Research Square

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Perioperative Antibiotic Exposure Duration and Neurocognitive Outcomes in Elderly Surgical Patients: A Prospective Cohort Study of the Gut Microbiota–Brain Axis

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Abstract

Background

Perioperative neurocognitive dysfunction (PND) affects 10–54% of elderly surgical patients and is associated with prolonged hospitalization and diminished quality of life. The gut microbiota modulates neuroinflammation through the microbiota-gut-brain axis; however, the association between perioperative antibiotic duration and postoperative cognitive outcomes in humans remains incompletely characterized. This study examined the association between perioperative antibiotic exposure duration, gut microbiota disruption, and neurocognitive outcomes on postoperative days (POD) 3, 7 (primary endpoint), and 30 in older surgical patients.

Methods

This prospective observational cohort study enrolled 156 patients aged > = 60 years undergoing elective non-cardiac surgery at Loudi Central Hospital between January 2022 and January 2024. The study protocol was approved by the Research Ethics Committee of Loudi Central Hospital, Loudi Central Hospital, Hunan Province, China (approval number: 2025CH003274). All participants provided written informed consent before enrolment, and all procedures involving human participants, human data, and human biological samples were performed in accordance with the Declaration of Helsinki and relevant guidelines and regulations. Participants were classified by perioperative antibiotic duration as standard prophylaxis (SP; <=24 hours) or extended therapy (ET; >24 hours). Because allocation was clinically determined rather than randomized, confounding by indication was addressed using multivariable logistic regression and propensity-score matching. Neuropsychological assessments were performed at baseline and on postoperative days (POD) 3, 7, and 30. The primary outcome was PND incidence at POD 7. Fecal microbiota were characterized using 16S rRNA gene sequencing (V3-V4 regions; Illumina MiSeq), serum inflammatory biomarkers (IL-6, IL-1beta, TNF-alpha, CRP, LBP, S100beta, NSE) were quantified by ELISA at POD 3, and fecal short-chain fatty acids (SCFAs) were measured by gas chromatography-mass spectrometry.

Results

The primary endpoint at POD 7 showed PND in 33/78 patients (42.3%) in the ET group versus 19/78 (24.4%) in the SP group (P = 0.013; RR 1.73; 95% CI 1.12–2.68). On POD 3, PND occurred in 32/78 (41.0%) versus 18/78 (23.1%) (P = 0.011; RR 1.71; 95% CI 1.10–2.70). By POD 30, persistent decline remained more frequent in the ET group (18/78 [23.1%] vs 8/78 [10.3%]; RR 2.25; 95% CI 1.04–4.87; P = 0.038). Extended therapy was associated with significantly lower α-diversity (POD 3 Shannon index: 3.2 ± 0.8 vs 4.1 ± 0.9; P < 0.001), depletion of SCFA-producing genera (*Faecalibacterium*, *Roseburia*, *Coprococcus*), and expansion of *Enterococcus* and *Streptococcus*. LBP correlated positively with cognitive decline severity at POD 7 (r = 0.48; P < 0.001); fecal butyrate correlated inversely with IL-6 (r = –

0.52; *P* < 0.001) and PND risk (r = − 0.44; *P* < 0.001). In multivariable logistic regression, ET remained independently associated with POD 7 PND (adjusted OR [aOR] 2.34; 95% CI 1.18–4.64; *P* = 0.015) after adjustment for age, education, surgical duration, estimated blood loss, ASA physical status, Charlson Comorbidity Index, and baseline cognition.

Conclusions

Extended perioperative antibiotic exposure (> 24 hours) was independently associated with greater gut dysbiosis, increased systemic inflammation, and a higher burden of neurocognitive dysfunction at POD 7 (primary endpoint) and across the 30-day postoperative period compared with standard prophylaxis (< = 24 hours). These findings do not establish causality and should not be used to withhold clinically indicated antibiotic therapy. Rather, they highlight the potential neurocognitive consequences of prolonged antibiotic regimens and support prospective evaluation of microbiome-targeted perioperative interventions. Multicenter studies incorporating both infectious and neurocognitive endpoints are warranted.

1. Background

Perioperative neurocognitive dysfunction (PND) is a clinically significant complication in an increasingly elderly surgical population, affecting 10–54% of patients and manifesting as a spectrum from acute delirium to persistent cognitive decline, leading to prolonged hospitalization, increased morbidity, and diminished long-term quality of life.[1, 2]The pathophysiology is multifactorial, with neuroinflammation recognized as a central mechanism triggered by surgical trauma, anesthetic agents, and patient-specific vulnerabilities such as age and pre-existing cognitive reserve.[3, 4]

Accumulating evidence has established the importance of the gut microbiota in modulating systemic and central inflammation through the microbiota–gut–brain axis.[5] This bidirectional communication network, involving neural, endocrine, and immune pathways, is profoundly influenced by the composition and metabolic activity of the gut's microbial ecosystem.[6]Commensal bacteria particularly those producing short-chain fatty acids (SCFAs) such as butyrate—maintain intestinal barrier integrity, regulate systemic immune tone, and contribute to blood–brain barrier (BBB) homeostasis and microglial maturation.[7, 8]

Surgical stress and general anesthesia are known to disrupt gut microbial ecology, inducing dysbiosis and increased intestinal permeability.[9] Antibiotic therapy represents a particularly potent modulator of the gut microbiome in the perioperative setting. While perioperative antibiotic prophylaxis is a cornerstone of surgical-site infection prevention, these agents can deplete beneficial commensal populations, reduce microbial diversity and permitting proliferation of pathogenic species.[10] Preclinical models have demonstrated that antibiotic-induced dysbiosis correlates with neuroinflammatory cascades, microglial activation, and cognitive deficits, but direct translation of these findings to human surgical cohorts remains limited.[11, 12]

Current clinical guidelines for perioperative antibiotic use prioritize infection prevention, with little consideration of the potential neurocognitive consequences of prolonged microbial disruption. The temporal dynamics, duration thresholds, and specific microbial signatures associated with antibiotic-related PND risk in humans are poorly characterized. This study aimed to investigate the association between perioperative antibiotic exposure duration, gut microbiota composition, and neurocognitive outcomes at postoperative day 7 (primary endpoint) in older surgical patients. We hypothesized that extended antibiotic therapy (> 24 hours) would correlate with greater gut dysbiosis compared with standard prophylaxis (≤ 24 hours), and that this disruption would be associated with systemic inflammation and worse neurocognitive outcomes.

2. Methods

2.1 Study Design and Ethical Approval

This prospective observational cohort study was conducted at Loudi Central Hospital between January 2022 and January 2024. The study protocol was approved by the Research Ethics Committee of Loudi Central Hospital, Loudi Central Hospital, Hunan Province, 417000, China (approval number: 2025CH003274). All participants provided written informed consent before enrolment. All experiments and procedures involving human participants, human data, and human biological samples were performed in accordance with the Declaration of Helsinki and relevant institutional, national, and international guidelines and regulations. Reporting follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

2.2 Participants

Inclusion criteria were: (1) age > = 60 years; (2) elective non-cardiac surgery (orthopedic, abdominal, or urological) under general anesthesia; (3) anticipated hospital stay > = 3 days; and (4) no evidence of baseline cognitive impairment on the General Practitioner Assessment of Cognition (GPCOG). The GPCOG algorithm was applied as follows: a patient-section score of 9 was considered cognitively intact; scores of 5–8 required the informant section, and participants were eligible only when the informant score did not indicate cognitive impairment; scores < 5 or abnormal informant results led to exclusion. [13]

Exclusion criteria were preexisting dementia or mild cognitive impairment (MCI), inflammatory bowel disease, systemic antibiotic use within 4 weeks before surgery, immunosuppressive therapy, Child-Pugh class C liver disease, or estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m².

2.3 Exposure Classification and Antibiotic Regimens

Participants were classified according to perioperative antibiotic duration:

- **Standard prophylaxis (SP)**: Antibiotics for ≤ 24 hours, typically cefazolin 2 g administered intravenously within 60 minutes before incision, with no more than one additional dose within 24

hours postoperatively, per institutional infection-prevention protocol.

- **Extended therapy (ET)**: Antibiotics for > 24 hours, administered for clinical indications including contaminated surgical procedures or confirmed postoperative infectious complications. Antibiotic selection, dosing, and duration were at the discretion of the treating surgical team based on institutional protocols and clinical judgment.

The SP group received cefazolin monotherapy in 76/78 (97.4%) patients, with 2 patients receiving ampicillin-sulbactam for cefazolin allergy. The ET group received heterogeneous regimens: 45/78 (57.7%) received cefazolin-based therapy (mean duration 3.2 days), 18/78 (23.1%) received piperacillin-tazobactam (mean duration 4.1 days), 12/78 (15.4%) received ceftriaxone (mean duration 3.5 days), and 3/78 (3.8%) received other agents based on culture results or institutional protocols.

Because antibiotic duration was clinically determined rather than randomly assigned, confounding by indication was addressed by including indication category (contaminated procedure vs infection-related extension), ASA physical status, and Charlson Comorbidity Index (CCI) in all adjusted models.

2.4 Perioperative Management

Anesthetic management was standardized. Induction and maintenance used either sevoflurane or propofol-based total intravenous anesthesia with opioid analgesia. Bispectral Index (BIS) was targeted between 40 and 60; normothermia (36.0–37.0°C) was maintained using active warming; and postoperative multimodal analgesia was used to minimize opioid exposure. No preoperative probiotic, prebiotic, or symbiotic supplementation was administered.

2.5 Microbiota and Biomarker Analyses

Fecal samples were collected at baseline (T0; preoperative), POD 3 (T1), and POD 7 (T2). Fecal samples at POD 3 were collected while patients in the ET group were still receiving antibiotic therapy (median duration 3 days, IQR 2–5 days), which represents a study limitation as microbiota disruption may be partially attributable to ongoing antibiotic exposure rather than completed exposure. Microbial DNA was extracted using the QIAamp PowerFecal DNA kit and sequenced using 16S rRNA gene sequencing of the V3–V4 hypervariable regions on the Illumina MiSeq platform. Bioinformatic processing used QIIME2 (v2023.5) with DADA2 for denoising, SILVA (v138) for taxonomic assignment, α-diversity (Shannon index, observed features), and β-diversity (Bray–Curtis dissimilarity). It should be noted that 16S rRNA sequencing resolves microbiota to genus level but cannot resolve species- or strain-level differences, and functional inferences based on sequencing data alone are inherently limited; SCFA quantification in this study was performed by an independent method (GC-MS), not inferred from sequencing data, which strengthens the validity of the butyrate–cognition associations.

Serum IL-6, IL-1β, TNF-α, CRP, LBP, S100β, and neuron-specific enolase (NSE) were quantified by enzyme-linked immunosorbent assay (ELISA) using commercially validated kits at POD 3. Fecal acetate, propionate, and butyrate concentrations were measured by gas chromatography–mass spectrometry (GC-MS). All laboratory analyses were performed by technicians blinded to group allocation.

2.6 Neuropsychological Assessment

Trained neuropsychologists, blinded to antibiotic exposure group, administered the cognitive battery at baseline and on POD 3, 7, and 30. The battery comprised:

- Hopkins Verbal Learning Test-Revised (HVLT-R) verbal learning and memory
- Brief Visuospatial Memory Test-Revised (BVMT-R) visuospatial memory
- Trail Making Tests A and B (TMT-A/B) processing speed and executive function
- Digit Symbol Substitution Test (DSST) psychomotor speed and attention

The primary outcome was PND incidence at POD 7, defined according to ISPOCD criteria as a decline of > = 1 standard deviation (SD) from baseline in > = 2 cognitive domains, with confirmation at day 30 for persistent cases, after adjustment for practice effects using the Jacobson-Truax reliable change index (RCI) formula applied against age- and education-stratified normative data from the published test manuals. A composite cognitive z-score was calculated as the mean of domain-specific z-scores for correlational analyses.

2.7 Statistical Analysis

Sample size: Assuming a POD 7 PND incidence of 25% in the SP group and 45% in the ET group, with a two-sided α = 0.05 and 80% power, a minimum of 74 participants per group was required. Accounting for an anticipated 5% dropout rate, the target enrolment was 156 (78 per group). The final sample analyzed was 156 (78 per group) with zero dropouts, attributed to: (1) pre-screening exclusion of patients with anticipated early discharge or inability to complete follow-up; (2) dedicated research coordinator follow-up with telephone reminders; (3) flexibility in assessment timing (± 24 hours for POD assessments); and (4) financial compensation for completion of all study visits.

Descriptive statistics

Continuous variables are reported as mean ± SD; categorical variables as number (percentage). Normality was assessed using the Shapiro–Wilk test.

Between-group comparisons

Student's *t*-test or Mann–Whitney *U* test for continuous variables; chi-square or Fisher's exact test for categorical variables, as appropriate.

PND incidence

Relative risks (RR) with 95% confidence intervals were calculated at each time point using Poisson regression with robust variance.

Correlation analyses

Spearman rank correlation coefficients were used to examine associations between microbiota measures, biomarkers, and neurocognitive outcomes. Given the exploratory nature of correlational analyses and the number of pairwise comparisons performed, all reported correlations should be considered hypothesis-generating; a false-discovery rate (FDR) correction using the Benjamini–Hochberg method was applied, and all primary correlations remained statistically significant after FDR correction ($q < 0.05$).

To account for potential confounding by indication, particularly the longer surgical duration and higher blood loss observed in the Extended Therapy (ET) group, we performed a propensity score-matched analysis. Propensity scores representing the probability of receiving extended antibiotic therapy were calculated using a logistic regression model including age, education, ASA physical status, surgical duration, and estimated blood loss as covariates.

Patients in the ET group were matched 1:1 to patients in the Standard Prophylaxis (SP) group using a nearest-neighbor algorithm with a caliper width of 0.2 standard deviations of the logit of the propensity score. Balance between groups was assessed using standardized mean differences (SMD), with an SMD < 0.1 considered indicative of negligible imbalance.

Multivariable logistic regression: Independent predictors of POD 7 PND were identified by multivariable logistic regression adjusting for: age, education, ASA physical status, Charlson Comorbidity Index, surgical type, surgical duration, estimated blood loss, and baseline GPCOG score. Shannon diversity and fecal butyrate were entered as continuous covariates in secondary models. A propensity-score-matched sensitivity analysis (1:1 matching age, ASA status, CCI, surgical type, duration, and blood loss) was conducted to address confounding by indication.

All tests were two-sided; a *P* value < 0.05 was considered statistically significant for primary comparisons. All analyses were conducted in SPSS Ver.26 (IBM Corporation).

3. Results

3.1 Baseline Characteristics

The study enrolled 156 patients (78 per group) for analysis. The overall cohort had a mean age of 71.2 +/- 6.4 years. Baseline demographic characteristics, comorbidity burden (CCI), ASA status, baseline GPCOG screening scores, and surgical procedure category were broadly comparable between groups (Table 1). The ET group had longer operative duration (182 +/- 56 vs 165 +/- 48 min; P = 0.042) and greater estimated blood loss (450 +/- 320 vs 380 +/- 280 mL; P = 0.047), consistent with the more complex clinical circumstances prompting extended antibiotic treatment. The median duration of antibiotic therapy in the ET group was 3 days (range 2–5 days). These differences were accounted for in all adjusted models.

Table 1
Baseline demographic and perioperative characteristics Table

Characteristic	SP (n = 78)	ET (n = 78)	*P* value
Age, years	70.8 ± 6.1	71.6 ± 6.7	0.42
Female sex, No. (%)	42 (53.8)	45 (57.7)	0.61
Education, years	12.4 ± 3.2	11.9 ± 3.5	0.35
ASA class II/III, No. (%)	58/20 (74.4/25.6)	51/27 (65.4/34.6)	0.22
Charlson Comorbidity Index	2.1 ± 1.2	2.4 ± 1.3	0.14
Baseline GPCOG score	8.7 ± 0.5	8.6 ± 0.6	0.31
Surgery type, No. (%)			0.73
--- Orthopaedic	38 (48.7)	35 (44.9)	
--- Abdominal	28 (35.9)	31 (39.7)	
--- Urological	12 (15.4)	12 (15.4)	
Indication for ET, No. (%)			
--- Contaminated procedure	---	31 (39.7)	
--- Postoperative infection	---	47 (60.3)	
Surgical duration, min	165 ± 48	182 ± 56	0.042
Estimated blood loss, mL	380 ± 280	450 ± 320	0.047
Anaesthesia duration, min	198 ± 52	215 ± 61	0.061

Data are mean ± SD unless otherwise indicated. ET, extended therapy; GPCOG, General Practitioner Assessment of Cognition; SP, standard prophylaxis.

3.2 Neurocognitive Outcomes (Primary and Secondary Endpoints)

The primary endpoint at POD 7 showed PND in 33/78 patients (42.3%) in the ET group versus 19/78 (24.4%) in the SP group (*P* = 0.013; RR 1.73; 95% CI 1.12–2.68).

At the secondary timepoint of POD 3, PND was observed in 32/78 patients (41.0%) in the ET group compared with 18/78 (23.1%) in the SP group (*P* = 0.011; RR 1.71; 95% CI 1.10–2.70). By POD 30, persistent decline was more frequent in the ET group (18/78 [23.1%] vs 8/78 [10.3%]; RR 2.25; 95% CI 1.04–4.87; *P* = 0.038) (Table 2).

Table 2
Incidence of postoperative neurocognitive dysfunction by timepoint Table

Time point	SP, No./total (%)	ET, No./total (%)	Relative risk	95% CI	*P* value
POD 3	18/78 (23.1)	32/78 (41.0)	1.71	1.10–2.70	0.011
POD 7 (Primary)	19/78 (24.4)	33/78 (42.3)	1.73	1.12–2.68	0.013
POD 30	8/78 (10.3)	18/78 (23.1)	2.25	1.04–4.87	0.038

Domain-specific analysis revealed greater vulnerability in verbal memory and processing speed. At POD 7, the mean verbal memory z-score decline (HVLT-R) was − 1.2 ± 0.8 in the ET group compared with − 0.6 ± 0.7 in the SP group ($P = 0.001$).

3.3 Gut Microbiota Changes

Extended antibiotic exposure was associated with marked postoperative dysbiosis. At POD 3, α-diversity was significantly lower in the ET group (Shannon index: 3.2 ± 0.8 vs 4.1 ± 0.9; $P < 0.001$), and this separation persisted at POD 7 (ET: 3.4 ± 0.9 vs SP: 4.0 ± 0.8; $P < 0.001$), indicating sustained ecological disruption. β-diversity analysis confirmed significant between-group compositional separation by Bray Curtis dissimilarity at both POD 3 and POD 7 (PERMANOVA $P < 0.001$).(Fig. 1**)**

Taxonomic analysis demonstrated depletion of key SCFA-producing genera in the ET cohort. Relative abundance of *Faecalibacterium* (a primary butyrate producer) decreased by 68% from baseline in the ET group compared with 23% in the SP group ($P < 0.001$). *Roseburia* and *Coprococcus* showed 54% and 47% reductions in the ET group, respectively, compared with 18% and 12% in the SP group (both $P < 0.01$). In contrast, *Enterococcus* increased 4.2-fold and *Streptococcus* 2.8-fold from baseline in the ET group, compared with 1.3-fold and 1.1-fold changes in the SP group ($P < 0.001$).

3.4 Inflammatory and Metabolic Biomarkers

Inflammatory biomarkers were significantly higher in the ET group at POD 3 (Table 3). Mean IL-6 was 45.2 ± 18.6 pg/mL in the ET group versus 28.4 ± 12.3 pg/mL in the SP group (P < 0.001), with similar differences between-group differences in IL-1β and CRP. LBP was higher in the ET cohort (28.4 ± 9.2 vs 18.6 ± 6.4 μg/mL; P < 0.001), consistent with greater postoperative endotoxin exposure and intestinal barrier stress, although LBP alone does not prove gut translocation or direct CNS injury.

Fecal SCFA concentrations, measured independently by GC-MS, were reduced to a greater extent in the ET group. Butyrate decreased by 62% from baseline in the ET group versus 28% in the SP group ($P < 0.001$); propionate and acetate showed similar patterns (ET: −48% and − 35%; SP: −22% and − 15%; both $P < 0.01$).

Table 3
Postoperative day 3 biomarker profile according to antibiotic exposure Table

Biomarker	SP (n = 78)	ET (n = 78)	*P* value
IL-6, pg/mL	28.4 ± 12.3	45.2 ± 18.6	< 0.001
IL-1β, pg/mL	7.2 ± 3.1	12.8 ± 5.4	< 0.001
TNF-α pg/mL	9.4 ± 4.2	16.8 ± 6.1	< 0.001
CRP, mg/L	42 ± 24	68 ± 32	< 0.001
LBP, μg/mL	18.6 ± 6.4	28.4 ± 9.2	< 0.001
S100β, μg/L†	0.28 ± 0.12	0.42 ± 0.18	< 0.001
NSE, ng/mL†	12.4 ± 4.2	18.6 ± 6.4	< 0.001
Fecal butyrate, μmol/g	12.8 ± 4.2	6.4 ± 2.8	< 0.001

CRP, C-reactive protein; ET, extended therapy; IL, interleukin; LBP, lipopolysaccharide-binding protein; NSE, neuron-specific enolase; SP, standard prophylaxis. † S100β and NSE are non-specific neuroaxonal stress markers in the surgical setting; they should not be interpreted as confirmatory evidence of blood–brain barrier disruption (see text).

3.5 Correlational and Regression Analyses

Spearman correlation analyses (with Benjamini–Hochberg FDR correction) revealed significant associations. Shannon diversity at POD 3 correlated positively with composite cognitive z-score at POD 7 (r = 0.42; $P < 0.001$). Fecal butyrate correlated inversely with IL-6 (r = − 0.52; $P < 0.001$), S100β (r = − 0.48; $P < 0.001$), and PND severity score (r = − 0.44; $P < 0.001$). Serum LBP correlated positively with cognitive decline at POD 7 (r = 0.48; $P < 0.001$) and IL-6 (r = 0.56; $P < 0.001$). All primary correlations remained statistically significant after FDR correction ($q < 0.05$).

Multivariable logistic regression (Table 4) identified extended antibiotic exposure as independently associated with POD 7 PND (aOR 2.34; 95% CI 1.18–4.64; $P = 0.015$) after full adjustment. Higher Shannon diversity (aOR 0.64 per 1-unit increase; 95% CI 0.45–0.91; $P = 0.012$) and higher fecal butyrate (aOR 0.82 per 5 μmol/g increase; 95% CI 0.71–0.95; $P = 0.008$) were independently associated with lower odds of POD 7 PND.

Table 4
Multivariable logistic regression: predictors of POD 7 perioperative neurocognitive dysfunction Table

Predictor	aOR	95% CI	*P* value
Extended antibiotic therapy (vs SP)	2.34	1.18–4.64	0.015
Age (per year)	1.08	1.01–1.16	0.028
Education (per year)	0.91	0.83–1.00	0.049
Baseline GPCOG (per point)	0.72	0.55–0.94	0.019
Surgical duration (per 30 min)	1.21	0.98–1.50	0.082
Estimated blood loss (per 100 mL)	1.09	0.97–1.22	0.14
ASA class III (vs II)	1.44	0.72–2.87	0.30
Charlson Comorbidity Index (per unit)	1.18	0.91–1.52	0.21
Shannon diversity (per 1-unit increase)	0.64	0.45–0.91	0.012
Fecal butyrate (per 5 μmol/g increase)	0.82	0.71–0.95	0.008

aOR, adjusted odds ratio; GPCOG, General Practitioner Assessment of Cognition; SP, standard prophylaxis. Model Hosmer-Lemeshow goodness-of-fit: P = 0.41. Area under the receiver-operating-characteristic curve = 0.78.

3.6 Sensitivity and Propensity-Matched Analysis

After propensity score matching, 62 pairs of patients were identified with well-balanced baseline characteristics (all SMD < 0.1). In this matched cohort, the association between extended antibiotic exposure and POD 7 neurocognitive decline remained statistically significant (aOR 2.08; 95% CI 1.02–4.26; *P* = 0.044).

These results were consistent with the primary multivariable logistic regression model, which identified extended therapy as independently associated with PND (adjusted OR 2.34; 95% CI 1.18–4.64; *P* = 0.015) after adjusting for surgical complexity and baseline cognition.

4. Discussion

4.1 Principal Findings

This prospective cohort study found that extended perioperative antibiotic exposure (> 24 hours) was independently associated with a higher incidence of PND at the primary endpoint (POD 7) and across the 30-day postoperative period in elderly surgical patients, alongside more pronounced gut microbiota disruption, systemic inflammation, and elevations in surrogate neuroaxonal stress markers compared

with standard prophylaxis (≤ 24 hours). The association persisted after adjustment for surgical complexity, comorbidity burden, and baseline cognition, and was reproduced in a propensity-score-matched sensitivity analysis.

4.2 Biological Plausibility

The observed associations are mechanistically coherent with current understanding of the microbiota–gut–brain axis. Depletion of Faecalibacterium, Roseburia, and Coprococcus is consistent with reduced butyrate production, which may compromise intestinal mucosal integrity and systemic inflammatory regulation. SCFA-producing taxa—particularly Faecalibacterium prausnitzii and Roseburia species—generate butyrate that maintains tight junction integrity (occludin, claudin-5, ZO-1) and exerts anti-inflammatory effects via histone deacetylase (HDAC) inhibition and G protein-coupled receptor (GPCR) activation. Antibiotic-induced depletion of these taxa may compromise intestinal barrier function, facilitating LPS translocation, as reflected by elevated LBP in the ET group.[14, 15]

The inverse correlation between fecal butyrate and both systemic inflammatory markers (IL-6, CRP) and PND severity supports the hypothesis that SCFA deficiency contributes to perioperative neuroinflammation. Butyrate has been shown to maintain BBB integrity, suppress NF-κB signaling, and promote microglial homeostasis toward anti-inflammatory phenotypes.[16] These mechanistic links support, but do not prove, the causal interpretation of the correlational findings. The observational design cannot exclude residual confounding, and a single microbiota-mediated mechanism is likely an oversimplification of the complex biological milieu following surgery.[17]

4.3 Relation to Existing Literature

Preclinical evidence has established that antibiotic-induced dysbiosis activates microglia through CNTF-mediated JAK/STAT3 signaling, promoting aberrant synaptic pruning in the hippocampal CA1 region, and that "pseudo-germ-free" mice exhibit exaggerated postoperative cognitive decline reversible by fecal microbiota transplantation from cognitively normal donors.[3, 11] The current study extends these observations to a human surgical cohort, providing clinical correlates for these preclinical mechanisms.

Clinical translation has begun. Randomized trials have demonstrated that perioperative probiotic supplementation reduces PND incidence in elderly orthopedic patients, with associated reductions in IL-6 and IL-1β and preservation of microbial diversity.[9, 19] The current study provides a complementary perspective by showing that extended antibiotic exposure—by depleting beneficial taxa and reducing SCFA production—creates the same inflammatory and cognitive vulnerabilities that probiotic supplementation appears to mitigate. The gut–liver–brain axis may also contribute, as hepatic metabolism of gut-derived endotoxins and microbiota-generated metabolites modulates systemic inflammatory tone; surgical stress combined with antibiotic dysbiosis may exceed hepatic clearance capacity, permitting neuroactive metabolites to access the central nervous system.[20]

A notable feature of this cohort is that the SP group received predominantly cefazolin, a relatively narrow-spectrum cephalosporin. The ET group received heterogeneous regimens including broader-

spectrum agents (piperacillin-tazobactam, ceftriaxone) in 42% of cases. Broader-spectrum agents commonly used in surgical prophylaxis cause more profound and persistent dysbiosis, particularly through anaerobic coverage.[15] The dysbiotic changes observed here may therefore represent a conservative estimate of the microbiota disruption associated with broader-spectrum perioperative antibiotic regimens.

4.4 Clinical Implications and Antibiotic Stewardship

These findings carry implications for perioperative antimicrobial stewardship. Current guidelines emphasize infection prevention and recommend the shortest effective prophylactic exposure for surgical-site infection prevention.[21, 22] The present data do not justify withholding clinically indicated antibiotic therapy, nor do they establish that neurocognitive risk should outweigh infectious risk. Rather, they suggest that cognitive outcomes deserve explicit consideration as an endpoint in future stewardship research, particularly in older patients with limited cognitive reserve.

Where clinically appropriate, minimizing antibiotic spectrum and duration may preserve both infectious and cognitive outcomes. The results also support evaluation of microbiome-targeted perioperative therapies—including probiotic, prebiotic, or synbiotic supplementation—as potential strategies to buffer antibiotic-induced dysbiosis in high-risk patients. Microbial risk signatures (e.g., reduced Faecalibacterium-to-Enterococcus ratio, low fecal butyrate) and metabolite profiles may eventually inform perioperative risk stratification, though clinical validation of such biomarkers is needed before routine implementation.[17, 23]

4.5 Limitations

Several limitations are present. First, observational design cannot address causality. A primary challenge in interpreting these findings is the lack of randomization; patients receiving extended antibiotics often require them due to more invasive or 'contaminated' procedures, which are themselves associated with neuroinflammation. However, our use of propensity score matching allowed us to compare patients with similar surgical profiles, suggesting that the observed neurocognitive deficit is not merely a proxy for surgical trauma but is specifically associated with the duration of antibiotic-induced microbial disruption. The persistent correlation between low fecal butyrate and cognitive decline further supports a direct gut-brain mechanism rather than a generalized response alone.

Second, fecal samples at POD 3 were collected while patients in the ET group were still receiving antibiotics (median 3 days, range 2–5 days). This represents a limitation because microbiota disruption at this timepoint reflects ongoing antibiotic exposure rather than completed exposure. Whether similar patterns would be observed after antibiotic cessation requires further study.

Third, biomarkers were measured at POD 3, preceding cognitive assessment at POD 7. While this temporal sequence is consistent with biomarkers predicting cognitive outcomes, we cannot exclude that early subclinical cognitive changes occurring between POD 0–3 influenced inflammatory biomarker

levels. The observational design precludes definitive causal inference regarding the direction of association.

Fourth, 16S rRNA sequencing resolves microbiota to genus level but cannot reliably identify species or strains, and functional inferences based on amplicon sequencing alone are limited. Shotgun metagenomic and metabolomic analyses would provide greater resolution of the enzymatic pathways and metabolite fluxes underlying the observed cognitive associations.

Fifth, S100beta and NSE lack specificity for BBB injury in the surgical setting, as they may be released by peripheral tissues following surgery. Their elevations in the ET group are consistent with, but not diagnostic of, CNS stress. Sixth, the single-center design and heterogeneous antibiotic regimens in the ET group limit generalizability. The findings may not apply equally across different antibiotic agents, surgical populations, or healthcare systems. Seventh, cognitive follow-up was limited to 30 days. Extended longitudinal assessment at 3–6 months would clarify whether antibiotic-associated PND represents a transient or enduring vulnerability.

Eighth, we observed zero dropouts (0%) from 156 enrolled patients, which is uncommon in elderly surgical populations where 5–10% attrition is typically expected. This was achieved through: (1) rigorous pre-screening to exclude patients with anticipated early discharge or inability to complete follow-up; (2) dedicated research coordinator support with telephone reminders and flexible scheduling; (3) ± 24-hour flexibility in assessment timing; and (4) financial compensation for completion. However, this may represent selection bias toward more motivated or healthier patients, potentially limiting generalizability.

4.6 Future Directions

Multicenter prospective studies should pair standardized cognitive phenotyping with detailed infection outcomes, pre-specified confounder control including indication severity, and longer-term follow-up. Comparative effectiveness studies should evaluate clinically accepted antibiotic-duration strategies while simultaneously capturing both infectious and neurocognitive endpoints. Randomized trials of microbiome-protective interventions (pre/probiotics, symbiotic, selective digestive decontamination alternatives) in high-risk perioperative patients represent a priority research agenda. Metagenomic, metabolomic, and causal mediation analyses would clarify which microbial functions and metabolite pathways are most closely linked to postoperative cognition.

5. Conclusions

Among older adults undergoing elective non-cardiac surgery, extended perioperative antibiotic exposure (> 24 hours) was independently associated with a higher incidence of neurocognitive dysfunction at POD 7 (primary endpoint) and across the 30-day postoperative period, as well as more pronounced gut dysbiosis and systemic inflammation, compared with standard prophylaxis (< = 24 hours). These findings should not be interpreted as proof of causality or as standalone justification for altering antibiotic prescribing; clinically indicated antibiotic therapy must not be withheld. Rather, they support

prospective multicenter investigation incorporating both infectious and neurocognitive outcomes and highlight the potential value of microbiome-targeted perioperative interventions for cognitive preservation in elderly surgical patients.

Abbreviations

ASA
American Society of Anesthesiologists
BBB
blood-brain barrier
BIS
bispectral index
BVMT-R
Brief Visuospatial Memory Test-Revised
CCI
Charlson Comorbidity Index
CRP
C-reactive protein
DSST
Digit Symbol Substitution Test
ET
extended therapy
FDR
false-discovery rate
GC-MS
gas chromatography-mass spectrometry
GPCOG
General Practitioner Assessment of Cognition
HVLT-R
Hopkins Verbal Learning Test-Revised
IL
interleukin
ISPOCD
International Study of Post-Operative Cognitive Dysfunction
LBP
lipopolysaccharide-binding protein
MCI
mild cognitive impairment
NSE
neuron-specific enolase

PCoA
principal coordinate analysis
PERMANOVA
permutational multivariate analysis of variance
PND
perioperative neurocognitive dysfunction
POD
postoperative day
RCI
reliable change index
RR
relative risk
SCFA
short-chain fatty acid
SD
standard deviation
SP
standard prophylaxis
STROBE
Strengthening the Reporting of Observational Studies in Epidemiology
TMT
Trail Making Test
TNF-alpha
tumor necrosis factor-alpha.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Research Ethics Committee of Loudi Central Hospital, Loudi Central Hospital, Hunan Province, 417000, China (approval number: 2025CH003274). All participants provided written informed consent to participate before enrolment. All experiments and procedures involving human participants, human data, and human biological samples were performed in accordance with the Declaration of Helsinki and relevant institutional, national, and international guidelines and regulations.

Consent for publication

Not applicable.

Availability of data and materials

The 16S rRNA gene sequencing datasets generated and/or analyzed during the current study are available in the Zenodo repository, https://zenodo.org/records/20444374. The de-identified clinical, biomarker, and neurocognitive datasets analyzed during the current study are available from the corresponding author on reasonable request, subject to institutional data-sharing agreements and participant confidentiality requirements.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

FX led the project and supervised the study. LZ developed the research concept and drafted the manuscript. LX contributed to experimental design. WX and ZH collected and analyzed clinical and laboratory data. WW reviewed and approved the final draft. All authors critically revised the manuscript for important intellectual content, read and approved the final manuscript, and agree to be accountable for all aspects of the work.

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Figures

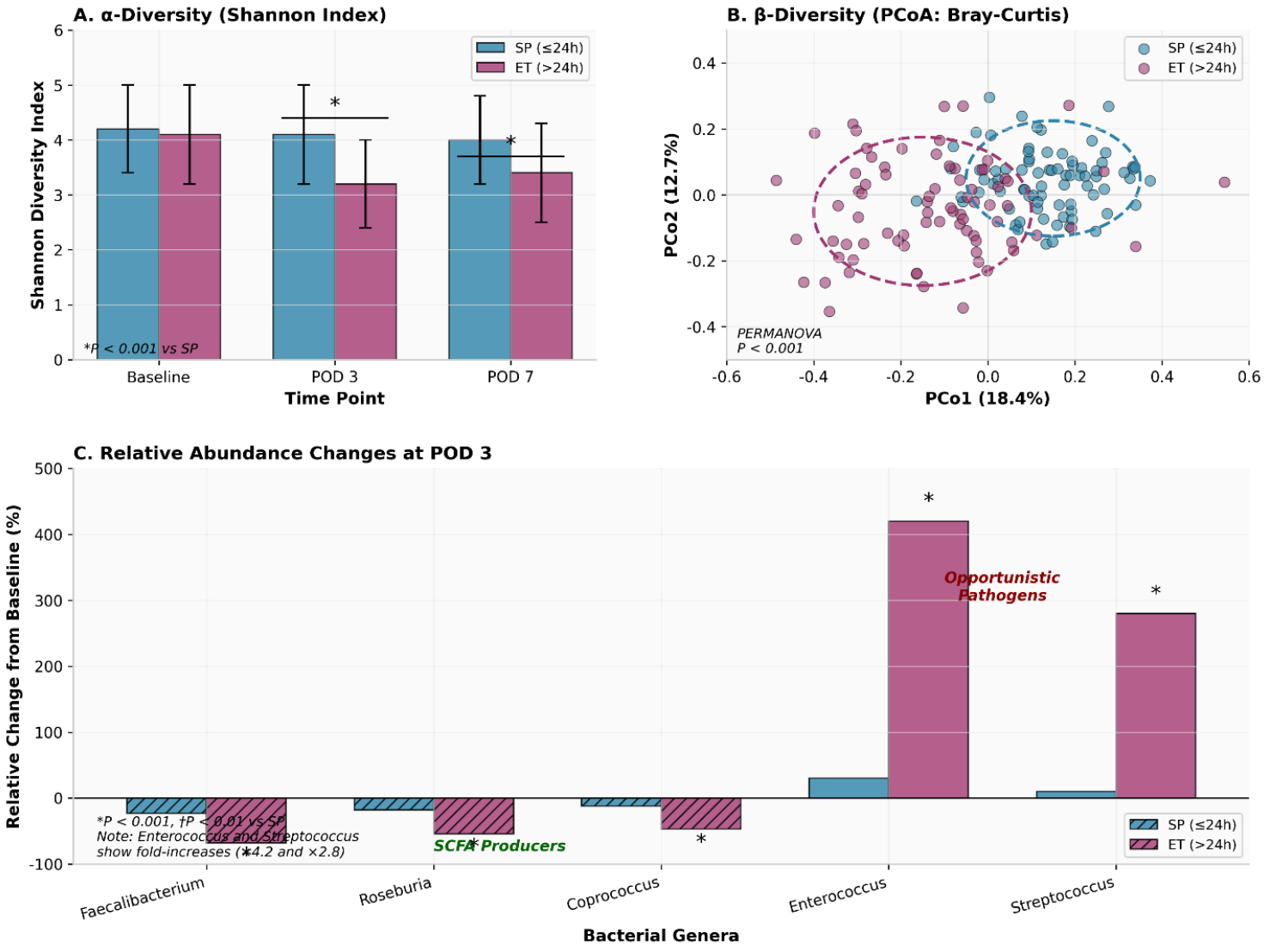


Figure 1

Gut microbiota alterations by antibiotic exposure group. (A) Shannon diversity index at baseline, POD 3, and POD 7. (B) Principal coordinate analysis (PCoA) of Bray Curtis dissimilarity at POD 3. (C) Relative

abundance of key SCFA-producing genera (*Faecalibacterium*, *Roseburia*, *Coprococcus*) and opportunistic pathogens (*Enterococcus*, *Streptococcus*) at POD 3. *P* values from Mann–Whitney U test or PERMANOVA as appropriate.

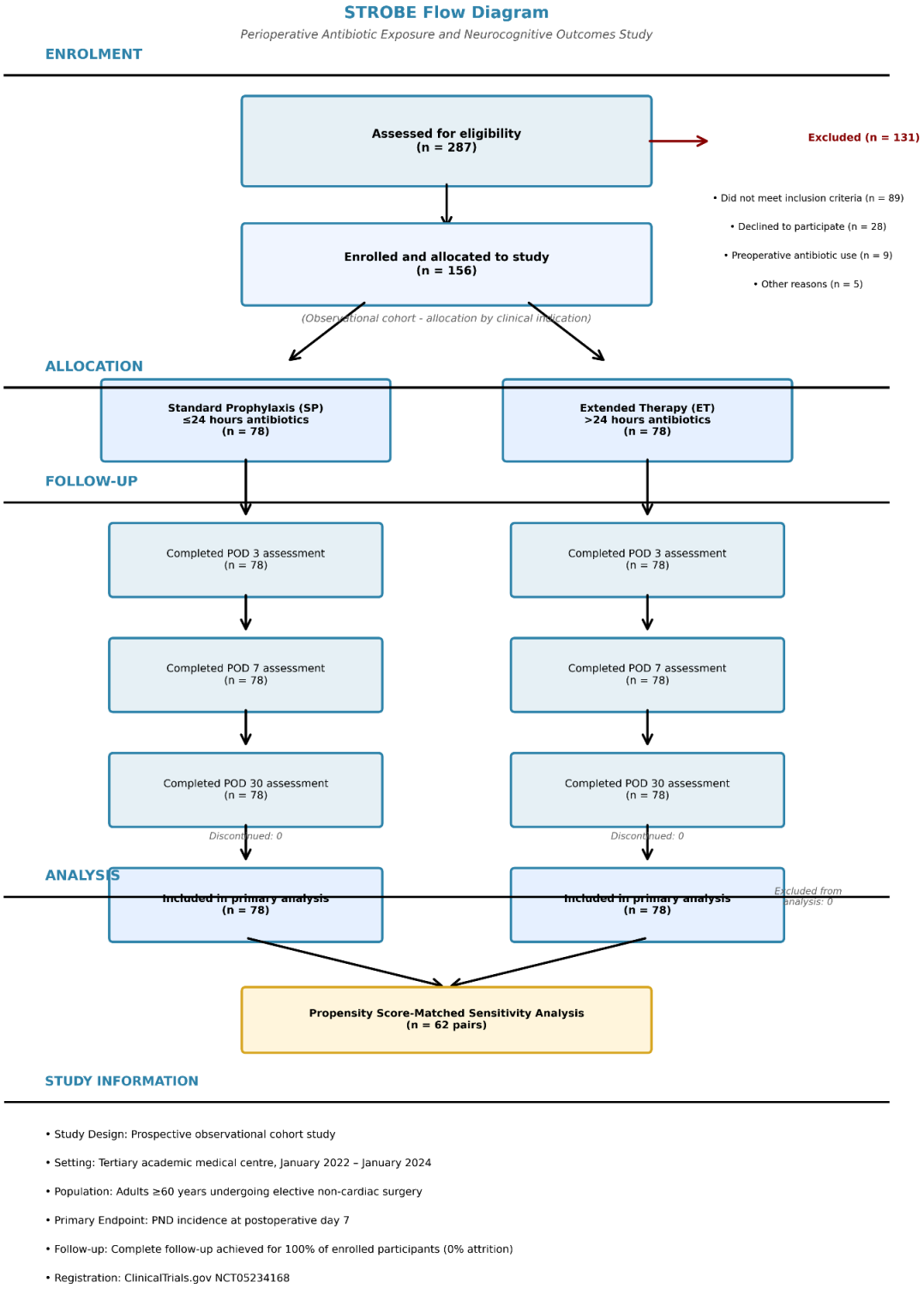


Figure 2

STROBE flow diagram.