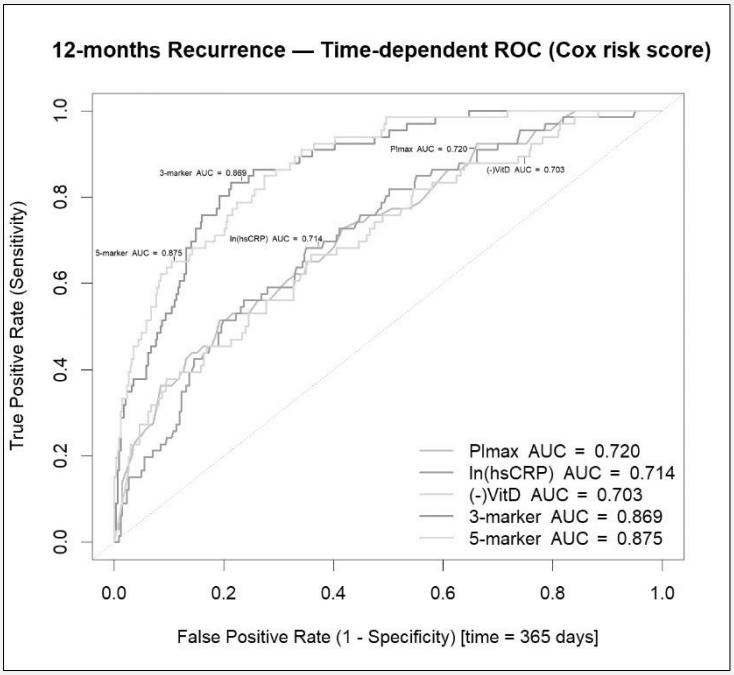


Figure 2. Time-dependent ROC curves for 12-month recurrence (Cox risk score).

Evaluation was performed at 365 days. Models include: PImax (single marker), ln(hs-CRP) (single marker), (-)VitD [25(OH)D per 1-SD decrease], 3-marker model [PImax + ln(hs-CRP) + 25(OH)D], and 5-marker model [3-marker plus age and NIHSS].
ROC: Receiver operating characteristic, AUC: area under the curve, NIHSS: National institutes of health stroke scale, PImax: Pulsatility index with affected-side preference.

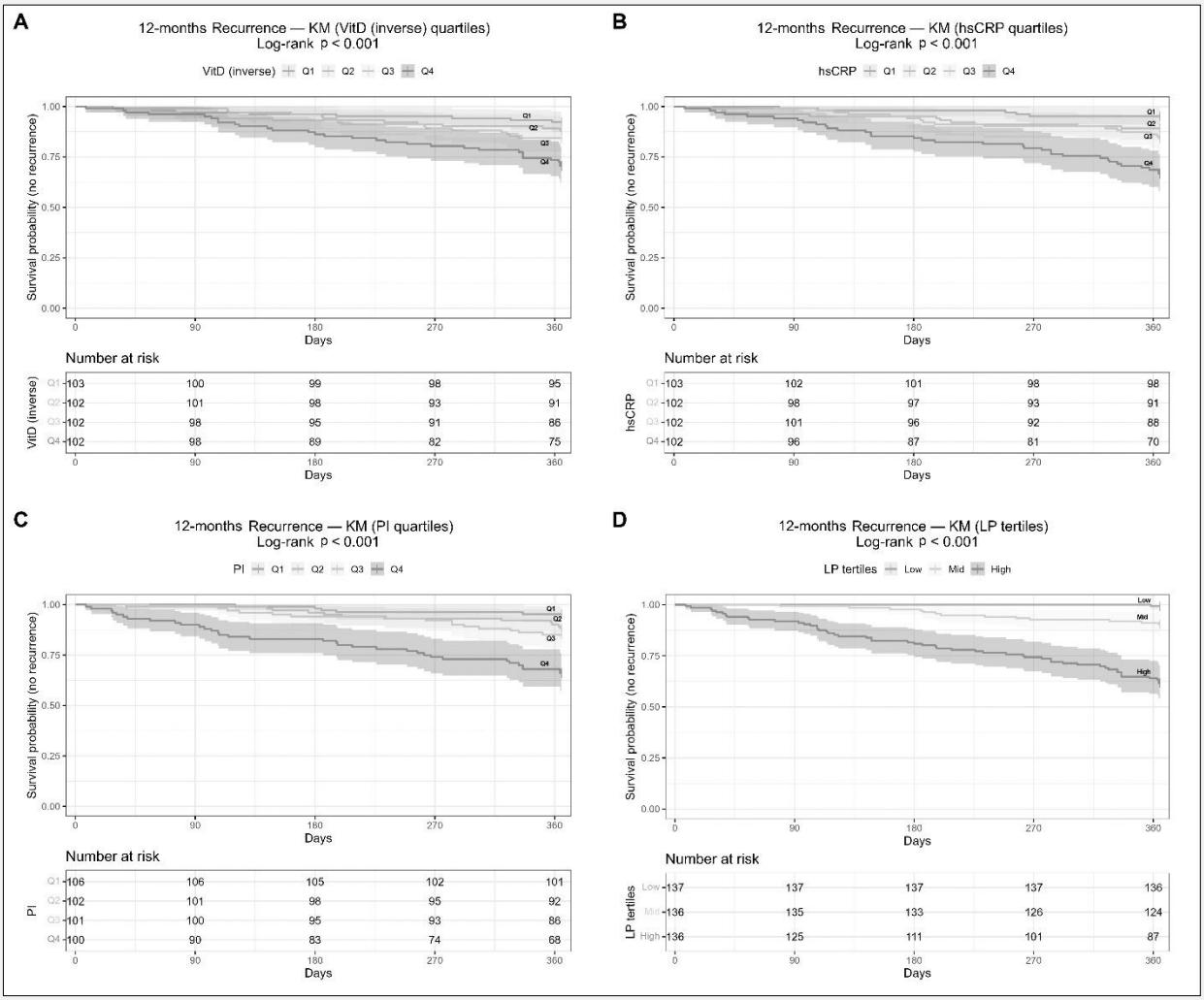


Figure 3. Kaplan-Meier curves for 12-month recurrence: single-marker quartiles and five-marker model risk tertiles.

A: Inverse vitamin D [(-)VitD] stratified by quartiles [higher values correspond to lower 25(OH)D].
B: hs-CRP stratified by quartiles (log-transformed and standardized).
C: PImax stratified by quartiles.
D: LP from the five-marker Cox model (25(OH)D, hs-CRP, PImax, age, NIHSS) stratified by tertiles (low, mid, high risk).
The horizontal axis shows time (days), the vertical axis represents recurrence-free survival probability. Shaded bands indicate 95% confidence intervals, numbers at risk are provided below each panel. Log-rank p < 0.001 for all comparisons.

Model calibration and decision analysis

To further evaluate the predictive stability and clinical application value of the three core indicators and their extended models, calibration and decision curves were plotted for both the three-indicator model (combining 25(OH)D, hs-CRP, and PImax) and the five-indicator model (further adjusted for traditional clinical variables including age and NIHSS score) (Figure 4). The predicted probabilities from both models demonstrated good agreement with the actual recurrence rates. The calibration slopes were both close to 1.0, with CITL values of 0.08 and 0.01, and Brier scores of 0.096 and 0.091, respectively, indicating good model calibration (Figure 4A).

Internal validation using 500 bootstrap iterations demonstrated minimal optimism. For the three-indicator model, the optimism-corrected Brier score was 0.099 (95% CI: 0.079 - 0.121) and calibration slope was 0.966 (95% CI: 0.729 - 1.235). The five-indicator model showed similar stability with optimism-corrected Brier

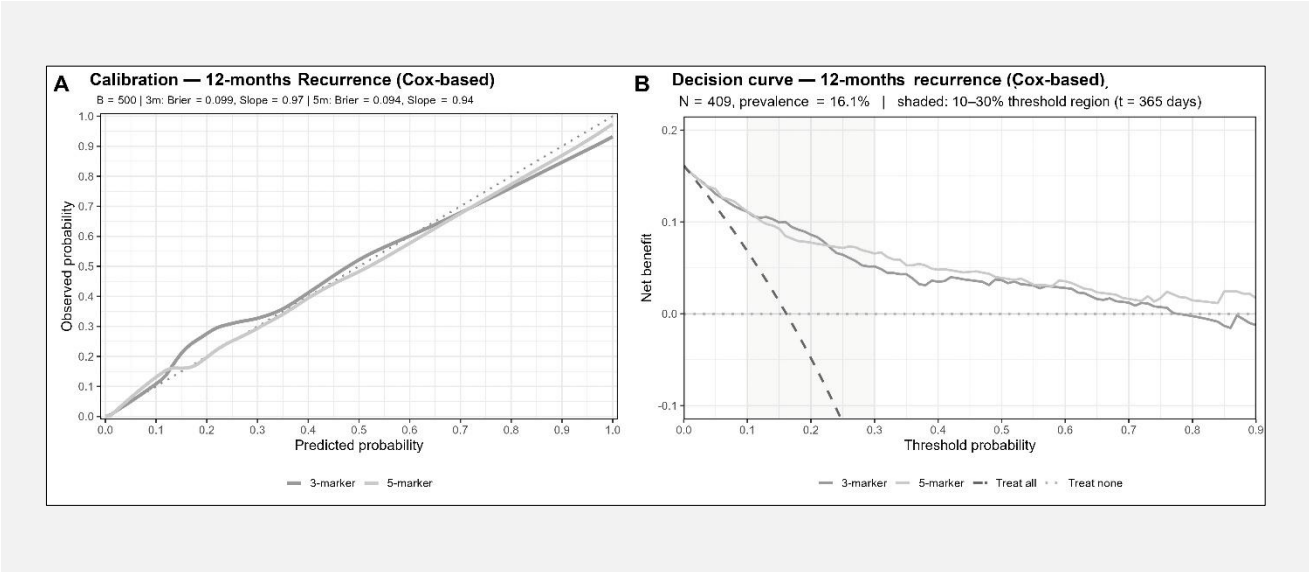


Figure 4. Calibration and decision analysis for 12-month recurrence.

A: Calibration curves for the 3-marker and 5-marker Cox models, showing agreement between predicted and observed probabilities (Brier score = 0.096 and 0.091; CITL = 0.08 and 0.01; slope = 1.0 for both).
B: DCA indicating greater net benefit for both models across threshold probabilities of 10% - 30%, compared to "treat all" or "treat none" strategies.

score of 0.094 (95% CI: 0.073 - 0.116) and calibration slope of 0.944 (95% CI: 0.732 - 1.178), confirming robust predictive performance with minimal overfitting.
In the DCA, both the three-indicator and five-indicator models demonstrated significant net benefit within the threshold probability range of 10% - 30% (which represents the clinically acceptable range for recurrence risk management), offering higher clinical utility compared to the "treat all" and "treat none" strategies (Figure 4B). Overall, the combined detection of vitamin D, hs-CRP, and the cerebral hemodynamic parameter PI demonstrated good differentiation ability and calibration performance for predicting recurrence risk, and its extended model further exhibited enhanced stability and generalizability.

DISCUSSION

This study included 409 patients with first-ever AIS. During the 12-month follow-up, low serum 25(OH)D levels, elevated hs-CRP, and elevated maximum PImax on the affected side were identified as independent risk factors for stroke recurrence. Multivariable Cox regression analysis demonstrated that these three indicators remained statistically significant after adjustment for traditional clinical variables such as age and NIHSS score (all $p < 0.05$). The combined prediction model based on 25(OH)D, hs-CRP, and PImax, also adjusted for age and NIHSS score, showed a C-index of 0.848 and an area under the ROC curve of 0.875, indicating good differentiation ability and clinical applicability.

Previous cohort studies have confirmed that vitamin D deficiency is associated with the risk of first-ever ischemic stroke and poor prognosis [15,16]. A dose-response meta-analysis by Vergatti et al. found that patients with 25(OH)D < 8.5 ng/mL (equivalent to approximately 21.25 nmol/L; conversion factor: 1 ng/mL = 2.5 nmol/L) at the time of the first stroke had a significantly increased risk of recurrence, while those with levels ≥ 9.3 ng/mL (approximately 23.25 nmol/L) had a reduced risk [26]. The findings of this study are consistent: the recurrence group had significantly lower 25(OH)D levels than the non-recurrence group ($p < 0.001$), suggesting that vitamin D deficiency may be involved in the occurrence of recurrent cerebrovascular events. Mechanistically, vitamin D influences vascular homeostasis by regulating multiple signaling pathways: it inhibits NADPH oxidase-mediated reactive oxygen species generation, thereby improving endothelial function, and simultaneously reduces the release of pro-inflammatory factors by suppressing the NF-κB pathway, participating in vascular pathophysiology [19,27]. Furthermore, vitamin D is involved in regulating calcium homeostasis in vascular smooth muscle cells, affecting cerebrovascular autoregulation [28]. Multiple studies have confirmed that low vitamin D levels are associated with stroke severity in the acute phase and poor early functional recovery, suggesting its potential influence on recurrence risk through long-term vascular remodeling and immunomodulatory mechanisms [29-31].
This study found that elevated hs-CRP levels were significantly associated with 12-month recurrence and maintained independent predictive value in multivari-

able analysis. As a sensitive marker of systemic inflammation, hs-CRP not only reflects the body's vascular inflammatory burden but also directly participates in vascular injury processes. Studies indicate that hs-CRP can promote plaque destabilization by inhibiting eNOS expression, promoting endothelial cell apoptosis, and enhancing platelet aggregation [18]. Multiple meta-analyses have confirmed that elevated hs-CRP is significantly associated with mortality, recurrence, and poor functional outcomes in ischemic stroke patients [20,21,32]. Research has shown that incorporating hs-CRP into prediction models significantly improves the predictive efficacy for recurrence in AIS [33]. The results of this study support hs-CRP as an effective biomarker for risk stratification in secondary prevention, with its elevation potentially indicating a persistent state of inflammatory activation, offering a potential target for anti-inflammatory therapy.

The PI is an important parameter in TCD measurements, reflecting cerebrovascular resistance and compliance. This study used the maximum PImax on the affected side as an observation indicator and found its increase to be independently associated with recurrence (HR = 2.53, $p < 0.001$). Previous studies have confirmed that an elevated PI is associated with poor functional outcomes after stroke and the burden of cerebral small vessel disease [34-36]. An increased PI typically suggests decreased small artery compliance and increased microcirculatory resistance [36]. Reduced vascular compliance is closely related to endothelial dysfunction and vascular wall remodeling. Under chronic inflammatory conditions, oxidative stress and inflammatory factors can promote vascular smooth muscle cell proliferation and extracellular matrix deposition, leading to increased arterial stiffness [23]. This alteration in vascular function reduces the reserve capacity of cerebrovascular autoregulation, making local brain tissue more susceptible to hypoperfusion during blood pressure fluctuations or inadequate collateral circulation [34]. Furthermore, some studies have shown that an elevated PI is associated with the degree of intracranial arterial stenosis and plaque instability, potentially reflecting the atherosclerotic burden and the extent of vascular remodeling [35]. Therefore, PImax, as a non-invasive hemodynamic indicator, may comprehensively reflect cerebrovascular structural and functional changes and the state of microcirculatory impairment.

The combined prediction model integrating metabolic (25(OH)D), inflammatory (hs-CRP), and hemodynamic (PImax) indicators achieved a C-index of 0.848, which was significantly superior to any single indicator (all $p < 0.05$). DCA evaluated clinical utility by quantifying net benefit across different risk thresholds. The threshold probability represented the minimum predicted risk at which a clinician would initiate intervention (e.g. a 10% threshold meant treating only patients with ≥ 10% predicted recurrence risk), while net benefit accounted for the trade-off between correctly identifying high-risk patients and unnecessarily treating low-risk patients. DCA showed that both the 3-marker and 5-marker models provided positive net benefit, superior to both "treat all" and "treat none" strategies, within the clinically relevant 10% - 30% threshold range. All indicators can be obtained through routine laboratory tests and TCD, procedures that are cost-effective and straightforward, indicating feasibility for implementation across different levels of healthcare institutions.

For clinical application, individual biomarker values (PImax, hs-CRP, and 25(OH)D) are put into the Cox model equation to calculate a predicted 12-month recurrence probability. Patients with predicted probabilities exceeding 0.78 (3-marker model) or 0.54 (5-marker model including age and NIHSS) are classified as high-risk and should be prioritized for intensive secondary prevention strategies.

This model enables baseline risk assessment during the acute phase of stroke, providing an objective basis for the early identification of high-risk patients. For high-risk populations, multidimensional intervention strategies such as vitamin D supplementation, intensified anti-inflammatory therapy, and optimized blood pressure management can be considered, thereby facilitating the rational allocation of medical resources and recurrence risk control. The model integrates key pathways of metabolism, inflammation, and hemodynamics, providing a theoretical basis for multi-target combined interventions.

Future research should validate the model's external validity through multicenter prospective cohorts. Serial measurements of vitamin D at multiple timepoints could help distinguish causal effects of vitamin D deficiency from transient acute-phase responses and elucidate whether dynamic changes in vitamin D levels during stroke recovery influence recurrence risk. Additionally, investigating the association between pre-stroke functional status, mobility indices, and vitamin D levels could better elucidate whether vitamin D deficiency represents a causal risk factor or a marker of general health status. Integrating high-resolution vessel wall imaging, CT perfusion-based radiomics, and metabolomics technologies could further elucidate the interactive mechanisms of the vitamin D-inflammation-cerebral hemodynamics axis. Interventional studies should focus on evaluating the dynamic effects of vitamin D supplementation and anti-inflammatory therapies on cerebral blood flow parameters. Furthermore, introducing machine learning algorithms to integrate more clinical and imaging biomarkers holds promise for optimizing the model's predictive performance and promoting its systematic application in stroke management.

This study has several limitations. First, as a single-center cohort, generalizability may be limited by institutional differences. Second, unmeasured confounding and potential bias from informative censoring cannot be entirely excluded. Third, biomarker measurements at a single baseline timepoint may not fully capture individual variability; we did not perform serial measurements to assess temporal changes, and seasonal variation in vi-

tamin D levels was not accounted for. Finally, external validation in independent cohorts would further confirm the model's generalizability.

CONCLUSION

This study confirms that vitamin D deficiency, systemic inflammation activation, and elevated cerebrovascular resistance are independently associated with mid- to long-term recurrence of ischemic stroke. The combined detection model based on 25(OH)D, hs-CRP, and PI-max demonstrates high differentiation ability in predicting recurrence risk. Integrating metabolic, inflammatory, and hemodynamic information can effectively identify high-risk populations for stroke recurrence, providing a basis for individualized risk stratification and precise secondary prevention.

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All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All subjects were approved by the Second Affiliated Hospital of Harbin Medical University.

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