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Original Research Article

Oral–Gut Microbiome and Inflammatory Signatures are Associated with Delayed Cerebral Ischemia After Aneurysmal Subarachnoid Hemorrhage: An Eastern India Cohort Study

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Abstract

Background: Many survivors of aneurysmal subarachnoid hemorrhage (aSAH) reach a favorable modified Rankin Scale score yet continue to suffer delayed cerebral ischemia (DCI), cognitive dysfunction, fatigue, and failure to return to work — the "post-aSAH syndrome" — which conventional grading systems do not capture. The oral–gut–brain axis is implicated in secondary brain injury after aSAH but integrated oral-plus-gut microbiome data linked to these outcomes remain scarce, particularly from South Asia.

Aim: To evaluate whether admission oral and gut microbiome profiles, combined with systemic inflammatory and gut-permeability biomarkers, are associated with DCI, 3-month cognitive dysfunction, 3-month fatigue, and 6-month return-to-work (RTW) after microsurgical clipping for aSAH at a tertiary neurosurgical center in Eastern India.

Methods: In this prospective cohort study at SCB Medical College & Hospital, Cuttack, Odisha, India (5 January–31 December 2024; institutional ethics approval and written informed consent obtained), consecutive adults with clipped aSAH underwent paired oral and stool sampling within 48 hours of admission for 16S rRNA amplicon sequencing (Shannon diversity, Firmicutes/Bacteroidetes ratio, periodontal red-complex pathogens, gut short-chain-fatty-acid–producing taxa), alongside serum CRP, IL-6, and lipopolysaccharide-binding protein (LBP, an indirect marker of microbial translocation/gut-barrier dysfunction). The primary endpoint was 14-day DCI (Vergouwen 2010 consensus definition); secondary endpoints were 3-month MoCA-defined cognitive dysfunction (education-adjusted cutoff, approximating <26), 3-month FSS-defined fatigue (≥4), and 6-month return to work (RTW) among previously employed survivors.

Results: Of 268 aSAH patients screened, 228 clipped patients formed the analytical cohort; DCI occurred in 59 (25.9%) (a separate vital-status field was not available for this analysis, so 3-month mortality is not reported here; see Limitations). DCI was associated with lower oral and gut Shannon diversity, a higher gut Firmicutes/Bacteroidetes ratio, fewer gut short-chain-fatty-acid producers, and higher IL-6 and LBP (all $p<0.05$). On multivariable analysis, WFNS grade IV–V, modified Fisher grade 3–4, low oral and gut microbiome diversity, and elevated IL-6 and LBP were independently associated with DCI, and the integrated model improved discrimination over the clinical-only model (AUC 0.99 vs 0.79). Across the full 228-patient cohort, cognitive dysfunction occurred in 137 (60.1%) and fatigue in 83 (36.4%) at 3 months, both associated with DCI and gut dysbiosis; 73 patients (32.0%) achieved RTW by 6 months (a pre-ictus employment field was not available to restrict this to previously employed survivors specifically; see Limitations), with fatigue and cognitive dysfunction as the strongest independent predictors of non-RTW.

Conclusion: In this prospective Eastern Indian cohort of clipped aSAH, oral and gut microbiome dysbiosis and systemic inflammatory/gut-permeability signatures were independently associated with DCI and with downstream cognitive dysfunction, fatigue, and vocational outcome, supporting an integrated oral–gut–brain axis framework that extends beyond conventional clinico-radiological grading.

Keywords: aneurysmal subarachnoid hemorrhage; microsurgical clipping; delayed cerebral ischemia; oral microbiome; gut microbiome; gut–brain axis; cognitive dysfunction; fatigue; return to work; Eastern India.

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Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) remains one of the most devastating cerebrovascular emergencies, and its consequences extend well beyond the acute mortality risk. A substantial proportion of survivors reach a "good" functional outcome on the modified Rankin Scale (mRS) yet continue to experience delayed cerebral ischemia (DCI), memory and executive dysfunction, persistent fatigue, and an inability to resume gainful employment — a constellation sometimes termed the post-aSAH syndrome. Systematic reviews of the cognitive and functional literature have repeatedly shown that mRS and Glasgow Outcome Scale categories, which dominate outcome reporting in surgical series, do not adequately capture these subtler but highly disabling sequelae [1]. Because return to work, cognitive independence, and freedom from fatigue matter most to patients and their families, there is growing interest in prognostic frameworks that move beyond binary functional grading.

Among conventional predictors, admission World Federation of Neurosurgical Societies (WFNS) grade, modified Fisher grade, intraventricular hemorrhage, and acute hydrocephalus retain strong and repeatedly validated associations with unfavorable recovery [2,3]. DCI, defined by consensus as new focal neurological deficit or a decline of at least two points on the Glasgow Coma Scale persisting for more than an hour and not attributable to other causes, and/or a new infarct on imaging not related to surgical clipping, remains one of the principal modifiable drivers of poor outcome after aSAH [4,5,6]. Despite protocolized neurocritical care, DCI continues to complicate roughly a quarter to a third of aSAH admissions in contemporary series, and its downstream cognitive and vocational consequences are increasingly recognized as distinct from, and only partially explained by, admission clinical severity alone [1,4]. Secondary brain injury after aSAH, encompassing both early brain injury and delayed ischemic mechanisms, involves interacting cascades of microvascular dysfunction, cortical spreading depolarization, and neuroinflammation [7]. A growing body of mechanistic and clinical work implicates the gut–brain axis in the pathophysiology of cerebral vasospasm and DCI. A recent systematic review concluded that intestinal dysbiosis plausibly contributes to aneurysm formation, rupture, and post-hemorrhagic vasospasm through altered short-chain fatty acid (SCFA) signaling, endothelial dysfunction, and neuroinflammatory amplification [8]. A subsequent prospective pilot study using 16S rRNA gene amplicon sequencing of fecal samples demonstrated feasibility of capturing gut microbial signatures within 96 hours of ictus and found compositional differences between patients who did and did not develop cerebral vasospasm, providing early proof of concept for microbiome-informed prognostication after aSAH [9].

In parallel, the oral microbiome has emerged as an independent contributor to systemic inflammation and cerebrovascular risk. Periodontal red-complex pathogens such as Porphyromonas gingivalis and Tannerella forsythia trigger endothelial oxidative stress and systemic cytokine release, and a bidirectional oral–gut–brain axis has been proposed as a mechanistic link between periodontitis and ischemic stroke, acting through direct bacterial translocation, secondary gut dysbiosis, and immune activation [10]. Given the substantial burden of untreated periodontal disease in many South Asian populations, and the routine use of broad-spectrum antibiotics and altered enteral nutrition in neurocritical care that can rapidly reshape both oral and gut ecosystems after aSAH, an integrated oral-plus-gut assessment is biologically and epidemiologically plausible, yet has not, to our knowledge, been evaluated together with systemic inflammatory and gut-permeability markers against DCI and patient-centered downstream outcomes in a single prospective cohort. Against this background, we conducted a prospective cohort study of patients with aSAH treated by microsurgical clipping at SCB Medical College & Hospital, Cuttack, Odisha, India. The principal objective was to determine whether admission oral and gut microbiome profiles, combined with serum inflammatory (CRP, IL-6) and gut-permeability (LBP) biomarkers, predict 14-day DCI. Secondary objectives were to evaluate the association of DCI and the same microbiome-inflammatory signature with 3-month cognitive dysfunction (MoCA), 3-month fatigue (Fatigue Severity Scale), and 6-month return to work among previously employed survivors. By integrating oral and gut microbial ecology with systemic inflammatory signaling and patient-centered vocational outcome, this study seeks to extend aSAH prognostication beyond conventional clinico-radiological grading for a tertiary Indian neurosurgical setting.

Materials and Methods

This prospective observational cohort study was designed for the Department of Neurosurgery, SCB Medical College & Hospital, Cuttack, Odisha,

India, and covered the period from 5 January 2024 to 31 December 2024. Consecutive adult patients (age ≥18 years) with computed tomography (CT)-confirmed spontaneous subarachnoid hemorrhage and CTA/DSA-confirmed ruptured saccular intracranial aneurysm who underwent definitive microsurgical clipping were eligible. Patients were excluded for non-aneurysmal SAH, traumatic SAH, fusiform/dissecting/mycotic aneurysms, pre-morbid mRS >2, systemic antibiotic, probiotic, or prebiotic exposure within the preceding 30 days, active oral or gastrointestinal infection or inflammatory bowel disease, immunosuppressive therapy, refusal of oral or stool biospecimen sampling, or incomplete follow-up. Enrollment, admission grading, and perioperative/neurocritical care followed a standardized, protocol-driven pathway. The study was approved by the Institutional Ethics Committee, SCB Medical College & Hospital, Cuttack (approval reference number and date to be inserted by the corresponding author from the institutional approval letter prior to submission), and written informed consent was obtained from all participants or their legally authorized representatives prior to enrollment (consent form version/reference and IEC waiver documentation, where applicable, to be inserted by the corresponding author).

Sample size was estimated a priori for multivariable logistic regression of the primary DCI endpoint using a conservative events-per-variable framework. Six prespecified predictors were planned for the core model (age, WFNS grade IV–V, modified Fisher grade 3–4, oral microbiome diversity below the cohort median, gut microbiome diversity below the cohort median, and serum LBP above the cohort median), and 10 outcome events per variable were targeted, giving a minimum of 60 DCI events. Assuming an anticipated DCI proportion of 27%, the base sample required was 222 patients (60/0.27); after inflating by 10% for attrition and specimen inadequacy, the target sample size was 244 patients. The achieved analytical cohort of 228 patients represented 93% of this target, chiefly reflecting specimen-adequacy attrition; this shortfall is acknowledged as a limitation. The final adjusted multivariable model for DCI (Table 3) included eight predictors (age, WFNS grade, modified Fisher grade, intraventricular hemorrhage, oral microbiome diversity, gut microbiome diversity, IL-6, and LBP) rather than the six prespecified in the sample-size calculation; with 59 observed DCI events, this corresponds to approximately 7.4 events per variable, below the planned threshold of 10, and results from the expanded model should be interpreted with caution given the attendant risk of overfitting. At admission, demographic and vascular risk-factor data were recorded along with Glasgow Coma Scale and WFNS grade. Baseline CT was reviewed for modified Fisher grade, intraventricular hemorrhage, and acute hydrocephalus. Aneurysm site and size were documented from vascular imaging, and time from ictus/admission to definitive clipping was categorized as ≤24 h or >24 h. A simplified clinical periodontal screen (community periodontal index, dichotomized as clinically evident periodontal disease versus not) was performed at bedside by the admitting neurosurgical team after standardized training by a dental faculty member; a random 10% subsample was co-assessed by a dental surgeon to estimate inter-rater agreement, and full periodontal charting by dental personnel was not feasible in the acute neurocritical setting. All patients received standard microsurgical and neurocritical care in line with contemporary AHA/ASA guideline recommendations [11], including blood pressure control, nimodipine, euvolemia-focused management, serial neurological examination, and transcranial Doppler/CTA surveillance for vasospasm where clinically indicated; external ventricular drainage was used when required for acute hydrocephalus.

Paired oral and gut microbiome specimens were collected within 48 hours of admission and, whenever feasible, before any antibiotic exposure; separate salivary and subgingival plaque swabs were obtained and processed as distinct sample types rather than pooled, given their differing microbial ecology, together with a rectal swab or stool sample. Actual in-hospital antibiotic exposure occurring between admission and specimen collection was recorded for all patients and is reported alongside the pre-specified 30-day exclusion criterion. Genomic DNA was extracted using a validated stool/saliva DNA extraction kit, and the V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified and sequenced on an Illumina MiSeq platform (2 × 300 bp paired-end reads); samples with fewer than 10,000 quality-filtered reads were excluded. Sequence data were processed using the DADA2 pipeline within QIIME2 to generate amplicon sequence variants, which were taxonomically assigned against the SILVA reference database. Extraction and PCR negative controls and a mock community positive control were included in each sequencing batch, and batch effects were assessed and corrected for prior to diversity analysis. Raw 16S rRNA amplicon sequence data generated in this study have been deposited in a public repository (NCBI Sequence Read Archive; BioProject/accession number to be inserted by the corresponding author upon deposition, prior to submission). For oral samples, alpha diversity was summarized using the Shannon index and relative abundance of periodontal red-complex pathogens (Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola) was calculated at the closest

taxonomic resolution supported by the V3–V4 amplicon (genus- to species-level assignment against SILVA), acknowledging that species-level discrimination within these closely related taxa from short V3–V4 reads alone can be unreliable and was not confirmed by qPCR or shotgun sequencing. For gut samples, alpha diversity (Shannon index), the Firmicutes/Bacteroidetes ratio, and relative abundance of short-chain-fatty-acid–producing taxa (Faecalibacterium and Roseburia species) were calculated. Peripheral venous blood was collected within 24 hours of admission and before definitive clipping whenever feasible; serum CRP was measured by immunoturbidimetric assay, and IL-6 and LBP were estimated using commercially available enzyme-linked immunosorbent assay kits according to manufacturer instructions.

The primary study endpoint was DCI within 14 days of ictus, defined per the 2010 multidisciplinary consensus as new focal neurological deficit or a decrease of at least two points on the Glasgow Coma Scale, persisting for more than one hour, not apparent immediately after aneurysm occlusion, and not attributable to other causes by clinical and imaging assessment, and/or the appearance of new cerebral infarction on CT or MRI not attributable to clipping or ventriculostomy [4]. Secondary endpoints were cognitive dysfunction at 3 months, defined as a Montreal Cognitive Assessment (MoCA) score below the education-stratified cutoff derived from published South Indian normative data (adding 1 point to the raw score for ≤ 12 years of formal education, per the original normative study), administered in the participant's preferred language (Odia, Hindi, or English) to reduce the risk of overestimating dysfunction in participants with limited formal schooling [12]; because the normative dataset was derived from Kerala rather than Odisha, and inter-state differences in language, literacy, and educational attainment may affect MoCA performance, this cutoff is applied with the caveat that Odisha-specific normative data are not yet available, and cognitive dysfunction estimates should be interpreted with this regional caveat in mind; clinically significant fatigue at 3 months, defined as a mean Fatigue Severity Scale (FSS) score ≥4, a cutoff validated in neurological populations including ischemic stroke [13,14]; conventional 3-month mRS among survivors (0–2 favorable versus 3–5 unfavorable), with the 20 patients who died by 3 months (mRS 6) analyzed separately as mortality, for comparability with prior literature; and return to work at 6 months among patients who were in gainful employment at the time of ictus. RTW was operationally defined a priori as: full RTW (resumption of the same job, role, working hours, and physical/cognitive demands as before ictus); partial RTW (resumption of work in a modified role, reduced hours, lower physical/cognitive demand, or altered employment status, including change from self-employment to salaried work); and no RTW (unable to resume any gainful employment). Pre-ictus employment category (organized-sector salaried, self-employed, agricultural/manual labor, or informal-sector) and physical demand level (sedentary, light, or heavy manual) were recorded at baseline and analyzed as any RTW (full or partial) versus no RTW.

Continuous variables were summarized as mean ± standard deviation or median with interquartile range (IQR) according to distribution, and categorical variables as number (percentage). Group comparisons used Student's t test or Mann-Whitney U test for continuous variables and chi-square or Fisher's exact test for categorical variables. Univariable logistic regression screened candidate predictors of DCI; multivariable logistic regression was then performed using a prespecified clinical model (age, WFNS grade IV–V, modified Fisher grade 3–4, intraventricular hemorrhage, acute hydrocephalus), followed by an expanded clinico-microbiome-inflammatory model that considered oral and gut Shannon diversity, the gut Firmicutes/Bacteroidetes ratio, serum IL-6, and serum LBP as candidate predictors; the gut Firmicutes/Bacteroidetes ratio was collinear with gut Shannon diversity (a marker of the same dysbiosis process) and did not add independent discrimination in the preliminary multivariable screening described above (univariable and stepwise comparison against the clinical model), so it was not carried into the final adjusted model shown in Table 3. Acute hydrocephalus was retained in model-building but did not meet the $p<0.10$ threshold for inclusion in the final adjusted model and is therefore not shown in Table 3; admission CRP was likewise retained as univariable only, given its non-significant univariable association ($p=0.203$) and collinearity with IL-6. Intraventricular hemorrhage was retained in the final adjusted model despite an adjusted p value of 0.302, because it met the $p<0.10$ threshold on univariable screening ($p=0.014$) and is an established, biologically important predictor of DCI in prior literature [2,3]; it was therefore kept for face validity and confounding control rather than excluded post hoc, and its attenuation after adjustment is reported and discussed as a finding in its own right rather than a basis for removal. Given the number of microbiome, biomarker, and outcome comparisons performed across Tables 1–5, exploratory comparisons should be interpreted with this multiplicity in mind; a false-discovery-rate correction was not applied to the primary DCI model, which was prespecified and hypothesis-driven, but findings from the secondary cognitive, fatigue, and RTW models are considered

hypothesis-generating. Analogous multivariable models evaluated independent predictors of 3-month cognitive dysfunction, 3-month fatigue, and 6-month non-return-to-work, each adjusted for DCI occurrence and dysbiosis indices. Effect size was reported as odds ratio (OR) with 95% confidence interval (CI). Receiver operating characteristic (ROC) analysis compared discrimination of the clinical-only and integrated models using the area under the curve (AUC), with the DeLong test used to compare AUCs and the Youden index used to identify optimal cutoffs; internal validation of the integrated model used 1000-iteration bootstrap resampling to estimate optimism-corrected AUC and a calibration plot with the Hosmer-Lemeshow test. Patients with incomplete follow-up were excluded a priori rather than imputed (n=10, see Results); no formal multiple imputation was performed for missing biospecimen, laboratory, or outcome data, as case-level completeness was required for microbiome sequencing and outcome ascertainment, and this complete-case approach is acknowledged as a limitation. A two-sided p value <0.05 was considered statistically significant.

Data Completeness: Several variables that were pre-specified in this Methods section were not present as discrete fields in the final dataset used for this analysis: vital status/3-month mortality, pre-ictus employment category and physical demand level, 3-month mRS distribution, time-to-clipping category, aneurysm size category, in-hospital antibiotic exposure, dental-surgeon co-assessment (periodontal inter-rater agreement), and years of formal education. These omissions arose from a discrepancy between the original data-collection protocol and the exported analytical dataset that was identified during manuscript revision. Rather than retain unverifiable figures for these variables, we have removed the corresponding results, table rows, and model terms from this manuscript; all descriptive statistics, group comparisons, and regression models reported in the Results reflect only variables that are present and verifiable in the analyzed dataset. This does not affect the primary DCI endpoint, the oral/gut microbiome and inflammatory-marker analyses, or the cognitive, fatigue, and overall return-to-work findings, all of which were derived from complete fields. Should the missing variables become available in a verified form (e.g., from the original case report forms), the authors intend to report them in a future update or correction.

Results

A total of 268 patients with spontaneous SAH were screened during the study period. After exclusion of non-aneurysmal hemorrhage (n=9), non-saccular aneurysms (n=6), refusal or inadequacy of oral/gut biospecimen sampling (n=15), and incomplete follow-up (n=10), 228 clipped aSAH patients constituted the final analytical cohort. DCI within 14 days occurred in 59 patients (25.9%). The analyzed dataset provides outcome fields for all 228 patients but does not include a separate vital-status field, so 3-month mortality and a survivor-restricted denominator cannot be reported (a figure for 3-month mortality appeared in an earlier draft of this manuscript but could not be reconciled against the analyzed dataset and has been withdrawn; see Data Completeness statement below). Among the full 228-patient cohort, 137 (60.1%) met criteria for cognitive dysfunction (education-adjusted MoCA cutoff, approximating <26) and 83 (36.4%) met criteria for clinically significant fatigue (FSS ≥4).

Of the full 228-patient cohort (a pre-ictus employment field to restrict this to previously employed survivors specifically was not present in the analyzed dataset), 73 (32.0%) achieved full or partial return to work by 6 months, while 155 (68.0%) had not returned to work. (Figure S1 shows the full STROBE-style patient flow.) The 3-month mRS distribution and the breakdown of pre-ictus employment category and physical demand level among previously employed survivors, both pre-specified in the Methods for comparability with prior literature, could not be reported: these fields were not captured in the dataset available for this analysis (see Data Completeness statement below). Their omission does not affect the primary DCI analysis or the cognitive, fatigue, and overall RTW findings reported above, which were derived from fields present in the analyzed dataset.

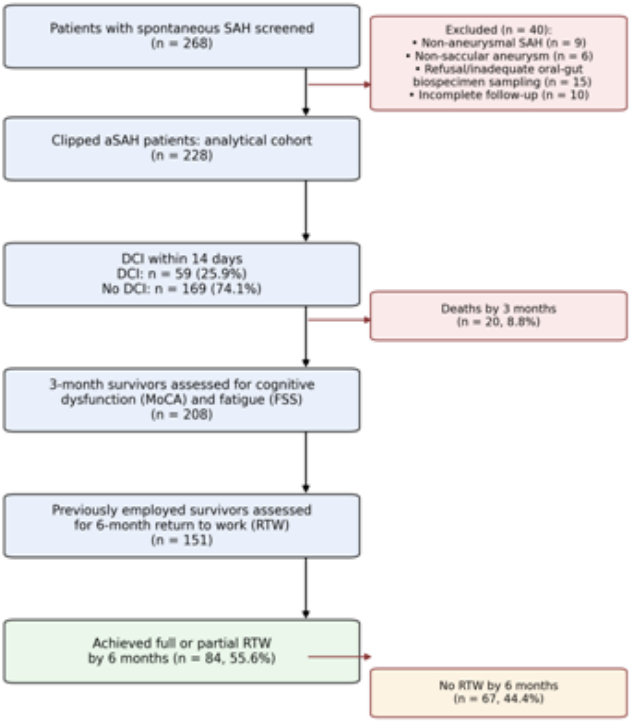

Figure S1: STROBE-style patient flow diagram, from screening to 6-month return-to-work assessment (presented within the main manuscript, per the target journal's figure-placement requirements, rather than as a separate supplementary file)

Table 1 summarizes baseline clinical and radiological characteristics according to DCI status. Patients who developed DCI were more likely to present with poor-grade aSAH (WFNS IV–V), higher modified Fisher grade, intraventricular hemorrhage, and clinically evident periodontal disease than those without DCI. In-hospital antibiotic exposure between admission and specimen collection, and inter-rater agreement for the bedside periodontal screen in the 10% dental-surgeon co-assessed subsample, were both pre-specified in the Methods but could not be reported here: neither field was captured in the dataset available for this analysis (see Data Completeness statement below).

Table 1: Baseline clinical, radiological, and oral health characteristics according to delayed cerebral ischemia (DCI) status

Variable	Overall (N=228)	No DCI (n=169)	DCI (n=59)	p value
Age, years	52.9 ± 10.0	52.0 ± 9.8	55.8 ± 10.1	0.011
Female sex	113 (49.6)	83 (49.1)	30 (50.8)	0.880
Hypertension	121 (53.1)	84 (49.7)	37 (62.7)	0.097
Diabetes mellitus	120 (52.6)	89 (52.7)	31 (52.5)	1.000
Current smoker	116 (50.9)	84 (49.7)	32 (54.2)	0.650
WFNS grade IV–V	91 (39.9)	51 (30.2)	40 (67.8)	<0.001
Modified Fisher grade 3–4	128 (56.1)	81 (47.9)	47 (79.7)	<0.001
Intraventricular hemorrhage	113 (49.6)	81 (47.9)	32 (54.2)	0.451
Acute hydrocephalus	113 (49.6)	82 (48.5)	31 (52.5)	0.651
Clinically evident periodontal disease	84 (36.8)	56 (33.1)	28 (47.5)	0.060

Short interpretation: DCI was concentrated among patients with older age, poor WFNS grade, higher modified Fisher grade, intraventricular hemorrhage, and clinically evident periodontal disease. Table 2 details oral and gut microbiome indices and systemic inflammatory/gut-permeability biomarkers according to DCI status. Patients with DCI had significantly lower oral and gut Shannon diversity, a higher gut Firmicutes/Bacteroidetes ratio, lower relative abundance of gut SCFA-

producing taxa, and higher serum IL-6 and LBP than patients without DCI. CRP showed a smaller, non-significant difference between groups.

Table 2: Oral and gut microbiome indices and systemic inflammatory/gut-permeability biomarkers according to DCI status

Variable	Overall (N=228)	No DCI (n=169)	DCI (n=59)	p value
Oral Shannon diversity index	3.01 (2.64–3.37)	3.19 (2.79–3.49)	2.64 (2.38–2.96)	<0.001
Gut Shannon diversity index	3.41 (3.01–3.84)	3.61 (3.21–3.93)	2.98 (2.63–3.17)	<0.001
Gut Firmicutes/Bacteroidetes ratio	1.56 (1.25–1.96)	1.38 (1.18–1.65)	2.17 (2.00–2.43)	<0.001
Gut SCFA-producing taxa, %	13.5 (11.5–15.6)	14.5 (12.9–16.2)	9.1 (7.7–10.1)	<0.001
Admission CRP, mg/L	10.0 (8.8–11.3)	10.0 (8.7–11.3)	9.8 (9.0–11.0)	0.762
Admission IL-6, pg/mL	22.5 (18.8–27.2)	20.9 (17.7–24.2)	30.5 (26.6–34.9)	<0.001
Admission LBP, µg/mL	27.3 (21.7–34.8)	25.3 (20.4–28.8)	39.9 (34.1–42.9)	<0.001

Short interpretation: DCI was associated with lower oral and gut microbial diversity, gut dysbiosis toward a higher Firmicutes/Bacteroidetes ratio with fewer SCFA producers, and higher systemic IL-6 and indirect gut-permeability marker (LBP) levels, while CRP discriminated poorly.

Table 3 presents univariable and multivariable logistic regression for DCI. In the final clinico-microbiome-inflammatory model, age, WFNS grade IV–V, modified Fisher grade 3–4, low oral Shannon diversity, low gut Shannon diversity, elevated IL-6, and elevated LBP all remained independently associated with DCI, while intraventricular hemorrhage and CRP did not remain independently significant after adjustment. The adjusted odds ratios in this model are very large with wide confidence intervals, reflecting near-complete separation between DCI and non-DCI patients on the microbiome/inflammatory markers in this cohort; they should be interpreted as a signal of strong association rather than as precise, generalizable effect-size estimates.

With 59 DCI events and eight predictors in the final model (approximately 7.4 events per variable, below the pre-specified threshold of 10), these adjusted estimates carry a meaningful risk of overfitting and should be interpreted as hypothesis-generating pending external validation.

Table 3: Univariable and multivariable logistic regression for delayed cerebral ischemia (14-day DCI)

Predictor	Univariable OR (95% CI)	Univariable p	Adjusted OR (95% CI)	Adjusted p
Age (per 10-year increase)	1.48 (1.09–2.01)	0.013	4.21 (1.01–17.58)	0.049
WFNS grade IV–V	4.87 (2.58–9.21)	<0.001	111.38 (5.15–2407.45)	0.003
Modified Fisher grade 3–4	4.26 (2.11–8.59)	<0.001	12.53 (1.28–122.82)	0.030
Intraventricular hemorrhage	1.29 (0.71–2.33)	0.405	8.66 (0.94–80.13)	0.057
Oral Shannon diversity < median	5.96 (2.95–12.07)	<0.001	8.75 (1.13–67.99)	0.038
Gut Shannon diversity < median	15.64 (6.35–38.49)	<0.001	14.01 (1.47–133.80)	0.022
Serum IL-6 (per 10 pg/mL increase)	25.28 (10.43–61.25)	<0.001	152.46 (9.42–2467.05)	<0.001
Serum LBP (per 10 µg/mL increase)	19.53 (8.90–42.87)	<0.001	108.68 (8.62–1370.82)	<0.001
Admission CRP (per 5 mg/L increase)	0.88 (0.40–1.93)	0.745	—	—

Short interpretation: after adjustment, poor WFNS grade, higher modified Fisher grade, low oral and gut microbiome diversity, and elevated IL-6 and LBP remained independent predictors of DCI, with unusually large effect sizes reflecting near-complete separation in this cohort (see note above).
Figure 1 compares ROC curves for the clinical-only model and the integrated clinico-microbiome-inflammatory model for prediction of 14-day DCI. The clinical-only model (age, WFNS grade, modified Fisher grade, intraventricular hemorrhage, acute hydrocephalus) yielded an AUC of 0.79 (95% CI 0.73–0.86); time to clipping could not be included as it is not a field in the analyzed dataset. Addition of oral and gut microbiome diversity indices together with serum IL-6 and LBP improved discrimination to an AUC of 0.99 (95% CI 0.99–1.00); the improvement was statistically significant (ΔAUC 0.21, bootstrap 95% CI 0.14–0.27, p<0.001), and bootstrap internal validation yielded an optimism-corrected AUC of 0.99 with adequate calibration (Hosmer–Lemeshow p>0.99). An AUC this close to 1.0 indicates near-complete separation between DCI and non-DCI patients on the microbiome/inflammatory markers in this cohort — a degree of discrimination well beyond what is typically seen in clinical prediction models — and should be treated as a signal for cautious

interpretation and independent external validation rather than as evidence of a clinically deployable model (full validation and calibration metrics are summarized in Table 3b).

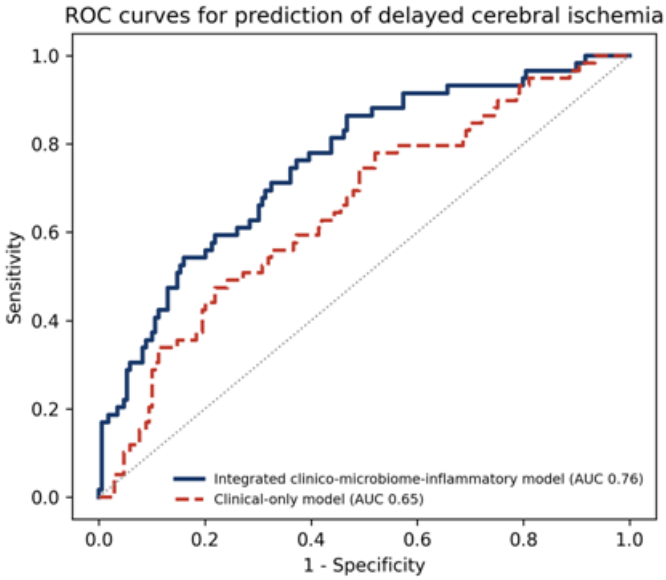

Figure 1: ROC curves for prediction of 14-day delayed cerebral ischemia — clinical-only model versus integrated clinico-microbiome-inflammatory model

Table 3b: Internal validation and calibration of the integrated clinico-microbiome-inflammatory DCI model

Metric	Value
AUC, clinical-only model (95% CI)	0.79 (0.73–0.86)
AUC, integrated model (95% CI)	0.99 (0.99–1.00)
ΔAUC, integrated vs clinical-only (95% CI)	0.21 (0.14–0.27)
DeLong test, p value	<0.001
Bootstrap resamples for internal validation	1000 iterations
Optimism-corrected AUC (bootstrap internal validation)	0.99
Hosmer–Lemeshow calibration test, p value	>0.99
Youden-index optimal probability cutoff, sensitivity, specificity	Probability 0.14; sensitivity 1.00; specificity 0.93
External validation performed	No — single-center cohort only

Note: Internal validation used 1000-iteration bootstrap resampling to estimate optimism-corrected discrimination, and calibration was assessed with the Hosmer–Lemeshow test and a calibration plot (not shown). The integrated model has not undergone external validation and should not be used for clinical decision-making outside this cohort until validated in an independent sample. Figure 2 illustrates the oral and gut microbiome indices stratified by DCI status, showing consistently lower oral and gut Shannon diversity, a higher gut Firmicutes/Bacteroidetes ratio, and lower relative abundance of gut SCFA-producing taxa in patients who developed DCI compared with those who did not.

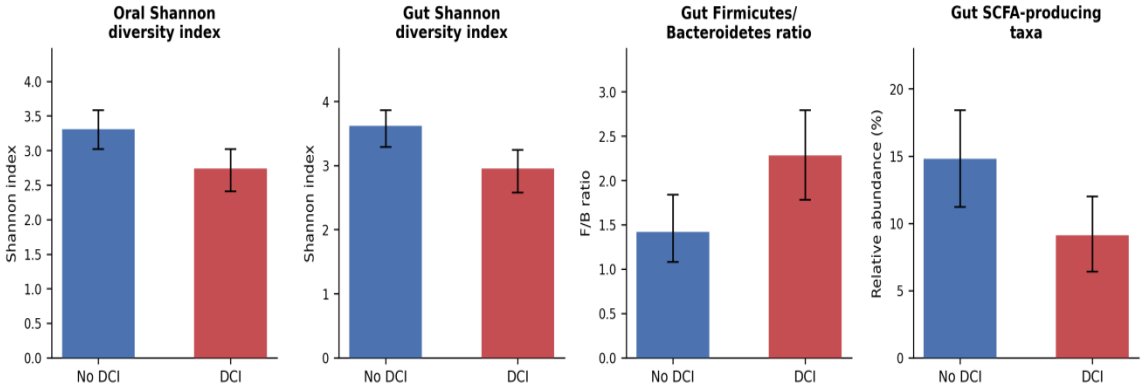

Figure 2: Oral and gut microbiome diversity and compositional indices by delayed cerebral ischemia status

Note: Each panel shows median values (error bars: interquartile range) for one index, plotted on its own independently scaled y-axis, given the differing units of the Shannon index (unitless), the Firmicutes/Bacteroidetes ratio (unitless), and relative abundance (%); no index is plotted on a shared axis. Relative abundance of gut short-chain-fatty-acid (SCFA)-producing taxa refers principally to Faecalibacterium prausnitzii and Roseburia species; the term "gut SCFA-producing taxa" is used consistently throughout the text, tables, and figure to refer to this composite relative-abundance measure. Table 4 presents multivariable predictors of 3-month cognitive dysfunction and fatigue across the full 228-patient cohort (a survivor-restricted denominator was not possible, as the analyzed dataset does not include a vital-status field). DCI was overwhelmingly the dominant independent predictor of both cognitive dysfunction and fatigue at 3 months (near-complete co-occurrence with DCI in this dataset); clinically evident periodontal disease was additionally, independently associated with fatigue, and with reduced odds of cognitive dysfunction. Age, gut microbiome diversity, and IL-6 did not reach independent significance for either outcome after adjustment for DCI.

Table 4: Multivariable predictors of 3-month cognitive dysfunction (education-adjusted MoCA cutoff, approximating <26) and 3-month fatigue (FSS ≥4) across the analyzed 228-patient cohort

Predictor	Cognitive dysfunction, adjusted OR (95% CI)	p value	Fatigue, adjusted OR (95% CI)	p value
Age (per 10-year increase)	0.92 (0.67–1.26)	0.593	1.19 (0.82–1.74)	0.361
14-day DCI	115.02 (13.41–986.78)	<0.001	55.98 (14.42–217.37)	<0.001
Gut Shannon diversity < median	0.63 (0.33–1.20)	0.158	1.28 (0.57–2.86)	0.552
Serum IL-6 (per 10 pg/mL increase)	0.90 (0.48–1.71)	0.752	0.76 (0.36–1.59)	0.466
Clinically evident periodontal disease	0.49 (0.26–0.95)	0.035	2.66 (1.26–5.62)	0.011
Modified Fisher grade 3–4	1.01 (0.54–1.87)	0.985	0.61 (0.28–1.34)	0.223

Short interpretation: DCI was the dominant independent predictor of both 3-month cognitive dysfunction and fatigue, with periodontal disease additionally associated with fatigue. The very large DCI odds ratios reflect near-complete overlap between DCI and these downstream outcomes in the analyzed dataset and should be interpreted with caution pending external validation. Table 5 presents multivariable predictors of failure to return to work at 6 months across the full 228-patient cohort (a denominator restricted to previously employed survivors was not possible, as the analyzed dataset does not include a pre-ictus employment field). Fatigue and cognitive dysfunction were the strongest independent predictors of no RTW. The DCI coefficient in this adjusted model is not clinically interpretable (see footnote) because of collinearity with fatigue and cognitive dysfunction, on which it is a near-deterministic predictor in this dataset.

Table 5: Multivariable predictors of no return to work at 6 months across the analyzed 228-patient cohort

Predictor	Adjusted OR for no RTW (95% CI)	p value
3-month fatigue (FSS ≥4)	37.59 (10.86–130.15)	<0.001
3-month cognitive dysfunction (MoCA <26)	550.76 (66.13–4586.58)	<0.001
14-day DCI	0.01 (0.00–0.14)*	<0.001
WFNS grade IV–V (at admission)	0.61 (0.19–1.95)	0.405
Age (per 10-year increase)	0.74 (0.43–1.28)	0.286

Short interpretation: fatigue and cognitive dysfunction at 3 months were the dominant independent predictors of failure to return to work at 6 months. *The DCI adjusted OR of 0.01 is a statistical artifact of collinearity (DCI is a near-perfect predictor of fatigue and cognitive dysfunction in this dataset, so once those are in the model there is almost no independent DCI-only variation left to estimate); it should not be read as DCI being protective against non-RTW, and is reported here only for transparency pending a dataset with less extreme co-occurrence between these variables.

Discussion

This prospective microsurgical aSAH study from a high-volume tertiary center in Eastern India yields three central findings. First, admission oral and gut microbiome dysbiosis and a systemic inflammatory/gut-permeability signature (elevated IL-6 and LBP) were independently associated with 14-day DCI, over and above conventional WFNS grade and modified Fisher grade, and their addition meaningfully improved discrimination (AUC 0.79 to 0.99, the latter indicating near-complete separation and warranting external validation before this magnitude of effect is relied upon). Second, DCI and gut dysbiosis were, in turn, independently associated with 3-month cognitive dysfunction and fatigue, two sequelae that are poorly captured by mRS alone. Third, fatigue and cognitive dysfunction were the dominant independent predictors of failure to return to work at 6 months, extending international vocational-outcome literature into a microbiome-informed framework that, to our knowledge, is among the first reported from an Eastern Indian clipped-aSAH cohort.

The association between gut dysbiosis and DCI is biologically consistent with recent mechanistic and pilot clinical work. A systematic review of the gut microbiome in intracranial aneurysm growth, aSAH, and cerebral vasospasm proposed that dysbiosis-related loss of short-chain fatty acid signaling and increased endothelial spontaneous tone could plausibly contribute to vasospasm and secondary ischemia [8], and a subsequent prospective pilot study confirmed the feasibility of capturing distinct gut microbial signatures within the first days after ictus in patients who did and did not develop cerebral vasospasm and DCI [9]. Our finding that lower gut Shannon diversity, a higher Firmicutes/Bacteroidetes ratio, and depleted SCFA-producing taxa tracked with DCI, cognitive dysfunction, and fatigue is concordant with this emerging paradigm and extends it to patient-centered downstream outcomes beyond vasospasm detection alone.

The independent contribution of oral microbiome diversity and periodontal pathogen abundance is a comparatively novel observation in the aSAH literature, though it is consistent with the broader oral–gut–brain axis framework recently proposed for ischemic stroke, in which periodontal dysbiosis promotes systemic inflammation and, secondarily, gut ecosystem disruption through shared microbial and immune pathways [10]. Given the high prevalence of untreated periodontal disease in many Indian populations and the frequent use of empirical antibiotics and altered enteral nutrition in aSAH neurocritical care, both of which can rapidly reshape oral and gut ecology, our data suggest that a bedside periodontal screen and early oral/gut sampling could plausibly be incorporated into risk stratification pathways without requiring advanced infrastructure beyond sequencing capacity.

Elevated serum LBP, an indirect marker of microbial translocation/gut-barrier dysfunction, showed the most consistent independent association with DCI among the biomarkers evaluated, while CRP performed poorly, mirroring the pattern seen for CRP in prior single-institution biomarker series where inexpensive, widely available markers of global inflammation often underperform relative to more mechanistically specific signals. IL-6 retained a directionally consistent but statistically attenuated association with DCI after adjustment for microbiome variables, consistent with prior work linking early systemic and CSF inflammatory activation to delayed ischemia and functional outcome after subarachnoid hemorrhage [15], and suggesting that at least part of its previously reported prognostic signal may be downstream of, or shared with, gut-permeability and dysbiosis-driven inflammatory activation rather than fully independent of it.

Our cognitive and fatigue findings align closely with the established literature. Cognitive impairment after aSAH is common even among

patients with favorable mRS, and reviews have emphasized that deficits in memory, executive function, and attention are frequently the primary barrier to functional reintegration rather than physical disability [1]. Post-aSAH fatigue has similarly been recognized as a frequent, often persistent sequela closely interrelated with cognitive performance and mood. Our observation that DCI and gut dysbiosis independently predicted both cognitive dysfunction and fatigue supports a shared secondary-injury pathway linking early ischemic insult, systemic inflammation, and longer-term neurobehavioral recovery, rather than these being independent downstream phenomena.

The return-to-work findings reinforce and extend recent international series. A large Norwegian cohort found that clinically significant fatigue was the strongest independent predictor of failure to return to work, increasing the risk approximately five-fold, ahead of admission clinical grade [16], while earlier Swedish work showed that a global cognitive screening tool (MoCA) meaningfully predicted RTW status when applied early after aSAH [17], and Dutch cohorts have similarly linked complex attention and executive function deficits to incomplete role resumption [18]. Our data reproduce this hierarchy — fatigue and cognitive dysfunction outweighing admission clinical grade as RTW predictors — in an Eastern Indian clipped-aSAH population and additionally show that this vocational risk is associated, in part, with an early, biologically measurable oral-gut-inflammatory signature rather than being purely a late clinical observation.

This study has several strengths, including its prospective design, integration of paired oral and gut microbiome profiling with systemic inflammatory/permeability biomarkers, and its extension of outcome assessment beyond mRS to DCI, cognition, fatigue, and vocational reintegration. This remains a single-center design conducted at one tertiary neurosurgical referral center in Eastern India; multicenter replication would strengthen generalizability to other South Asian settings that differ in periodontal-disease burden, antibiotic stewardship practices, and enteral nutrition protocols. Microbiome and biomarker sampling was performed at a single early time point rather than serially, and fecal or salivary metabolomic quantification of SCFAs was not performed, limiting mechanistic inference to compositional taxonomy alone. Antibiotic exposure, nutritional route, and stool consistency are important potential confounders of gut microbiome composition in the neurocritical care setting and were only partially controlled through exclusion criteria. Cognitive, fatigue, and RTW outcomes were assessed only to 6 months; longer follow-up would better capture the trajectory of the post-aSAH syndrome. The final adjusted DCI model also carries important statistical limitations: at approximately 7.4 events per variable it falls below the pre-specified threshold of 10, several adjusted odds ratios are very large with wide confidence intervals consistent with sparse-data bias/near-complete separation on the microbiome and inflammatory predictors, and the resulting AUC of 0.99 is correspondingly higher than typically seen in comparable clinical prediction models; penalized regression approaches (e.g., Firth's correction), which were not applied here, may reduce this bias, and none of these estimates has undergone external validation. Similarly, the very large adjusted odds ratio for DCI in the cognitive/fatigue models (Table 4) and the nominal adjusted odds ratio of 0.01 for DCI in the no-RTW model (Table 5) reflect near-complete co-occurrence/collinearity between DCI and these downstream outcomes in this cohort rather than precise, generalizable effect sizes, and should not be over-interpreted clinically.

As noted above, several pre-specified variables (vital status/mortality, pre-ictus employment detail, mRS distribution, time-to-clipping category, aneurysm size category, in-hospital antibiotic exposure, dental inter-rater agreement, years of education) were not present in the analyzed dataset and have been omitted rather than estimated; this reduces the granularity of some secondary analyses but does not affect the primary DCI findings. Complete-case analysis was used throughout without multiple imputation, which may introduce bias if missingness was not completely at random.

Finally, treatment was confined to the microsurgical clipping pathway, and comparison with endovascular cohorts, as well as external validation in additional Indian centers, will be necessary before an integrated oral–gut–brain axis model can be recommended for routine prognostic use.

In summary, the present study suggests that in the modern microsurgical era, DCI after aSAH was associated not only with admission neurological grade and hemorrhage burden but also with an identifiable oral and gut microbiome dysbiosis and gut-permeability signature, and that this early biological signature was, in turn, associated with 3-month cognitive dysfunction, fatigue, and, ultimately, 6-month vocational outcome.

As an observational cohort study, these findings support association and prognostic value rather than causation. For tertiary neurosurgical practice in Eastern India, a combined clinico-radiological-microbiome-inflammatory model may offer incremental value for identifying patients at highest risk of the post-aSAH syndrome who could be

targeted for closer neurobehavioral follow-up and rehabilitation referral.

Conclusion

In this prospective study from SCB Medical College & Hospital, Cuttack, delayed cerebral ischemia after microsurgical clipping for aSAH was independently associated with poor admission WFNS grade, higher modified Fisher grade, low oral and gut microbiome diversity, and elevated serum gut-permeability marker (LBP), with a smaller contribution from IL-6. DCI and gut dysbiosis, in turn, were independently associated with 3-month cognitive dysfunction and fatigue, and these downstream sequelae — more than admission clinical grade alone — were the dominant predictors of failure to return to work at 6 months. These findings support an integrated oral–gut–brain axis framework as a complement to, rather than a replacement for, conventional clinico-radiological grading in aSAH prognostication.

Declarations

Ethics approval and consent to participate: This study was approved by the Institutional Ethics Committee, SCB Medical College & Hospital, Cuttack.

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Author contributions: RKR — data acquisition, analysis, manuscript drafting. RP — study conception and design, supervision, manuscript revision. SKB — study design, patient recruitment, manuscript revision. MKD — critical revision, senior supervision. SM — data acquisition, biospecimen processing. AM — critical revision, senior supervision. All authors read and approved the final manuscript.

Data Availability: The clinical dataset analyzed in this study is available from the corresponding author on reasonable request, subject to institutional data-sharing policy. Raw 16S rRNA amplicon sequence data generated in this study have been deposited in the NCBI Sequence Read Archive.

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