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Plasma endotoxin level independently predicts unfavorable 90-day outcome in acute ischemic stroke patients with type 2 diabetes mellitus

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RESEARCH ARTICLE

ABSTRACT

The prognostic utility of plasma endotoxemia, marked by elevated lipopolysaccharide (LPS) and often associated with gut barrier dysfunction, remains undefined in acute ischemic stroke (AIS) patients with type 2 diabetes. In this single-center prospective cohort of 300 AIS patients with diabetes, we measured early admission plasma LPS and assessed its correlation with stroke severity and inflammatory markers, and its independent association with 90-day unfavorable functional outcome (modified Rankin Scale 3–6). Compared to the good outcome group, patients with poor outcomes had significantly higher LPS levels. Log-transformed LPS levels positively correlated with baseline NIHSS score, high-sensitivity C-reactive protein, and white blood cell count. After multivariable adjustment for age, NIHSS, HbA1c, atrial fibrillation, estimated glomerular filtration rate, and other covariates, each standard deviation increase in log-LPS was independently associated with an increased risk of 90-day unfavorable outcome (odds ratio 1.86, 95% CI 1.39–2.48). A clear dose-response relationship was observed, with the risk of poor outcome and mortality rising across LPS quartiles. Adding LPS to a basic clinical prediction model improved the area under the curve from 0.79 to 0.83, enhanced risk reclassification, and provided greater net clinical benefit across a range of decision thresholds. In conclusion, early admission plasma endotoxemia is an independent predictor of 90-day functional outcome in AIS patients with diabetes and improves conventional risk stratification. This finding positions LPS as a clinically accessible biomarker that is associated with poor recovery in acute ischemic stroke.

KEYWORDS

plasma lipopolysaccharide, endotoxemia, acute ischemic stroke, type 2 diabetes, prognosis, inflammation, functional outcome

1. INTRODUCTION

Acute ischemic stroke is one of the neurological diseases with the heaviest global burden of death and disability, and patients with concomitant type 2 diabetes have a higher risk of stroke onset, more severe disease, and significantly increased rates of 90-day unfavorable functional outcome and mortality [1, 2]. Traditional prognostic assessment of stroke is usually based on age, NIHSS score, blood pressure, glycemic control, and reperfusion therapy [3], but in this population with a high metabolic burden and inflammatory load, these indicators may not fully reflect the true risk [4].

In recent years, the gut microbiota and their metabolites have been considered important links connecting metabolism, immune inflammation, and cardio-cerebrovascular outcomes, among which endotoxemia resulting from endotoxins produced by intestinal bacteria entering the circulation is closely related to chronic low-grade inflammation, endothelial dysfunction, and the progression of atherosclerosis [5, 6]. Lipopolysaccharide (LPS), the predominant endotoxin in human circulation, is primarily derived from the cell walls of

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Gram-negative bacteria. In conditions like type 2 diabetes, gut dysbiosis and increased intestinal permeability are well-documented, potentially contributing to the translocation of microbial products, including LPS, into the circulation, a state often referred to as metabolic endotoxemia [7, 8]. However, it is important to acknowledge that LPS may also originate from other sources such as the oral cavity, respiratory tract, or subclinical infections.

Current studies on stroke prognosis mainly focus on traditional inflammatory markers such as CRP, white blood cells, or a few cytokines, and few have systematically evaluated the role of LPS in this metabolically high-risk population of acute stroke with diabetes [9]; whether LPS can independently predict 90-day unfavorable functional outcome and improve the discrimination and decision net benefit of clinical models still lacks sufficient prospective clinical evidence in this population. In this study, patients with acute ischemic stroke and concomitant type 2 diabetes were enrolled, early admission plasma LPS levels were prospectively measured, and NIHSS score and inflammatory markers were combined to evaluate the relationships of plasma endotoxemia (LPS) with stroke severity and systemic inflammation; furthermore, multivariable models were used to explore the independent predictive effect and dose-response relationship of LPS on 90-day unfavorable functional outcome, and to analyze the incremental value of LPS for improving the discrimination, reclassification, and decision net benefit of clinical prediction models, with the aim of providing clinical evidence for stroke risk stratification and potential interventions from the perspective of microbe-host immune interactions.

2. MATERIALS AND METHODS

2.1. Study design

This study was a single-center, prospective, observational cohort study, and the enrollment period was from March 1, 2024 to February 28, 2025. It was conducted after approval by the hospital ethics committee (approval number: 2024-009), and all participants or their legal representatives signed written informed consent. The study objective was to evaluate the independent predictive value of early admission gut microbiome–derived endotoxemia levels for 90-day functional outcomes in acute ischemic stroke patients with concomitant diabetes, and to verify the dose-response relationship and the incremental value for clinical prediction models. The personnel responsible for endotoxin measurement and those responsible for outcome assessment were mutually blinded, and data management used pre-defined electronic case report forms and a double-check procedure.

2.2. Study population

Inclusion criteria: consecutive enrollment of participants who met the following conditions: age ≥18 years; acute ischemic stroke confirmed by imaging; time from onset to admission ≤24 h and time from onset to first blood sampling ≤24 h; previously clearly defined type 2 diabetes or glycated hemoglobin at admission ≥6.5%; baseline modified Rankin Scale (mRS) score ≤2 points [10]; signed informed consent. Exclusion criteria: participants with any of the following conditions were excluded: intracranial hemorrhagic stroke or subarachnoid hemorrhage; active infection within 7 days before admission requiring systemic antibacterial therapy or meeting the Sepsis-3 diagnostic criteria at admission; major surgery or multiple trauma within the past 1 month; inflammatory bowel disease, short bowel syndrome, or definite severe structural intestinal disease; decompensated liver cirrhosis or Child–Pugh class C hepatic insufficiency; end-stage renal disease receiving dialysis; active malignant tumor receiving systemic antitumor therapy; long-term immunosuppressive therapy (equivalent prednisone ≥10 mg day^{-1} for ≥2 weeks) or post-organ transplantation status; pregnancy or lactation; inability to complete the 90-day follow-up.

2.3. Baseline data and follow-up

2.3.1. Collection of baseline clinical and imaging data. After admission, age, sex, body mass index, history of hypertension, atrial fibrillation, coronary artery disease, and smoking were recorded, and systolic and diastolic blood pressure on the first day of admission were measured. Baseline NIHSS score was assessed by neurologists who had received uniform training [11]. All participants underwent cranial CT or MRI to confirm ischemic stroke, and CTA or MRA was performed when necessary to assess the presence of large vessel occlusion; imaging data were independently interpreted by two neuroradiologists, and discrepancies were adjudicated by a third senior physician.

2.3.2. Recording of diabetes- and stroke-related variables. After admission, diabetes duration, antidiabetic treatment regimen (with or without insulin use, with or without oral antidiabetic drugs), fasting plasma glucose on the first day of admission, glycated hemoglobin, estimated glomerular filtration rate, and lipid profile were recorded. Reperfusion therapy modalities, including intravenous thrombolysis and mechanical thrombectomy, were recorded, as well as the time from onset to treatment initiation.

2.3.3. Assessment of 90-day functional outcome. At 90 ± 7 days, mRS scores were assessed by evaluators who were blinded to the laboratory tests using standardized in-person visits or telephone follow-up [12], and all-cause mortality was recorded. To ensure consistency, 10% of the participants were randomly selected for reassessment by a second evaluator, and the Cohen κ coefficient was calculated to evaluate agreement. Participants for whom follow-up information could not be obtained were defined as lost to follow-up; in the main analysis, available-case analysis was performed, and in sensitivity analyses, missing outcomes were handled using multiple imputation.

2.4. Measurement of endotoxin and related biomarkers

2.4.1. Blood sample collection and processing procedures. The first venous blood sample was collected within 2 h after admission, and the time from onset to blood sampling did

not exceed 24 h. Peripheral venous blood was collected into pyrogen-free 3.2% sodium citrate anticoagulation tubes, gently inverted to mix, and transported on ice. Within 30 min from blood collection, plasma was separated by centrifugation at 1,500 g for 15 min at 4 °C, followed by centrifugation at 10,000 g for 10 min to prepare platelet-poor plasma. The plasma was aliquoted into pyrogen-free polypropylene cryovials according to predefined numbering and stored at −80 °C, and all measurements were completed after a single thawing at the time of testing. Specimens with visible hemolysis or severe lipemia were not included in the endotoxin testing process.

2.4.2. Methods for measuring endotoxin and inflammatory markers. Plasma endotoxin was quantified using a recombinant Factor C fluorescence assay (PyroGene™ Recombinant Factor C Endotoxin Detection System, Lonza Group Ltd., Basel, Switzerland) on a microplate reading system (Synergy H1, BioTek Instruments, Winooski, VT, USA), calibrated with a five-point standard curve (0.005–5.0 EU/mL). All samples were tested in duplicate wells, and those with a coefficient of variation exceeding 10% were retested [13]; samples beyond the linear range were remeasured after dilution at the specified multiple. Each plate included a blank control and low- and high-level quality control materials, and the acceptable ranges were determined jointly according to the manufacturer's quality control procedures and the laboratory's internal quality control scheme. Inflammatory markers included high-sensitivity C-reactive protein, which was measured by an immunoturbidimetric assay, and white blood cell count, which was measured using an automated hematology analyzer; differential leukocyte counts were reported according to standardized laboratory procedures.

2.4.3. Stratification of endotoxemia and definition of thresholds. In the statistical analysis, endotoxin was natural log-transformed and modeled as a continuous variable, and at the same time stratified by quartiles for comparison. The threshold for "high endotoxin" used for clinical discrimination was determined by the Youden index on ROC curves with 10-fold cross-validation, and this threshold was used to construct a binary variable for model comparison; the threshold was determined solely for statistical discrimination and did not replace modeling with endotoxin as a continuous variable.

2.5. Definition of observational indicators and outcome variables

The primary exposure variable was early admission plasma endotoxin level (EU/mL), log-transformed and standardized per standard deviation increment. Secondary exposure variables were high-sensitivity C-reactive protein and white blood cell count. The primary outcome was 90-day unfavorable functional outcome, defined as mRS 3–6 [14]; secondary outcomes included 90-day all-cause mortality and the 90-day mRS as an ordinal distribution. Baseline stroke severity was represented by the admission NIHSS score, and reperfusion therapy was represented by an indicator variable.

2.6. Statistical analysis

2.6.1. Sample size estimation and statistical software. The main analysis was based on multivariable Logistic regression to evaluate the independent association between endotoxin and 90-day unfavorable functional outcome. According to the empirical rule of events per parameter, 8 pre-specified covariates (age, sex, baseline NIHSS, systolic blood pressure, glycated hemoglobin, atrial fibrillation, estimated glomerular filtration rate, and reperfusion therapy) were planned to be included in the model, with at least 10 events per parameter as the lower limit. Based on the literature and historical data from our center, the proportion of 90-day unfavorable outcome in acute ischemic stroke patients with concomitant diabetes was expected to be approximately 35% [15]. Under this assumption, to ensure ≥80 events and to account for approximately 10% loss to follow-up, the target enrollment sample size was set at 300 cases, which would yield about 105 events, corresponding to ≥13 events per parameter [16]. Statistical analyses were performed using *R* 4.3.2, with a two-sided $\alpha = 0.05$ as the level of significance.

2.6.2. Descriptive analysis and between-group comparisons. After testing the distribution of continuous variables, they were described as mean and standard deviation or median and interquartile range, and categorical variables were described as frequency and percentage. The *t*-test or Mann–Whitney *U* test was used to compare continuous variables between two groups, and the χ^2 test or Fisher's exact test was used to compare categorical variables. To reduce the influence of extreme values, endotoxin was log-transformed and mildly Winsorized at the 1 and 99% percentiles; all comparisons reported two-sided *P* values and 95% confidence intervals.

2.6.3. Multivariable regression and analysis of dose–response relationship. The main model was a multivariable Logistic regression, in which log-transformed and standardized endotoxin was included as a continuous independent variable and the outcome was 90-day unfavorable functional outcome. Covariates were determined in advance based on biological plausibility and clinical importance and were entered into the model simultaneously, without data-driven stepwise selection. Multicollinearity was assessed using the variance inflation factor, and covariates were handled when VIF>5. Restricted cubic splines with knots at the 5th, 35th, 65th, and 95th percentiles were used to test the linearity assumption of the relationship between endotoxin and the outcome and to plot adjusted dose–response curves. Two sensitivity analyses were performed: (①) modeling the outcome as an ordinal mRS category using a proportional odds model; (②) excluding patients who died within 7 days after admission to assess the impact of early death. Missing data were handled using multiple imputation by chained

equations to generate 10 imputed datasets, and estimates were combined according to Rubin's rules.

2.6.4. Construction and performance evaluation of prediction models. First, a basic clinical model was constructed, including age, sex, baseline NIHSS, systolic blood pressure, glycated hemoglobin, atrial fibrillation, estimated glomerular filtration rate, and reperfusion therapy. Endotoxin was then added to the basic model, and the differences in performance between the two models in terms of discrimination, calibration, and reclassification were compared. Discrimination was evaluated using the area under the ROC curve, and differences in AUC were compared by the DeLong method; calibration was evaluated using calibration curves and the Brier score, and the calibration slope was reported; reclassification was evaluated using the category-free net reclassification index and integrated discrimination improvement. Internal validation was performed using 1,000 bootstrap resamples, and the optimism-corrected AUC and calibration slope were reported. To assess potential clinical net benefit, decision curve analysis was conducted and the changes in net benefit across a range of threshold probabilities were presented.

3. RESULTS

3.1. Study population and baseline characteristics

A total of 973 patients with acute ischemic stroke were screened, of whom 311 met the inclusion criteria and were enrolled. Among them, 300 patients completed endotoxin measurement and 90-day follow-up and were included in the analysis, while 11 patients were lost to follow-up (Fig. 1). Results of the Mann–Whitney U test and χ^2 test showed that, compared with the 90-day good functional outcome group, patients in the poor outcome group were older, had lower proportions of male sex and current smoking, higher proportions of hypertension and atrial fibrillation (AF), longer diabetes duration, more frequent insulin use, higher levels of fasting plasma glucose (FPG) and HbA1c, lower estimated glomerular filtration rate (eGFR), higher baseline

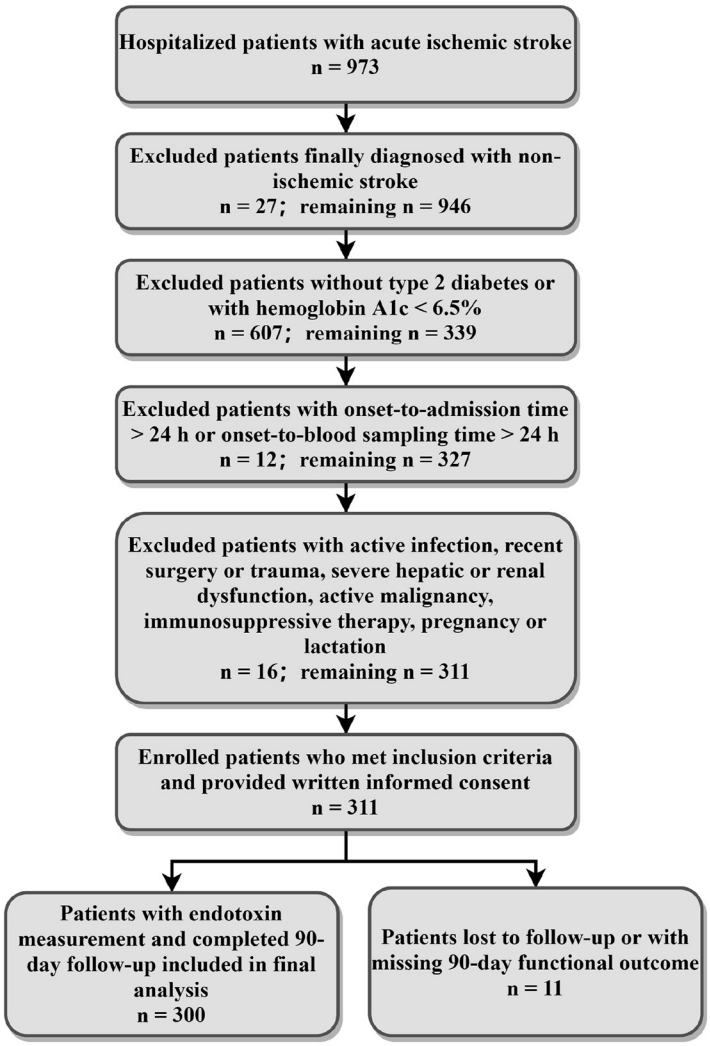

Fig. 1. Flow chart of patient screening and follow-up

NIHSS scores, and higher proportions of large vessel occlusion (LVO) and mechanical thrombectomy (MT), as well as increased levels of lipopolysaccharide (LPS), high-sensitivity C-reactive protein (hs-CRP), and white blood cell (WBC) count (all $P < 0.05$) (Table 1).

3.2. Association between early admission endotoxin levels and stroke severity and inflammatory status

Spearman rank correlation analysis showed that early admission log-transformed LPS levels were positively correlated with stroke severity (baseline NIHSS) and inflammatory markers (hs-CRP and WBC), and all correlations were statistically significant ($P < 0.01$) (Table 2).

Table 2. Correlation between early admission plasma endotoxin levels and stroke severity and inflammatory markers

Index	Sample size (n)	r_s	P
Baseline NIHSS score	300	0.368	<0.001
hs-CRP, mg L^{-1}	300	0.412	<0.001
WBC count, 10^9 L^{-1}	300	0.214	0.001

Note: Endotoxin levels were log-transformed for correlation analysis.

3.3. Independent association between endotoxemia and 90-day functional outcome

Multivariable binary and ordinal Logistic regression analyses showed that early admission log-transformed LPS (per 1 SD increase) was independently and positively associated with 90-day poor functional outcome (main analysis OR = 1.86, 95% CI 1.39–2.48, $P < 0.001$). Higher Age, Baseline NIHSS, and HbA1c and the presence of AF were also associated with an increased risk of poor outcome, whereas higher eGFR was associated with a decreased risk of poor outcome (all $P < 0.05$). Male, SBP, and reperfusion therapy were not significantly associated with outcome (all $P > 0.05$). Ordinal Logistic regression sensitivity analysis treating 90-day mRS 0–6 as an ordinal outcome yielded results that were generally consistent with the main analysis in terms of effect direction and significance (Table 3).

3.4. Dose-response relationship between endotoxin levels and 90-day functional outcome

Binary Logistic regression and trend tests confirmed that, with increasing quartiles of early admission LPS levels, the incidences of 90-day poor functional outcome and all-cause death increased in a stepwise manner, indicating a

Table 1. Baseline characteristics of patients stratified by 90-day functional outcome (n, %)/M [IQR]

Variable	Overall population (N = 300)	Good outcome group (n = 195)	Poor outcome group (n = 105)	Test statistic	P
Age	67.2 [60.1, 74.6]	64.9 [58.5, 71.2]	71.3 [65.4, 78.1]	$Z = -4.215$	<0.001
Male	172 (57.33%)	122 (62.56%)	50 (47.62%)	$\chi^2 = 4.923$	0.027
BMI, kg m^{-2}	26.3 [24.1, 28.7]	26.1 [24.0, 28.2]	26.6 [24.4, 29.4]	$Z = -1.334$	0.182
SBP, mmHg	151 [140, 164]	149 [138, 161]	154 [143, 169]	$Z = -1.755$	0.079
DBP, mmHg	84 [77, 90]	83 [76, 89]	85 [78, 92]	$Z = -1.021$	0.307
Hypertension	230 (76.67%)	142 (72.82%)	88 (83.81%)	$\chi^2 = 4.176$	0.041
AF	61 (20.33%)	28 (14.36%)	33 (31.43%)	$\chi^2 = 14.862$	<0.001
CAD	77 (25.67%)	46 (23.59%)	31 (29.52%)	$\chi^2 = 1.874$	0.171
Current smoking	100 (33.33%)	78 (40.00%)	22 (20.95%)	$\chi^2 = 13.827$	<0.001
Diabetes duration, years	11.4 [6.2, 17.8]	10.6 [5.8, 16.9]	12.7 [7.1, 18.9]	$Z = -2.102$	0.036
Insulin use before admission	121 (40.33%)	72 (36.92%)	49 (46.67%)	$\chi^2 = 3.987$	0.046
Oral antidiabetic drugs before admission	232 (77.33%)	153 (78.46%)	79 (75.24%)	$\chi^2 = 0.456$	0.499
FPG, mmol L^{-1}	8.5 [7.1, 10.0]	8.2 [7.0, 9.7]	9.0 [7.5, 10.6]	$Z = -2.487$	0.013
HbA1c, %	7.8 [7.1, 8.7]	7.7 [7.0, 8.5]	8.1 [7.3, 9.0]	$Z = -2.006$	0.045
eGFR, mL $min^{-1}1.73$ m^2	78.6 [64.3, 92.5]	81.4 [68.2, 94.8]	72.9 [58.7, 88.1]	$Z = 2.987$	0.003
LDL-C, mmol L^{-1}	2.70 [2.10, 3.26]	2.65 [2.08, 3.18]	2.79 [2.15, 3.41]	$Z = -0.846$	0.398
Baseline NIHSS score	8 [4, 13]	5 [3, 9]	13 [8, 18]	$Z = -7.543$	<0.001
LVO	139 (46.33%)	81 (41.54%)	58 (55.24%)	$\chi^2 = 5.832$	0.016
IVT	155 (51.67%)	104 (53.33%)	51 (48.57%)	$\chi^2 = 0.889$	0.346
MT	81 (27.00%)	42 (21.54%)	39 (37.14%)	$\chi^2 = 7.216$	0.007
LPS, EU/mL	0.46 [0.30, 0.71]	0.39 [0.25, 0.59]	0.63 [0.43, 0.92]	$Z = -6.238$	<0.001
hs-CRP, mg L^{-1}	4.6 [1.8, 9.8]	3.6 [1.4, 7.8]	6.7 [2.9, 12.4]	$Z = -3.842$	<0.001
WBC count, 10^9 L^{-1}	7.9 [6.5, 9.4]	7.6 [6.3, 9.0]	8.4 [6.9, 10.1]	$Z = -2.361$	0.018

Note: Data are presented as median [interquartile range] or n (%). BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; AF: atrial fibrillation; CAD: coronary artery disease; FPG: fasting plasma glucose; HbA1c: glycated hemoglobin; eGFR: estimated glomerular filtration rate; LDL-C: low-density lipoprotein cholesterol; NIHSS: National Institutes of Health Stroke Scale; LVO: large vessel occlusion; IVT: intravenous thrombolysis; MT: mechanical thrombectomy; LPS: lipopolysaccharide; hs-CRP: high-sensitivity C-reactive protein; WBC: white blood cell. mRS 0–2 at 90 days indicates good outcome, and mRS 3–6 indicates poor outcome.

Table 3. Multivariable regression analysis of early admission endotoxemia and 90-day poor functional outcome

Variable	Binary Logistic regression		Ordinal Logistic regression	
	OR (95% CI)	*P*	OR (95% CI)	*P*
LPS (per 1-SD increase, log-transformed)	1.86 (1.39–2.48)	<0.001	1.73 (1.34–2.24)	<0.001
Age (per 10 y increase)	1.29 (1.05–1.60)	0.014	1.24 (1.03–1.49)	0.017
Male	0.74 (0.46–1.19)	0.205	0.78 (0.52–1.18)	0.248
Baseline NIHSS (per 1 point increase)	1.18 (1.11–1.26)	<0.001	1.17 (1.10–1.24)	<0.001
SBP (per 10 mmHg increase)	1.07 (0.95–1.20)	0.220	1.05 (0.96–1.15)	0.270
HbA1c (per 1% increase)	1.19 (1.01–1.41)	0.040	1.17 (1.02–1.35)	0.028
AF	1.73 (1.01–2.96)	0.042	1.68 (1.04–2.72)	0.031
eGFR (per 10 mL $min^{-1}1.73\ m^2$ increase)	0.88 (0.78–0.99)	0.033	0.90 (0.81–0.99)	0.037
Reperfusion therapy (IVT or MT)	0.69 (0.42–1.13)	0.141	0.72 (0.48–1.09)	0.119

Note: The ordinal Logistic regression model assumed proportional odds, and the proportional odds assumption was verified by the Brant test.

significant dose-response relationship ($P < 0.001$) (Table 4). Based on multivariable Logistic regression combined with restricted cubic spline analysis, when early admission LPS level was modeled as a continuous variable, the risk of 90-day poor functional outcome increased approximately linearly with increasing LPS; after the reference around the 25th percentile, the OR continued to rise and the range of the 95% confidence interval entirely above 1 gradually widened, and the test for nonlinearity was not statistically significant (P_nonlin = 0.247), suggesting a predominantly linear dose-response relationship (Fig. 2).

3.5. Incremental value of the prediction model and clinical net benefit

Based on a continuous risk reclassification framework and using 1,000 bootstrap resamples to estimate 95% CI and *P* values, adding LPS to the basic clinical model yielded a category-free NRI of 0.21 and an IDI of 0.03 for predicting 90-day poor functional outcome (both $P < 0.01$), indicating significant improvement in risk reclassification and discrimination (Table 5). When nonparametric ROC analysis with DeLong's test was used to compare the AUCs of the two models, the AUC of the basic clinical model for predicting 90-day poor functional outcome was 0.79, which increased to 0.83 after adding LPS ($P = 0.021$), suggesting an improvement in model discrimination (Fig. 3A). In decision curve analysis, at threshold probabilities of approximately 0.27–0.55, the net benefit of both prediction models was higher than that of the "no model" and "treat all" strategies, and the extended model including LPS showed higher net benefit at most thresholds (Fig. 3B).

4. DISCUSSION

In this prospective cohort study of patients with AIS and concomitant type 2 diabetes, we demonstrated that early admission plasma lipopolysaccharide (LPS) a key marker of plasma endotoxemia serves as an independent and dose-dependent predictor of 90-day unfavorable functional outcome. Our principal findings are fourfold: First, LPS levels were significantly higher in patients with poor functional recovery and correlated positively with both stroke severity (NIHSS) and systemic inflammatory markers (hs-CRP, WBC). Second, after comprehensive adjustment for established clinical and metabolic risk factors, LPS remained independently associated with a nearly two-fold increased

Table 4. 90-day outcomes stratified by LPS quartiles

LPS quartile group	LPS, EU/mL	Poor functional outcome	All-cause death	OR for poor functional outcome (95% CI)	OR for all-cause death (95% CI)
Q1:0.12–0.29	0.21 [0.17, 0.26]	16 (21.33%)	3 (4.00%)	1.00 (reference)	1.00 (reference)
Q2:0.30–0.44	0.36 [0.32, 0.41]	22 (29.33%)	5 (6.67%)	1.53 (0.73–3.22)	1.71 (0.39–7.45)
Q3:0.45–0.64	0.53 [0.48, 0.59]	28 (37.33%)	7 (9.33%)	2.20 (1.07–4.53)	2.47 (0.61–9.94)
Q4:0.65–1.15	0.82 [0.71, 0.96]	39 (52.00%)	12 (16.00%)	3.99 (1.96–8.16)	4.57 (1.23–16.94)

Note: Q1 was used as the reference group. P for trend of poor functional outcome <0.001 (trend test of LPS quartile group in binary Logistic regression).

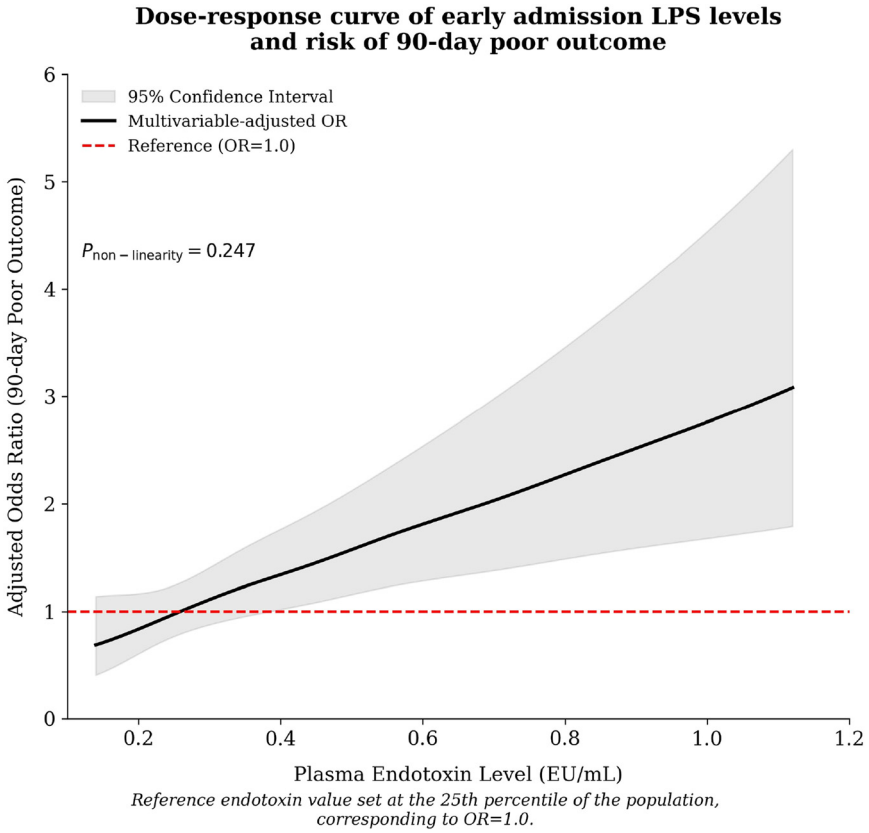

Fig. 2. Dose-response curve of early admission LPs levels and risk of 90-day poor outcome

Table 5. Reclassification indices for predictive performance of the model with endotoxin compared with the basic clinical model

Index	Estimate	95% CI	*P*
NRI	0.21	0.07–0.36	0.003
IDI	0.03	0.01–0.06	0.008

risk of poor outcome per standard deviation increase. Third, a clear, approximately linear dose-response relationship was observed, with the incidence of unfavorable outcomes and mortality rising progressively across LPS quartiles. Finally, incorporating LPS into a basic clinical prediction model yielded statistically significant improvements in discrimination, risk reclassification, and clinical net benefit, underscoring its incremental prognostic value.

The association between elevated LPS and more severe neurological deficits at admission aligns with the concept of a dysregulated gut-brain axis in acute stroke. Patients with type 2 diabetes exhibit a well-documented predisposition to gut dysbiosis, increased intestinal permeability, and baseline metabolic endotoxemia [17–19]. The acute stroke event likely acts as a second hit, where sympathetic activation and altered gut perfusion further compromise intestinal barrier integrity [9, 19]. LPS can also originate from other sites, such as subclinical infections, indwelling catheters, the oral cavity, or the respiratory tract [20]. To mitigate this, we excluded patients with active clinical infections at admission. Furthermore, the observed strong, dose-dependent association of LPS with outcome even after adjustment for systemic inflammatory markers (hs-CRP, WBC) suggests its role may extend beyond being a simple marker of general infection [21, 22]. Nonetheless, future studies incorporating direct markers of intestinal permeability (e.g., zonulin, intestinal fatty acid-binding protein) or specific bacterial translocation (e.g., 16S rDNA in blood) alongside LPS measurements are needed to definitively confirm the gut as the primary source in this acute stroke setting.

Crucially, our study extends beyond correlation to demonstrate independent prognostic value. The persistence of the LPS-outcome association after adjusting for NIHSS, glycemic control (HbA1c), atrial fibrillation, and renal function suggests that plasma endotoxemia (LPS) contributes risk information distinct from traditional prognosticators. The observed dose-response relationship reinforces the biological plausibility of this link and argues against a mere epiphenomenon. In the metabolically vulnerable context of diabetes, characterized by endothelial dysfunction and a chronic low-grade inflammatory state [23, 24], the additive burden of acute endotoxemia may tip the balance towards worse recovery by sustaining inflammation, impairing microvascular reperfusion, and hampering neurorepair mechanisms long after the initial ischemic insult [22].

From a clinical perspective, the most translational finding is the incremental predictive value LPS adds to a standard clinical model. The significant improvements in the area under the curve (AUC), net reclassification index

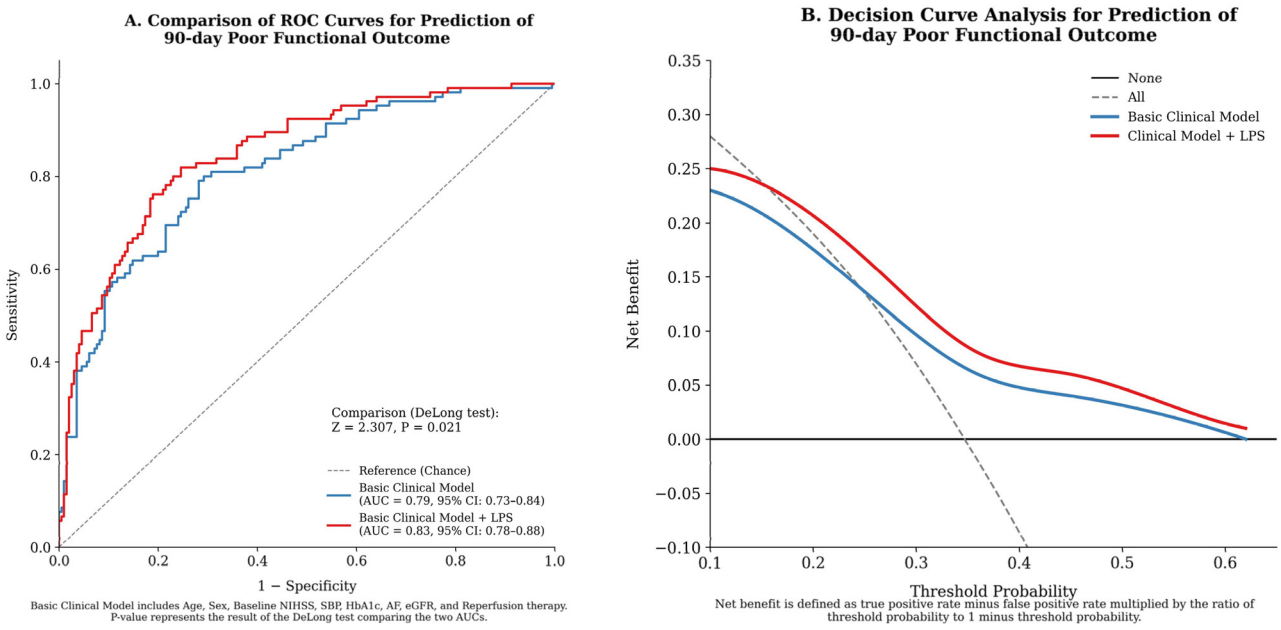

Fig. 3. Predictive performance and clinical net benefit of models with and without LPS. 3A: Comparison of ROC Curves for Prediction of 90-day Poor Functional Outcome. 3B: Decision Curve Analysis for Prediction of 90-day Poor Functional Outcome

(NRI), and integrated discrimination improvement (IDI), though modest in magnitude, indicate that LPS meaningfully refines risk stratification. Decision curve analysis further confirmed that using the LPS-enhanced model for clinical decisions would provide greater net benefit across a range of clinically relevant threshold probabilities [25]. This suggests that measuring early endotoxemia could help identify a high-risk subgroup of diabetic stroke patients who, despite similar age and stroke severity scores, harbor a heightened inflammatory burden driven by gut barrier dysfunction. Such stratification could be valuable for prognostication and for future research targeting adjuvant therapies aimed at modulating the gut microbiome or attenuating endotoxin-mediated inflammation.

Our study must be interpreted in light of its limitations. First, its single-center design and moderately sized, homogeneous cohort may limit generalizability to other ethnic or healthcare settings. Second, the observational nature cannot establish causality; despite rigorous multivariable adjustment, residual confounding from unmeasured factors (e.g., diet, prior antibiotic use, detailed gut health metrics) is possible. Third, we measured LPS at a single early time point. Serial measurements could reveal dynamic patterns with greater prognostic insight and clarify the impact of acute stroke management on endotoxemia. Fourth, the absence of concurrent gut microbiome sequencing or intestinal barrier markers (e.g., zonulin, intestinal fatty acid-binding protein) limits our ability to delineate the specific microbial sources or the precise barrier defects leading to LPS elevation [26, 27]. Nonetheless, our findings position plasma LPS as a clinically accessible biomarker that captures a deleterious inflammatory phenotype relevant to stroke recovery in diabetic patients. Finally, our outcomes were limited to 90-day function and mortality; future studies should assess longer-term endpoints, including cognitive decline and stroke recurrence.

Clinical outcomes included only 90-day functional status and all-cause mortality, while important endpoints such as medium- and long-term cognitive function, post-discharge infections, and stroke recurrence were not yet included. The current results support the potential value of LPS as a prognostic biomarker [28, 29], but they are still some distance away from directly formulating intervention strategies targeting the gut microbiota–endotoxin axis, and need to be further validated in future mechanistic studies and randomized controlled trials.

5. CONCLUSION

In conclusion, this study provides novel clinical evidence that plasma endotoxemia (LPS), quantified by early plasma LPS, is a significant and independent predictor of short-term functional outcome after acute ischemic stroke in patients with type 2 diabetes. Its association with stroke severity, systemic inflammation, and poor recovery, coupled with its ability to improve conventional risk models, positions LPS as a clinically accessible biomarker that is associated with poor recovery in acute ischemic stroke. These findings underscore the importance of considering host-microbiome interactions in stroke pathophysiology and open avenues for future research into gut-targeted therapeutic strategies aimed at improving outcomes in this high-risk population.

Declaration of interests: 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.

Ethical statement: This study was conducted in accordance with the ethical standards of the institutional and national research committees and the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of Department of Neurology, People's Hospital of Longhua, Shenzhen city, 518109, China (Approval No. 2024-009). Informed consent was obtained from all participants prior to inclusion in the study.

Data availability: All data are available on request from corresponding author.

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