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Incremental value of a gut microbiome score (M-score) for predicting stroke-associated pneumonia beyond the clinical A^2DS^2 score: A nested case–control study

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RESEARCH ARTICLE

ABSTRACT

Stroke-associated pneumonia (SAP) is a major complication of acute ischemic stroke. The brain–gut–lung axis suggests that post-stroke gut dysbiosis may offer independent risk information beyond clinical scores like A^2DS^2. The aim of this study was to evaluate the incremental predictive value of early post-stroke gut microbiome biomarkers for SAP and to construct a simplified microbiome risk score (M-score). In a prospective nested case–control study, we identified 63 SAP cases from a cohort of 551 patients with acute ischemic stroke and stool samples were collected 24–72 h post-admission. Cases were matched 1:2 (age, sex) to 126 controls. Gut microbiota was profiled via 16S rRNA sequencing. Three differentially abundant genera (prevalence ≥20%, FDR $q < 0.10$) were used to construct an M-score via bootstrapped regression. Independent associations were assessed with conditional logistic regression. Incremental value over the A^2DS^2 score was evaluated using AUC, continuous net reclassification improvement (NRI), and the Brier score. SAP cases exhibited reduced α-diversity and distinct β-diversity (both $P < 0.01$). Cases had higher *Enterococcus* and *Streptococcus* and lower Faecalibacterium levels (all $q < 0.05$). The M-score was independently associated with SAP (adjusted OR per 1-SD: 1.76, 95% CI: 1.30–2.39, $P = 0.001$). Adding the M-score to A^2DS^2 significantly improved prediction: AUC increased from 0.76 to 0.84 ($\Delta AUC = 0.08$, $P = 0.009$), NRI was 0.31 (95% CI: 0.12–0.51), and the Brier score decreased from 0.18 to 0.16. Results were robust in sensitivity analyses. A gut microbiome score based on three genera provides significant independent and incremental predictive value for SAP over the A^2DS^2 score, enabling more precise early risk stratification after stroke.

KEYWORDS

acute ischemic stroke, stroke-associated pneumonia, gut microbiome, predictive model, risk stratification

1. INTRODUCTION

Acute ischemic stroke (AIS) represents a leading cause of death and disability worldwide. In this vulnerable population, stroke-associated pneumonia (SAP) emerges as the most critical in-hospital complication, occurring in up to one-third of patients [1]. The development of SAP is a pivotal determinant of poor outcome, being independently associated with increased mortality, prolonged hospitalization, and significantly worse long-term functional recovery [2, 3]. Consequently, the accurate early identification of patients at the highest risk is a clinical imperative to guide intensified monitoring and potential preventive strategies.

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Current clinical practice relies on risk assessment scores based on readily available patient characteristics. Among these, the A^2DS^2 score, incorporating age, atrial fibrillation, dysphagia, sex, and stroke severity, has achieved widespread validation for SAP prediction [4]. Systematic reviews confirm its utility, yet also highlight a critical limitation: its predictive performance, while significant, remains suboptimal, with areas under the curve (AUC) frequently below 0.80 and imperfect calibration [5, 6]. This leaves a substantial proportion of patients, particularly those with intermediate scores, in a diagnostic grey zone where risk stratification is imprecise. Efforts to augment these clinical models with blood-based biomarkers have yielded only marginal improvements [7], underscoring the need to explore novel, independent biological pathways that contribute to post-stroke susceptibility to infection.

The burgeoning concept of the brain-gut-lung axis provides a compelling framework for this exploration. AIS triggers a profound systemic stress response, characterized by sympathetic overactivation and immune dysregulation, which directly compromises intestinal barrier integrity and motility [8, 9]. This "gut-lysis" facilitates the translocation of microbial products and even live bacteria, potentially priming the lungs for infection via cross-talk between the mucosal immune systems, a pathway supported by both clinical observations and animal models [10, 11]. Consequently, the gut microbiome is positioned not merely as a bystander but as a dynamic mediator of post-stroke systemic immunity, positioning it as a promising source of novel risk biomarkers [12].

Initial investigations support this hypothesis. Patients who develop SAP exhibit distinct gut microbial signatures early after stroke onset, characterized by reduced overall community diversity (alpha-diversity), expansion of opportunistic pathogens like *Enterococcus and Streptococcus*, and depletion of commensals with immunomodulatory functions, such as the butyrate-producing genus *Faecalibacterium* [13, 14]. These patterns of dysbiosis echo findings in other critical illnesses associated with infection risk [15, 16]. However, the existing evidence landscape has notable shortcomings. Studies are often cross-sectional, include heterogeneous time windows, and lack prospective evaluation of the microbiome before the standardized diagnosis of SAP [17]. Critically, while associations are reported, no study has yet quantified whether gut microbiome features provide incremental predictive value over established clinical scores like A^2DS^2. The rigorous assessment of a biomarker's clinical utility requires more than an association; it demands a demonstration of improved discrimination (AUC), enhanced calibration, and superior net reclassification when integrated into existing models. Furthermore, for potential clinical translation, there is a pressing need to move beyond complex microbial community descriptions to derive simplified, interpretable risk scores, as demonstrated in other fields [18, 19], but lacking for SAP [20].

To bridge this translational gap, we implemented a prospective nested case-control design within a consecutively enrolled AIS cohort. This study was expressly designed to: (1) define the early (24–72 h) gut microbiota signature antecedent to SAP; (2) construct and internally validate a parsimonious Microbiome Risk Score (M-score); and (3) perform a head-to-head comparison to evaluate if the M-score yields statistically significant and clinically meaningful improvements in model discrimination (AUC), calibration, and net reclassification over the clinical benchmark (A^2DS^2 score).

2. MATERIALS AND METHODS

2.1. Study design and ethical approval

This was a single-center, prospective nested case–control study conducted within a consecutively enrolled cohort of acute ischemic stroke patients. This design ensures that all exposure data (gut microbiome) was collected prospectively and prior to the outcome. From May 1, 2023, to April 30, 2025, we recruited eligible patients at admission and collected stool samples within a standardized early window (24–72 h post-admission). Patients who subsequently developed SAP within 7 days of stroke onset were defined as cases. For each case, two controls were selected from the cohort using risk-set sampling, matching on age and sex, and ensuring they were at risk (i.e., free of SAP) at the time of the case's diagnosis. Laboratory personnel performing DNA extraction, sequencing, and primary bioinformatics were blinded to all clinical outcomes and case-control status. The study protocol was approved by the Institutional Review Board/Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen University (Zhuhai, China) (Approval No. hmu202301213). Written informed consent was obtained from all participants or their legal representatives.

2.2. Study participants

Inclusion criteria were: age ≥18 years; diagnosis of acute ischemic stroke confirmed by cranial MRI or CT within 7 days of symptom onset; ability to provide a stool sample within the 24–72-h post-admission window; and provision of written informed consent. Exclusion criteria included: use of systemic antibiotics, oral or enteral probiotics, or antimicrobial traditional medicines within 4 weeks prior to admission; presence of active infection or pneumonia on admission; history of major gastrointestinal surgery, inflammatory bowel disease, recent bowel preparation, or parenteral nutrition; severe hepatic (Child-Pugh C) or renal failure (eGFR <15 mL/min/1.73 m^2 or on dialysis), active malignancy, or long-term immunosuppressive therapy; requirement for invasive mechanical ventilation at admission; and inability to obtain an adequate stool sample within the prespecified time window.

2.2.1. Diagnostic criteria for stroke-associated pneumonia. Stroke-associated pneumonia was defined according to international consensus criteria as a new pulmonary infection occurring within 7 days after stroke onset. Diagnosis required both imaging evidence (new or progressive

infiltrates, consolidation, or cavitation on chest X-ray or CT) and clinical evidence (at least two of the following: fever >38.0 °C or hypothermia <36.0 °C; leukocytosis >12 × 10^9/L or leukopenia <4 × 10^9/L; new or increased purulent sputum; new or worsening cough, dyspnea, or tachypnea with respiratory rate >22 breaths/min). All imaging was independently reviewed by two board-certified chest radiologists blinded to clinical and microbiome data. Suspected SAP events were adjudicated by an independent endpoint committee comprising two senior neurologists and one pulmonologist, all blinded to microbiome data. Disagreements were resolved by consensus or, if needed, by a third independent specialist.

2.2.2. Selection and matching of cases and controls. All cohort participants who met the SAP criteria were included as cases. For each case, two controls were selected using risk-set sampling from those who were alive, hospitalized, and had not developed SAP by the index date (the SAP diagnosis date of the matched case). Controls were individually matched to their corresponding case on age (±5 years) and sex. This matching strategy was chosen to control for fundamental demographic factors while preserving variability in key clinical risk factors such as stroke severity and dysphagia, thereby allowing assessment of the microbiome's independent association in adjusted analyses. All analyses used the stool sample collected at the single, early timepoint (24–72 h post-admission).

2.2.3. Sample size and power considerations. The sample size was determined by the requirements for developing a stable multivariable prediction model. Our primary aim was to construct a combined model (A^2DS^2 plus microbiome score) with no more than five candidate predictors. Following the rule of thumb of at least 10 events per variable, we targeted a minimum of 50 SAP events. Based on a historical SAP incidence of approximately 10% at our center, we estimated that a cohort of 500–600 participants would yield sufficient events. For the nested case–control analysis, we aimed to include at least 50 cases and 100 matched controls (1:2 ratio). This sample size provides >80% power (two-sided $\alpha = 0.05$) to detect a difference in the area under the curve (AUC) of 0.10 between the clinical and combined models, assuming an AUC of 0.75 for the clinical model, using the method of the DeLong et al. [21] for correlated ROC curves.

2.3. Clinical data collection and A^2DS^2 score

Trained research nurses collected baseline clinical data within 24 h of admission using standardized electronic case report forms. Data included demographics, vascular risk factors (hypertension, diabetes, atrial fibrillation, etc.), past medical history, medication use (including proton pump inhibitors), and stroke characteristics (NIH Stroke Scale score, results of formal swallowing screening, stroke subtype). The A^2DS^2 score was calculated for each participant according to the original publication, summing points for age ≥75 years (1 point), atrial fibrillation (1), dysphagia (2), male sex (1), and NIHSS score (0–4 = 0, 5–9 = 3, 10–14 = 5, ≥15 = 7). The primary outcome (SAP) was actively surveilled daily by research staff reviewing medical charts, laboratory results, and physician notes, with final ascertainment at day 7 post-stroke or hospital discharge, whichever came first.

2.4. Stool sample collection, processing, and storage

To capture the early post-stroke gut microenvironment, stool samples were collected within 24–72 h of hospital admission. Collection was performed by trained nurses using a sterile, single-use collection kit. Approximately 3–5 g of fresh stool were collected, avoiding contact with urine or disinfectants. Samples were immediately transferred to sterile cryovials, stored in a 4 °C portable cooler within 5 min of collection, and transported to the laboratory for processing within 2 h. Upon receipt, samples were aliquoted and stored at −80 °C until DNA extraction. All samples were de-identified with a unique barcode before freezing. To monitor for environmental contamination, a negative control (sterile swab) was processed alongside each batch of patient samples during collection and extraction.

2.5. DNA extraction, 16S rRNA gene amplification, and sequencing

Microbial genomic DNA was extracted from approximately 200 mg of stool using the QIAamp PowerFecal Pro DNA Kit (Qiagen), which includes mechanical bead-beating for efficient lysis of Gram-positive bacteria. DNA concentration and purity were assessed spectrophotometrically (NanoDrop) and fluorometrically (Qubit dsDNA HS Assay). The hypervariable V3–V4 region of the 16S rRNA gene was amplified using primer pair 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) with attached Illumina adapter sequences and unique dual-index barcodes. Amplicon libraries were purified, quantified, pooled in equimolar ratios, and sequenced on an Illumina NovaSeq 6000 platform using 2 × 250 bp paired-end chemistry. Each sequencing run included extraction blanks and a mock microbial community standard (ZymoBIOMICS Microbial Community Standard) for quality control.

2.6. Bioinformatic processing of sequencing data

Raw demultiplexed sequencing reads were processed using QIIME 2 (version 2023.9). Primer sequences were trimmed with cutadapt. Denoising, paired-end read merging, and chimera removal were performed with DADA2, yielding amplicon sequence variants (ASVs). Taxonomy was assigned to ASVs using the SILVA 138.1 reference database (99% OTUs). Sequences classified as mitochondria, chloroplasts, or unassigned at the kingdom level were removed. Potential contaminant ASVs were identified and filtered using the decontam *R* package (prevalence method) with extraction blank controls. Samples with fewer than 10,000 reads after processing were excluded. For α-diversity

analysis, samples were rarefied to an even depth of 20,000 reads. For all downstream statistical analyses (differential abundance, score construction), the raw ASV count table was transformed using a centered log-ratio (CLR) transformation after adding a pseudocount of 1 to all values, to address the compositional nature of the data. β-Diversity was assessed using Bray–Curtis dissimilarity on CLR-transformed data.

2.7. Construction of the microbiome risk score (M-score)

Biomarker selection and score construction followed a prespecified, multistep process to minimize overfitting. First, only bacterial genera with a prevalence of at least 20% across all samples were considered. Second, CLR-transformed abundances of these genera were compared between cases and controls using the Wilcoxon rank-sum test; *P*-values were adjusted for multiple testing with the Benjamini–Hochberg false discovery rate (FDR), and genera with $q < 0.10$ were retained. Third, all genera meeting the FDR threshold were ranked by the absolute value of their Cohen's d effect size, and the top three genera were selected for score construction. Finally, a non-conditional logistic regression model (with SAP as outcome) was fitted using the CLR values of the three selected genera as predictors. The regression coefficients from this model were shrunk toward zero via a bootstrap procedure (1,000 iterations) to reduce optimism. The final M-score for each participant was calculated as $M = \beta_0 + (\beta_1 \times z_1) + (\beta_2 \times z_2) + (\beta_3 \times z_3)$, where z_i represents the standardized (mean = 0, SD = 1) CLR value of each genus, β_i are the shrunken coefficients, and β_0 is the intercept.

2.8. Study variables and endpoints

The primary endpoint was the occurrence of SAP within 7 days of stroke onset. Primary exposure variables were α-diversity indices (Shannon index, Observed ASVs), the three selected genera, and the composite M-score. The clinical baseline model (Model-C) used the pre-calculated A^2DS^2 score as a single continuous predictor. In adjusted association analyses, clinical covariates included age, sex, admission NIHSS score, dysphagia status, chronic pulmonary disease, and atrial fibrillation.

2.9. Statistical analysis plan

All analyses were performed using *R* software (version 4.3.2). A two-sided *P*-value <0.05 was considered statistically significant, except for microbiome-wide tests where an FDR $q < 0.10$ was used. Baseline characteristics in the matched case–control set were summarized using standardized differences. α-Diversity indices were compared with the paired Wilcoxon signed-rank test. β-Diversity (Bray–Curtis) was visualized via principal-coordinates analysis and tested using PERMANOVA (adonis2 function, 999 permutations) with case–control status as the predictor.

The independent association of microbiome features with SAP was assessed using conditional logistic regression, stratified by the matched sets (age/sex). Models were run unadjusted and then adjusted for the prespecified clinical covariates (NIHSS, dysphagia, chronic pulmonary disease, atrial fibrillation). Odds ratios with 95% confidence intervals were reported for a 1-SD increase in α-diversity indices and the M-score.

To evaluate predictive performance and incremental value, we constructed two non-conditional logistic regression models (with age and sex included as covariates to account for the matching): Model-C (clinical), containing only the A^2DS^2 score, and Model-CM (combined), containing the A^2DS^2 score plus the M-score. Both models underwent internal validation with optimism correction via 1,000 bootstrap resamples. We reported the optimism-corrected C-statistic (AUC), calibration intercept, and calibration slope; calibration was further visualized with bootstrap-corrected calibration curves. Discrimination between models was compared using the DeLong test for correlated ROC curves.

The improvement offered by the M-score was quantified using three metrics: the change in AUC (ΔAUC) with 95% confidence interval and *P*-value from the DeLong test; the continuous net reclassification improvement (NRI); and the optimism-corrected Brier score, with a lower score indicating better overall accuracy.

Two pre-specified sensitivity analyses were conducted to assess robustness. First, the primary analysis was repeated after excluding any participant who received systemic antibiotics between admission and the index date. Second, the M-score in Model-CM was replaced individually with each of its three component genera to evaluate the contribution of the composite score versus individual taxa.

3. RESULTS

3.1. Enrollment of study participants and baseline characteristics

During the study period, 812 patients with acute ischemic stroke were assessed for eligibility. After applying inclusion and exclusion criteria, 586 were enrolled in the prospective cohort. Following stool collection and stringent quality control of sequencing data, 551 participants constituted the final microbiome cohort, from which 63 patients (11.4%) developed SAP. Using risk-set sampling, 126 controls were matched (1:2) to these cases on age and sex, forming the final nested case–control population for analysis ($n = 189$) (Fig. 1).

As intended by the matching strategy, age and sex were well-balanced between SAP cases and controls (standardized difference, |Std Diff| < 0.10). Cases exhibited the expected clinical profile of higher SAP risk, with significantly greater stroke severity (mean NIHSS score: 11.3 vs. 7.8; Std Diff = 0.56), a higher prevalence of dysphagia (46.0% vs. 25.4%; Std Diff = 0.44), and consequently higher A^2DS^2 scores (5.8 vs. 4.3; Std Diff = 0.69). Notable imbalances (|Std Diff| > 0.20) were also present for atrial fibrillation (22.2% vs. 14.3%), chronic pulmonary disease (15.9% vs. 11.1%), and use of sedatives/analgesics (27.0% vs. 17.5%). These and all other baseline characteristics are detailed in Supplementary Table S1.

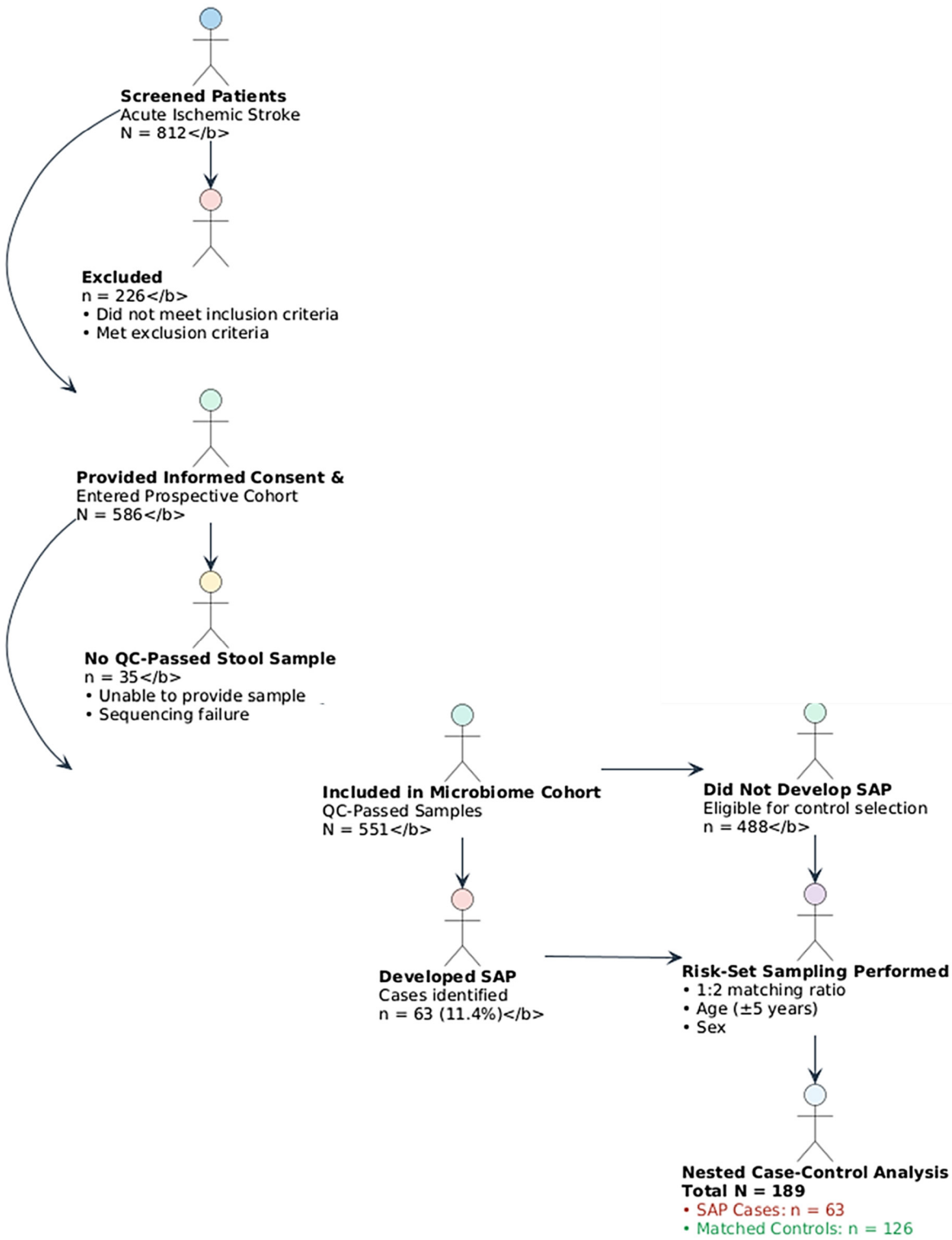

Fig. 1. Flowchart of participant screening and nested case–control selection

3.2. Gut microbiota diversity and overall structural differences

Analysis of early post-stroke gut microbiota revealed significant ecological disruption in patients who developed SAP. Compared to controls, SAP cases demonstrated a marked reduction in α-diversity, with significantly lower median values for both the Shannon index ($P < 0.01$) and Observed ASVs richness ($P < 0.01$). Furthermore, the overall microbial community structure (β-diversity) was significantly different between the two groups (PERMANOVA on Bray-Curtis distance: $R^2 = 0.031$, $P = 0.003$), indicating a distinct separation in gut ecosystem composition prior to

pneumonia diagnosis. Visualizations of these α- and β-diversity differences are provided in Supplementary Fig. S1.

3.3. Differences in gut microbial composition and construction of microbiota biomarkers

Following the pre-specified biomarker discovery protocol, three bacterial genera met the criteria of prevalence ≥20% and differential abundance at a false discovery rate (FDR) $q < 0.10$. SAP cases showed significantly elevated CLR-transformed abundances of *Enterococcus* and *Streptococcus*, while the beneficial genus Faecalibacterium was depleted (all $q < 0.05$) (Fig. 2).

These three genera were integrated into a single Microbiome Risk Score (M-score). The score was constructed by weighting the standardized CLR values of each genus by

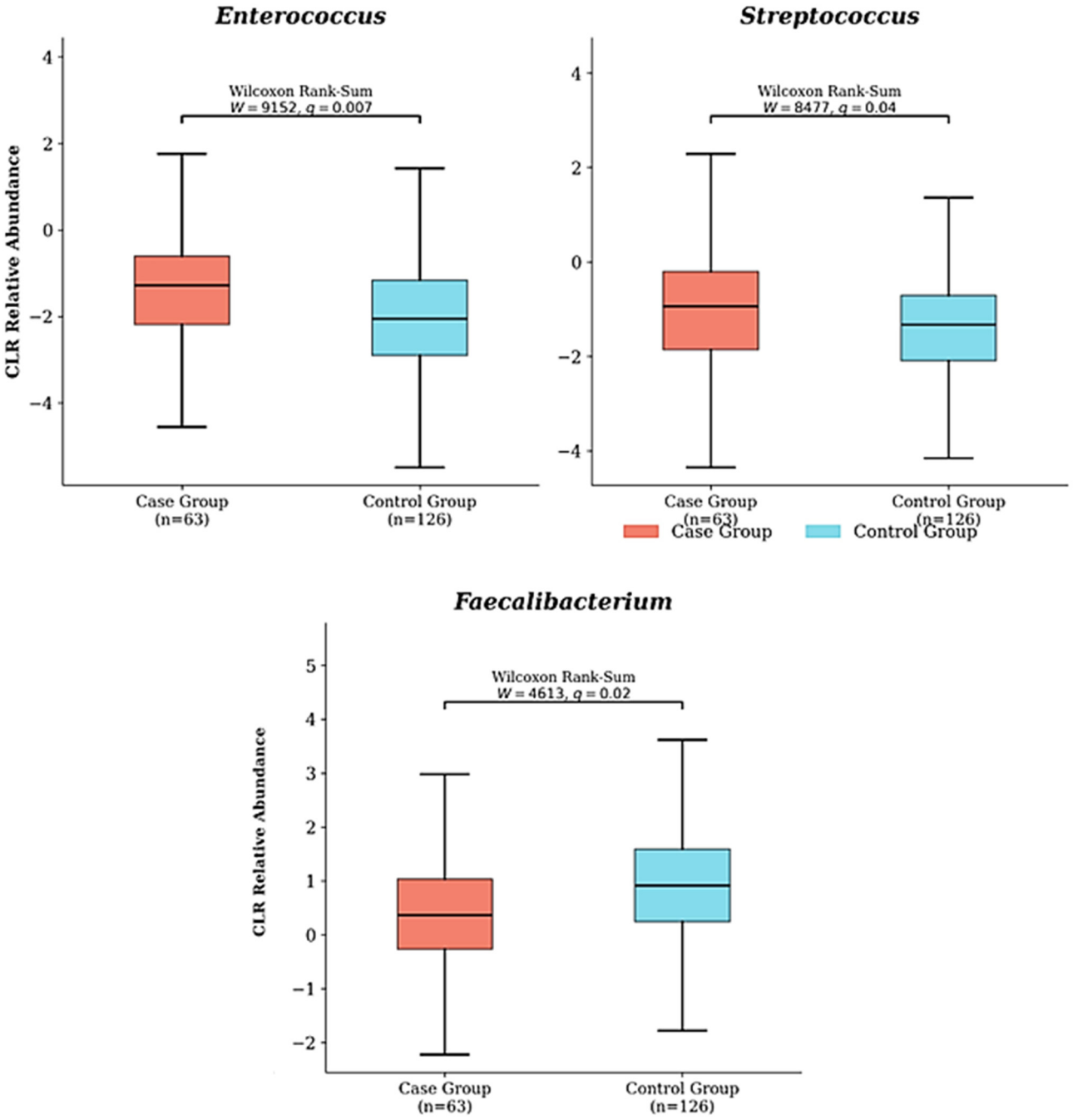

Fig. 2. Abundances of the three bacterial genera comprising the microbiome risk score (M-score). Box plots display the centered log-ratio (CLR)-transformed abundances of Enterococcus, Streptococcus, and Faecalibacterium in stroke-associated pneumonia (SAP) cases and matched controls. These genera were identified as differential features through a pre-specified, multistep selection process (prevalence ≥20%, FDR-corrected $q < 0.10$; see Methods 2.7 for full details) and were integrated into the M-score. Center lines represent medians; box limits represent the interquartile range (IQR); whiskers extend to 1.5 × IQR. $^{***}q < 0.001$; $^{**}q < 0.01$ (Wilcoxon rank-sum test with Benjamini-Hochberg FDR adjustment)

coefficients derived from a bootstrap-shrunken logistic regression model. The final coefficients and intercept are presented in Table 1.

3.4. Independent association between gut microbiota biomarkers and SAP

We assessed the independent association of microbiome features with SAP using conditional logistic regression, adjusting for key clinical confounders (NIHSS score, dysphagia, chronic pulmonary disease, atrial fibrillation). For each one-standard-deviation increase, the M-score was strongly associated with higher odds of SAP (adjusted odds ratio [aOR] = 1.76, 95% CI: 1.30–2.39, $P = 0.001$). Similarly, each SD increase in the Shannon index was associated with reduced odds (aOR = 0.74, 95% CI: 0.56–0.97, $P = 0.031$). The association for Observed ASVs was not significant after adjustment (aOR = 0.83, $P = 0.147$) (Table 2).

3.5. Discriminative ability and incremental value of the combined predictive model

The addition of the M-score to the clinical A^2DS^2 score significantly improved model performance. The model containing only the A^2DS^2 score (Model-C) had an AUC of 0.76. The combined model (Model-CM: A^2DS^2 + M-score) achieved a significantly higher AUC of 0.84 (ΔAUC = 0.08, DeLong test $P = 0.009$) (Fig. 3A).

The incremental value of the M-score was further confirmed by improvements in calibration and risk reclassification. The continuous Net Reclassification Improvement (NRI) was 0.31 (95% CI: 0.12–0.51), indicating superior risk stratification. Critically, the optimism-corrected Brier score, a measure of overall prediction error where lower values indicate better accuracy, improved from 0.18 to 0.16. The calibration intercept moved closer to the ideal value of 0, and the calibration slope moved closer to 1, indicating superior agreement between predicted probabilities and observed outcomes across all risk levels, as visualized in the calibration plot (Fig. 3B, Table 3).

Table 1. Construction of the microbiome score (M-score) and final coefficients

Feature name (genus level)	Final coefficient (β)
Enterococcus	0.58
Streptococcus	0.37
Faecalibacterium	−0.63
Intercept (β_0)	−0.71

Note: M-score calculation formula: $M = \beta_0 + \Sigma (\beta_i \times z_i)$, where z_i is the standardized CLR value (mean 0, standard deviation 1) of the corresponding genus.

Table 2. Conditional logistic regression analysis of α-diversity indices and the M-score for the risk of SAP

Exposure variable (per 1-SD increase)	Unadjusted OR (95% CI)	*P*-value	Adjusted OR (95% CI) †	*P*-value
Shannon index	0.68 (0.52–0.89)	0.004	0.74 (0.56–0.97)	0.031
Observed ASVs	0.79 (0.62–1.01)	0.060	0.83 (0.64–1.07)	0.147
M-score	1.93 (1.46–2.57)	<0.001	1.76 (1.30–2.39)	0.001

†Adjusted for admission NIHSS score, abnormal swallowing screening, chronic pulmonary disease, and atrial fibrillation. Matching factors (age, sex) are controlled by the conditional model.

3.6. Sensitivity analyses

The robustness of the primary findings was confirmed in pre-specified sensitivity analyses (see Supplementary Table S2 for complete results). First, after excluding the 22 participants who received systemic antibiotics between admission and the index date, the association between the M-score and SAP risk remained strong (adjusted OR = 1.71, 95% CI: 1.24–2.35; $P = 0.002$), and the improvement in model discrimination over the A^2DS^2 score persisted (ΔAUC = 0.07, $P = 0.021$).

Second, when the composite M-score was replaced in the model by each of its constituent genera individually, the resulting increases in AUC and net reclassification improvement (NRI) were consistently smaller than those achieved by the full M-score. Notably, Streptococcus alone did not yield a statistically significant improvement in discrimination (ΔAUC = 0.03, $P = 0.137$), underscoring the added predictive value of integrating multiple microbial features into a single composite score.

4. DISCUSSION

In this prospective nested case-control study, we demonstrate that gut dysbiosis within 72 h of acute ischemic stroke onset provides significant independent and incremental predictive information for the risk of stroke-associated pneumonia. The gut microbiome's predictive capacity was best captured by a composite Microbiome Risk Score (M-score) integrating the expansion of opportunistic genera (*Enterococcus, Streptococcus*) and depletion of a beneficial butyrate-producer (*Faecalibacterium*). When added to the established A^2DS^2 clinical score, the M-score substantially improved model discrimination, calibration, and reclassification performance. These findings advance the field from descriptive correlations to a quantitative, clinically-focused risk assessment strategy and provide a strong rationale for incorporating gut microbiome evaluation into early post-stroke risk stratification.

Our first key finding is that the early post-stroke gut ecosystem is profoundly altered in patients destined to develop SAP, exhibiting reduced α-diversity and a distinct community structure independent of stroke severity. This aligns with the conceptual framework of the brain-gut-lung axis, where stroke-induced sympathetic activation and immune dysregulation compromise gut barrier integrity [8, 9].

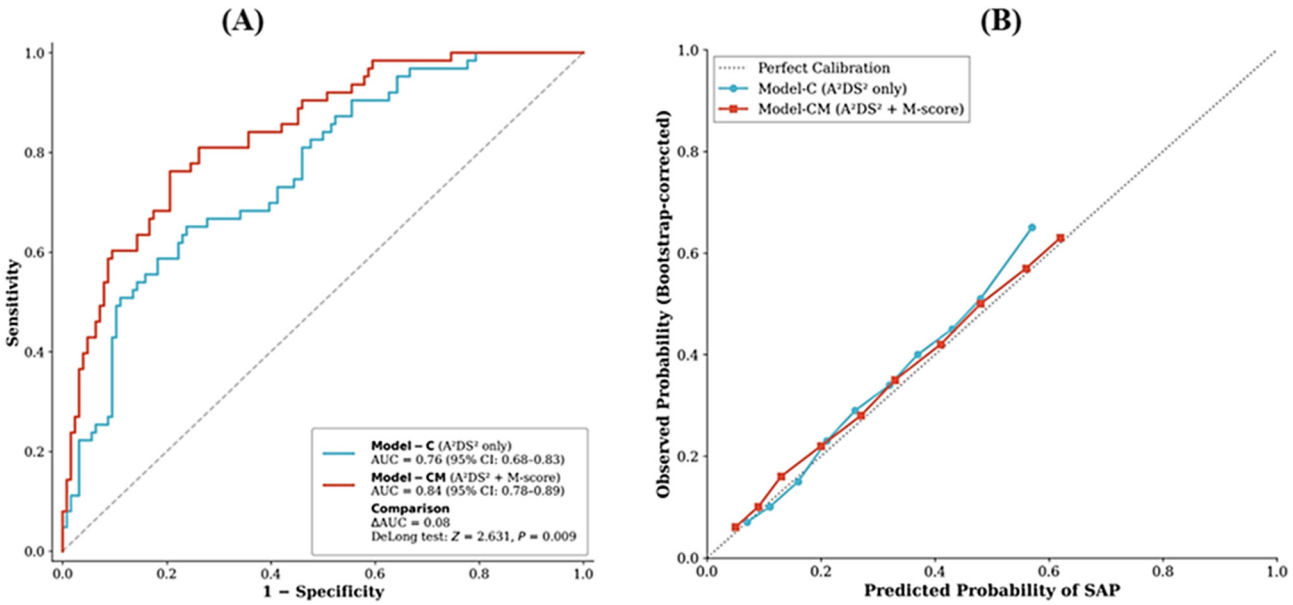

Fig. 3. Incremental predictive performance of the microbiome risk score. (**A**) ROC curves for the clinical model (A^2DS^2 score) and the combined model (A^2DS^2 + M-score). (**B**) Bootstrap-corrected calibration curves

Table 3. Comparison of incremental predictive value and calibration metrics

Metric	Model-C (A^2DS^2)	Model-CM (A^2DS^2 + M-score)	Difference or Net Improvement
Continuous NRI (95% CI)	–	0.31 (0.12–0.51)	0.31
Brier score (optimism-corrected)	0.18	0.16	−0.02
Calibration intercept, α (corrected)	−0.13	−0.05	0.08
Calibration slope, β (corrected)	1.23	1.04	−0.19

NRI: Net Reclassification Improvement; CI: Confidence Interval. Model-C: Clinical model containing the A^2DS^2 score. Model-CM: Combined model containing the A^2DS^2 score and the microbiome risk score (M-score). The Brier score is a measure of overall prediction error (lower values indicate better accuracy). Calibration metrics (intercept α, slope β) were derived from bootstrap-corrected internal validation (1,000 iterations). An ideal model has an intercept of 0, a slope of 1, and a Brier score of 0.

This "gut-lytic" state weakens colonization resistance, creating an environment permissive for pathogen expansion. While previous studies have reported associations between low gut diversity and poor outcomes in critical illness [15] and post-stroke infection [13], our work extends this by prospectively linking very early dysbiosis, assessed prior to SAP diagnosis, to subsequent *pneumonia* risk in a rigorously matched and adjusted analysis. This suggests the gut microbial state is not merely a passive marker of illness severity but an active, identifiable contributor to a susceptible host phenotype [12].

The biological plausibility of this link is strengthened by the specific taxa identified. The M-score encapsulates a high-risk microbial configuration: enrichment of *Enterococcus* and Streptococcus, and loss of *Faecalibacterium*. *Enterococcus* and *Streptococcus* are classic opportunistic pathogens known to expand during host stress, translocate across impaired mucosal barriers, and provoke inflammatory responses [22]. Faecalibacterium, a major butyrate producer, is critical for maintaining epithelial health, regulatory T-cell function, and overall immune homeostasis [23]. Its depletion signifies a loss of these protective, anti-inflammatory functions. Previous work in critical care has linked similar microbial shifts to hospital-acquired pneumonia and acute respiratory distress syndrome [10, 16]. Our study synthesizes these signals into a single, quantifiable score, moving beyond isolated associations to represent the net "infection-susceptible" burden of the gut ecology, a concept supported by similar multi-taxa approaches in other diseases [18, 19].

The most significant contribution of this study is the demonstration of incremental predictive value. The A^2DS^2 score, while useful, has inherent limitations in discrimination and calibration, as highlighted in systematic reviews [5, 6], and reflects host vulnerability but not the state of the gut-lung axis. By adding the M-score, we achieved a statistically and clinically meaningful improvement: a 0.08 increase in AUC, a significant net reclassification improvement (NRI = 0.31), and better-calibrated risk estimates. This represents a critical advance. While others have suggested the gut microbiome's potential for SAP prediction [14, 20], none have quantitatively demonstrated its added value over a standard clinical score using robust metrics like the NRI and Brier score. Our approach of augmenting an existing clinical tool, rather than building a complex new model from scratch, offers a more pragmatic and

translatable pathway for clinical implementation, as it aligns with established workflows.

5. LIMITATIONS AND FUTURE DIRECTIONS

Our findings must be interpreted in light of several limitations. First, external validation in independent, multicenter cohorts is required to confirm generalizability. Second, while we adjusted for key confounders, residual confounding and the observational design preclude causal inference; the dysbiosis we identified may be a marker of host vulnerability. Third, the use of 16S rRNA sequencing limits resolution to the genus level; future metagenomic and metabolomic studies are needed to pinpoint species and functional pathways. Finally, the current need for sequencing limits immediate clinical application; developing rapid assays for the M-score taxa is a prerequisite for practical deployment.

Future research should therefore prioritize large-scale validation, mechanistic multi-omics studies, and ultimately, randomized trials to test whether microbiome-informed interventions can prevent SAP and improve outcomes.

6. CONCLUSION

In conclusion, early gut microbiome dysbiosis after acute ischemic stroke, quantified by a simple M-score based on three bacterial genera, is an independent risk factor for SAP. This score provides significant incremental predictive value when combined with the standard A^2DS^2 clinical score, improving the identification of high-risk patients who might benefit from intensified preventive measures. These results underscore the gut microbiome's role as a key component of post-stroke pathophysiology and provide a validated, quantitative framework for integrating microbial biomarkers into clinical risk stratification.

Authors contributions: **YZ:** Conceptualization, Data curation, Formal analysis, Funding acquisition, writing – original draft, **QD:** Data curation, Formal analysis, editing draft, reviewing the draft., **WL:** Investigation, Methodology, Project administration, editing draft, reviewing the draft.

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SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1556/030.2026.02907.

ABBREVIATIONS

SAP	Stroke-associated pneumonia
AIS	Acute ischemic stroke
AUC	Areas under the curve
FDR	False discovery rate
CLR	Centered Log-Ratio
aOR	Adjusted odds ratio
NRI	Net Reclassification Improvement
CL	Confidence Interval

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