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Systemic bacteria-derived extracellular vesicle as a predictive biomarker for post-operative delirium

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Systemic bacteria-derived extracellular vesicle as a predictive biomarker for post-operative delirium

Directed by Professor Bon-Nyeo Koo

The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Medical Science

Sujung Park

December 2023

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ABSTRACT

Systemic bacteria-derived extracellular vesicle as a predictive biomarker for post-operative delirium

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(Directed by Professor Bon-Nyeo Koo)

Delirium often manifests postoperatively, particularly among older patients. Characterized by unpredictable progression, it leads to disturbances in awareness, orientation, memory, and behavior. Occurring in 20-25% of patients aged over 60, postoperative delirium results in prolonged hospital stays, higher mortality rates, increased economic burdens, and greater needs for post-discharge care. Approximately a third of susceptible patients could benefit from preventative measures. However, the complexity of delirium, along with its unknown underlying mechanisms, hindered the progress of diagnosis, treatment, and research.

Dysbiosis, or imbalances in gut microbiota, has been associated with various neurological and mood disorders. The gut-brain axis, a communication pathway between gut microbiota and the brain, extends its influence beyond merely gut functionality. Recent studies have identified the human microbiome's role in postoperative delirium. Extracellular vesicles are essential for cell-to-cell communication and are emerging as promising biomarkers for disease prediction and quick assessment. Furthermore, bacteria-derived extracellular vesicles (BEVs) can cross the blood-brain barrier and may have a key role in immune regulation. This study was aimed at investigating the effects of BEVs in blood and gut

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microbiota composition on postoperative delirium, with the intention of establishing a predictive model for postoperative delirium.

This study included a total of 128 patients, all of whom were over 70 years old and scheduled for spinal surgery. We collected stool and serum samples immediately before starting the operation, and assessed delirium at least twice daily during postoperative hospitalization. Next-generation sequencing was utilized to analyze the bacterial taxa based upon 16s rRNA gene sequencing using the preoperative fecal samples as well as the BEVs. Comparative analysis of significant bacterial taxonomies between patients with non-delirium and delirium, and random forest classifier were employed to predict postoperative delirium status using the preoperative samples.

Baseline characteristics of the 88 patients in the training set were comparable between the two groups. As for the analyses of BEVs, patients with delirium had significantly reduced diversity of BEVs, compared to those with non-delirium; lower richness (measured by observed ASVs and Chao1) and evenness (measured by Shannon H and inversed Simpson). The clinical outcomes were significantly differentiated with 15 bacterial taxa in blood; EVs from bacteria belonging to *Bacilli* and *Alphaproteobacteria* were more abundant in non-delirium, whereas more BEVs from *Gammaproteobacteria* were detected in delirium group. However, significant diversity of gut microbial community and bacterial taxa was not observed between the two groups. Using inference of functional pathways based upon 16s rRNA gene sequencing, the gut environment of non-delirium group was significantly enriched with 16 functional pathways, mainly composed of TCA cycle and nucleotide-related pathways. To understand the potential prognostic factors to predict postoperative delirium, the significant BEVs were subjected to random forest classifier, resulting in 78.41% accuracy. In addition, the prediction model was validated with independence cohort, of

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which 80% of patients were correctly classified.

This research highlights the importance of circulating BEVs in the prediction of postoperative delirium, and their potential application to medical decision for the prevention and management of the patients.

Key words : delirium ; bacteria-derived extracellular vesicle ; gastrointestinal microbiome ; precision medicine

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Systemic bacteria-derived extracellular vesicle as a prognostic marker for post-operative delirium

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(Directed by Professor Bon-Nyeo Koo)

I. INTRODUCTION

Delirium, characterized by an unpredictable progression, is a form of acute cognitive impairment[1]. It is characterized by disruptions and fluctuation in awareness, orientation, memory, cognition, and behavior. Postoperative delirium is common in older patients after the surgery, predominantly between postoperative day 2-5[2]. In patients aged over 60 years, the postoperative delirium occurs in 20-25% of cases[3,4]. Spinal surgery accounted for 11.5% of overall prevalence of postoperative delirium[5], along with longer hospital stay, increased mortality rate within 30 days after the surgery[6,7], higher economic costs[5], and higher risk of needing care hospital upon discharge[8,9]. As the frequency of surgical procedures in elderly patients rises, postoperative delirium has garnered increased attention. However, limited information is available regarding biomarkers associated with postoperative delirium.

Delirium can be mistaken for depression due to inactivity or slow mentation. Its presentation can vary significantly between individuals, ranging from hyperactive to hypoactive states. The heterogeneous phenotype of delirium, along with unclear pathophysiological mechanisms, makes it difficult to diagnose, treat, and research. However, it can be prevented in about one third of patients at risk by screening people with risk factors and educating them in advance before surgery [10]. Thus, the investigation of preoperative contributors to delirium status after surgery is critically important to predict clinical outcomes, and improve patient care management with interventions.

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The gastrointestinal tract is a complex habitat, typically harboring vast number of microorganisms including bacteria, virus and fungi. This microbiome is continually influenced and shaped by its host and surrounding environment, while simultaneously impacting the host's function, health, and susceptibility to diseases. In animal studies, the gut microbiome has been progressively acknowledged as a significant contributor to postoperative delirium[11]. Routine bowel preparation prior to surgery not only modified the gut microbiota composition in patients with gastric cancer, but also escalated the occurrence of postoperative delirium[12]. Recent studies suggest that changes in gut microbiota following surgery may play a role in the onset of postoperative delirium[13,14].

The gut-brain axis, which refers to the communication between the gut microbiota and brain, has recently been validated by an increasing number of research studies[15–17]. For example, growing body of research has indicated that gut microbial imbalance can directly affect cognitive dysfunction such Alzheimer's disease (AD), caused by an increase in neurotoxins and neuroinflammatory molecules and a decrease in tryptophan and norepinephrine-producing bacteria[18–20]. Specifically, the abundance of the anti-inflammatory genus *Faecalibacterium* was decreased in patients with cognitive impairment[21]. It is proposed that treatment to change the gut microbiota could be beneficial in modifying the neuropathology related to AD and its progression[22].

In cases of postoperative delirium, significant variations in gut microbiome composition were noted when mice subjected to abdominal surgery were categorized into delirium and non-delirium phenotypes[11]. At the genus level, there were notable reductions in the levels of *Ruminiclostridium*, Ruminococcaceae UCG 014, and *Desulfovibrio* in mice experiencing postoperative delirium when compared to mice without postoperative delirium. Also, Hu Liu et al.'s study showed that significant associations between the pathogenesis of postoperative delirium and composition of the gut microbiota[23]. In the postoperative delirium cohort, there were high levels of *Proteobacteria, Enterobacteriaceae, Escherichia shigella, Klebsiella, Ruminococcus, Roseburia, Blautia, Holdemanella, Anaerostipes, Burkholderiaceae, Peptococcus, Lactobacillus, and Dorea.*

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In contrast, the control cohort exhibited abundance in *Streptococcus equinus and Blautia hominis.* These findings underscore the pivotal role of the gut microbiome in the manifestation of postoperative delirium.

Enclosed within a phospholipid bilayer membrane, extracellular vesicles (EVs) are particles ranging from 20-400nm and can be detected in all bodily fluids including plasma, saliva, cerebrospinal fluid, feces, and urine[24,25]. The EVs are expelled from the cell after the outer membrane forms vesicles, and encloses cellular proteins, lipids, bacterial DNA and RNA[26]. They play a crucial role in cell-to-cell communication or pathogenesis promotion. They can enter the bloodstream and engage with numerous host organs for regulators of the immune system[27]. When bacteria-derived extracellular vesicles (BEVs) in urine of normal subjects were compared to those with autism spectrum disorder, the percentage of BEVs derived from *Pseudomonas, Sphingomonas, Agrobacterium, Achromobacter,* and *Roseateles* decreased in autism[28]. The taxonomy identified from urinary EVs included that of gut microbiota previously reported in other studies. In research on allergic airway diseases in children, the BEVs derived from *Agrobacterium* phylum and *Sphingomonadaceae* family were significantly higher in atopic asthma and chronic rhinitis than those in control group[29]. The research suggested that BEVs can be a biomarker for prediction or rapid assessment of disease.

The BEVs have been identified as potent carriers capable of crossing the blood-brain barrier and transporting signaling molecules to the central nervous system (CNS). The BEVs play a role in controlling inflammation in the nervous system and also help manage tissue injury and healing[30]. As a result, they have an impact on the onset, progression, and potentially the recovery from various diseases affecting the central nervous system[31,32]. These include autoimmune conditions, neurodegenerative disorders, strokes, traumatic brain injuries, and infectious diseases of the CNS[31,33,34]. Recent studies focused on creating a diagnostic tool for other diseases by utilizing microbiome data from serum BEVs in combination with clinical or pathological information[35,36]. Therefore, based on these researches, we planned this study to apply circulating BEVs to develop biomarkers

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that can be used to screen patients with delirium.

Understanding the significance of the gut-brain axis mechanism in delirium is crucial, as it may facilitate the exploration of rational early treatment approaches[37,38]. The mechanism involves direct and indirect pathways between cognitive and emotional centers in the brain with peripheral intestinal functions[37]. The gut microbiota has been recognized as a modulator of immune cells within the gut-brain communication system[39–41]. The gut microbiota, or the community of microorganisms living in our intestines, plays a crucial role in this communication system[42]. Imbalances in the microbiota, known as dysbiosis, have been linked to various conditions such as depression or anxiety and disease associated with neuroinflammation[43–45]. In the realm of psychiatry, there is currently active research exploring the use of probiotics to treat and prevent dysbiosis[40,46]. In our research, we explored the relationship between the composition of gut microbiota and BEVs in the blood during delirium and developed a predictive model for postoperative delirium.

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II. MATERIALS AND METHODS

1. Ethics approval

The study was approved by the local institutional review board (Severance Hospital 4-2019-0654; ClinicalTrials.gov Identifier: NCT04120272). Written informed consent was obtained for the study. All procedures conformed to the standards in the Declaration of Helsinki.

2. Participants

We enrolled 128 patients aged >70 years who were scheduled for spine surgery (i.e., spinal fusion, laminectomy, discectomy, or transforaminal lumbar interbody fusion) between October 2019 and March 2023 (Figure 1). They were screened within one week before the surgery. Their medical comorbidities, surgical history, and years of education were recorded. Patients were excluded if they had literacy problems, language difficulties, hearing or visual impairment, history of current medication for neuropsychiatric disease, neurologic disease (e.g., stroke, seizures, or dementia), and diagnosis of cognitive impairment.

3. Preoperative assessment

The assessment included demographic characteristics (e.g., age, gender, education level), medical information (e.g., medical history, the American Society of Anesthesiologists physical status classification, Charlson Comorbidity Index) and the evaluation of cognitive function using mini-mental status examination (MMSE) and Montreal Cognitive Assessment (MoCA). The MMSE was employed to evaluate various cognitive domains including orientation, memory, language, attention, calculation, praxis, and visuospatial skills. With a maximum attainable score of 30, a higher score denotes better cognitive

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function. Conversely, the MoCA is a screening tool specifically for Mild Cognitive Impairment (MCI). Both the MMSE and MoCA were administered by trained professionals before the surgery. In addition, the Geriatric Depression Scale (GDS) was utilized to evaluate the level of depression[47]. Disease or disorder diagnosed at least 10% of either group were analyzed to understand comorbidity, resulting in hypertension, bone and joint diseases (including osteoarthritis, osteoporosis and rheumatoid arthritis), hyperlipidemia, diabetes mellitus, respiratory diseases (including asthma, chronic obstructive pulmonary disease, bronchiectasis, emphysema, bronchitis and pulmonary fibrosis), and cardiovascular diseases (including coronary artery disease, myocardial infarction, arrythmia, angina, atrial fibrillation and cardiomegaly).

4. Sampling of blood and stool

Five grams of stool in each patient was sampled immediately before the surgery using sterilized collection tools (N-Swab transport™, NFS-2, Noble Bio, Hwaseong, Korea), instantaneously dispensed into sterile cryotubes, and then promptly stored in a -80°C freezer until DNA extraction. Blood was drawn from radial artery right before surgery. All blood samples were relocated to a separator tube, then spun at 3000 rpm at a temperature of 4°C for 15 minutes. The clear, liquid portion above the sediment (supernatant) was collected and preserved at -80°C.

5. Anesthetic management

All the surgical procedures were performed in the prone position. The Wilson frame was used with the head and neck in the neutral position. The type of surgery was laminectomy, discectomy, and spinal fusion. Anesthesia was induced with propofol (1–1.5 mg kg^{-1}), remifentanil (0.05–0.2 μg kg^{-1} min^{-1}), and rocuronium (0.6 mg kg^{-1}). Anesthesia was maintained by inhalation or intravenous anesthesia. During surgery, the concentration of

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sevoflurane, desflurane, or propofol was adjusted to achieve a SedLine® patient state index (PSI) of 25–50, which is the suggested range for ensuring safety and efficacy of guiding anesthetic administration in general surgical patients by the manufacturer. Vasoactive drugs such as norepinephrine and ephedrine were used to maintain the mean blood pressure within 80–120% of the baseline during the surgery. The lungs were ventilated with a 50% oxygen/air mixture.

6. Assessment of postoperative delirium

We used the Confusion Assessment Method to assess delirium at least twice daily during hospitalization. The Nursing Delirium Screening Scale (Nu-DESC) was also used to assess delirium in patients. Patients were classified as either having non-delirium or delirium status. The identification of delirium was validated through consultation with a intensive care unit physician and was determined by the presence of four key clinical indicators: (1) sudden changes and fluctuation, (2) lack of attention, (3) disorganized thinking, and (4) changes in consciousness level. Patients with delirium were evaluated regarding duration of neurological symptoms, subtype of delirium, and severity of cognitive impairment using the Korean version of the Delirium Rating Scale (DRS) at the time when delirium appeared.

7. Genomic DNA extraction from fecal samples

Total genomic DNAs was extracted from 0.2 g of fecal sample using Maxwell® RSC PureFood GMO and Authentication Kit (Promega, USA), following the manufacturer's instructions. The concentration of the DNA was determined by a UV-vis spectrophotometer (NanoDrop 2000c, USA), and the quantification of DNA was performed using a QuantiFluor® ONE dsDNA System (Promega, USA). All extracted DNAs were stored at –20 °C until their use in further experiments.

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8. Analysis of gut environment

The microbiota composition was analysed by 16S rRNA amplicon sequencing using Illumina Miseq (Illumina, Inc., USA). For sequencing, the V3-V4 region of the bacterial 16S rRNA gene was amplified using primer set F319 and R806. The entire process was conducted following the Illumina manufacturer's protocol.

The gut microbiota analysis was performed with QIIME 2 2022.02 pipeline[48]. Paired end sequence data were demultiplexed using MiSeq Reporter and joined using the q2-vsearch Plugin. Merged sequences were quality filtered using the q2-quality-filter plugin followed by denoising with Deblur (via q2-deblur) [49]. Taxonomy was assigned to ASVs using the q2-feature-classifier classify-sklearn naïve Bayes taxonomy classifier against the SILVA DB v138 [50]. Functional pathways were analyzed using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) 2 v2.3.0 beta, based upon 16S rRNA gene sequences. This allowed us to anticipate the metagenome content up to MetaCyc to infer functional pathways.

9. Analysis of bacterial extracellular vesicles

Nanovesicles were separated from the samples through the differential centrifugation, genomic DNAs were extracted, then 16S rRNA sequencing was performed using Illumina MiSeq (Illumina, USA). Through this process, the gut microbiota was classified, and the correlation between the clinical characteristics and the rRNA abundance derived from a specific microorganism was made (MD healthcare, Seoul, Korea).

Bacterial EVs were boiled using a heat block for 40 min at 100 °C and then the remaining particles and waste were removed by centrifugation at 18,312g for 30 min at 4 °C. The DNA was extracted from supernatants using a DNeasy PowerSoil Pro kit (QIAGEN, Germany). The DNA of bacterial EVs in each sample was quantified by QIAxpert

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(QIAGEN, Germany). V3-V4 regions of the 16S rDNA gene was amplified with primers; 16S_V3_F (5′-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3′) and 16S_V4_R (5′-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3′). The library preparation was performed using PCR products and each amplicon was sequenced by MiSeq.

Illumina results including both the nucleotide sequence of reads and a quality score (Q-score) associated to each nucleotide in each read were imported to QIIME2 (https://qiime2.org/2021.4/). After V3-V4 primer were removed, forward and reverse reads were truncated at 200 and 260 bases based on Q-scores, respectively. DADA2 defaults the action regarding chimeras to “consensus” and pooling to “independent”. DADA2 algorithm is used for modeling and correcting Illumina-sequenced amplicon errors and then used in the identification of ASVs. In the study, a Naïve Bayesian classifier was pre-trained on the SILVA 138 database and then used for the taxonomic annotation of our samples. Samples with less than 1000 read counts were not considered for downstream analyses.

10. Statistical analysis

As a step of preprocessing data, missing values were imputed with values predicted by proximity matrix from random forest (rfImpute() of randomForest R package). For alpha diversity, the 16s rRNA sequence reads were rarefied to standardize sequencing depth of each sample into minimum number of reads (Rarefy(depth = min()) of GUniFrac R package). Then, the alpha diversity in each group was measured by observed ASVs (specnumber() of vegan R package) and Chao1 (estimate(permutations=100) of vegan R package) for richness, and Shannon H (diversity(index = "simpson") of vegan R package) and Inverse Simpson (diversity(index = "invsimpson") of vegan R package) for evenness. For beta diversity, the sequence reads were normalized to a relative abundance, the fraction

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of the taxon observed relative to the sum of all observed taxa corresponding to the sample in the feature table to analyze diversity between groups that was visualized by non-metric dimensional scaling (NMDS) based on the Bray-Curtis dissimilarity (metaMDS(distance = "bray") of vegan R package), and statistically tested by analysis of similarity (ANOSIM) (anosim(distance = "bray", permutations = 9999) of vegan R package). Significant bacterial taxa of each group were subjected to generate circular cladograms by GraPhlAn to understand what signature bacteria lineages are associated with each clinical outcome[51]. The significant functional pathways inferred by PICRUSt2 were visualized by bubble charts with mean fold changes[52,53]. All variables of the two groups were statistically tested by Welch's t-test (t.test(var.equal = FALSE) of stats R package) for continuous and Chi-squared test (chisq.test() of stats R package) for categorical data (Figure 1).

The variables that significantly differentiated between two groups, and did not show redundancy were used to build decision trees by random forest machine learning algorithm (randomForest(importance=TRUE, proximity=TRUE) of randomForest R package). To optimize the random forest model, the number of trees and variables were decided upon the lowest Out-Of-Bag (OOB) score (Figure 1). To estimate the ability of the random forest model to accurately identify clinical outcomes, calculated were error rate, accuracy, sensitivity, specificity, predicted positive and negative value (confusionMatrix() of caret R package). In addition, partial dependence plots (partial_dependence() of edarf R package) were constructed to understand how different values of a particular feature impact the prediction of random forest model.

For the integration of significant factors from blood and stool samples, the strength and direction of the association between the factors were measured by Spearman's correlation coefficients and p values, and visualized with correlogram (cor_mat(method = "spearman") and cor_plot() of rstatix R package).

All data were analyzed and visualized using R with 5% significance level[53].

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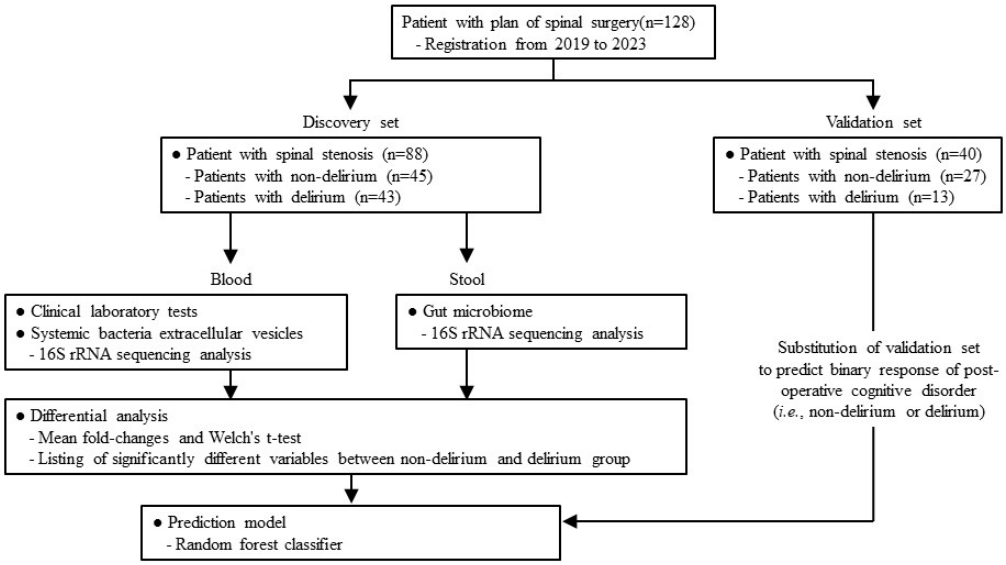

Figure 1 Study flowchart

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III. RESULTS

1. The basic characteristics of patients with non-delirium and delirium were comparable.

The baseline demographic characteristics of the 88 patients between non-delirium and delirium group were not significantly differentiated in terms of preoperative MMSE or MoCA score of cognitive function test, and Charlson comorbidity index (Table 1, Table A1). In preoperative laboratory results, the number of WBC (White blood cell) and MCHC (Mean Corpuscular Hemoglobin Concentration) were significantly increased in delirium group than non-delirium group (Table 2). However, considering the normal range of WBC (4.5 – 11.0 x 10^3/μL) and MCHC (32 – 36g/dL), the statistical significances between the two groups may not be related to inflammation, infection or dysfunction to carry oxygen to tissues. Thus, the two groups involved in this research had a similar characteristic of demographic, anthropometric and basic clinical laboratory tests.

2. Diversity of and distinct lineages of bacterial taxa rescued from preoperative blood are significantly associated with postoperative delirium status.

Recently, BEVs have been considered to deliver messages from gut environment to extraintestinal organs including brain[54,55]. To understand the BEVs as a potential prognostic factor of postoperative delirium status, we analyzed the sequences of the 16S rRNA gene obtained from blood samples, associated with clinical outcomes. For diversity of BEVs within group (Figure 2A), patients with delirium have lowered levels of richness (measured by observed ASVs and Chao1) and evenness (measured by Shannon H and Inverse Simpson), compared to non-delirium group. A nonmetric multidimensional scaling (NMDS) ordination using all ASVs was used to understand the diversity of BEVs between the groups, resulting in significantly different systemic BEV composition between non-delirium and delirium (Figure 2B). To investigate the BEVs, associated with

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postoperative delirium status, significantly different bacterial taxa (Figure A1) were visualized with cladogram (Figure 2C). At class level of analysis, BEVs from *Bacilli* and *Alphaproteobacteria* were more abundant in non-delirium, whereas more BEVs from *Gammaproteobacteria* were detected in delirium group (Figure 2C).

These data revealed that BEVs in blood sampled from patients before surgery are significantly associated with postoperative delirium status; lower diversity of systemic BEVs dominated with EVs from *Gammaproteobacteria* are significantly associated with postoperative delirium, whereas patients with non-delirium are significantly associated with more diverse systemic BEVs enriched with EVs from *Bacilli* and *Alphaproteobacteria.*

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Table 1. Clinical characteristics of delirium and non-delirium patients

	Delirium (N=43)	non-delirium (N=45)	P value
Sex			0.710
- male	14 (32.6%)	12 (26.7%)	
- female	29 (67.4%)	33 (73.3%)	
Age (years)	75.6 ± 3.8	75.3 ± 3.9	0.702
Height (cm)	157.3 ± 7.8	157.6 ± 7.9	0.866
Weight (kg)	60.3 ± 9.8	60.6 ± 9.4	0.911
Body mass index (kg/m^2)	24.4 ± 3.6	24.3 ± 2.7	0.886
ASA-PS			0.479
- I	0 (0.0%)	0 (0.0%)	
- II	14 (32.6%)	18 (40.0%)	
- III	28 (65.1%)	27 (60.0%)	
- IV	1 (2.3%)	0 (0.0%)	
CCI			0.199
≥ 4	34 (79.1%)	29 (64.4%)	
< 4	9 (20.9%)	16 (35.6%)	
Surgical experience	38 (88.4%)	40 (88.9%)	0.999
Benzodiazepine medication	6 (14.0%)	1 (2.2%)	0.101
MMSE	27.5 ± 1.6	27.7 ± 1.9	0.593
MoCA	23.7 ± 2.8	24.1 ± 2.5	0.473
GDS	4.1 ± 4.2	3.6 ± 3.8	0.515

Data are presented as mean ± standard deviation or the number of patients (percentage).

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ASA-PS, the American Society of Anesthesiologists-physical status classification system; CCI, Charlson Comorbidity Index; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; GDS, Geriatric Depression Scale.

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Table 2. Preoperative laboratory results of delirium and non-delirium patients

	Delirium (N=43)	non-delirium (N=45)	P value
WBC ($10^3/\mu\ell$)	6.8 ± 1.7	6.2 ± 1.2	0.047
Hemoglobin (g/dL)	13.1 ± 1.5	13.1 ± 1.4	0.773
Platelet count ($10^3/\mu\ell$)	229.9 ± 49.2	241.6 ± 49.2	0.268
MCV (fL)	92.8 ± 4.2	92.1 ± 6.3	0.505
MCH (pg)	31.2 ± 1.5	30.5 ± 2.6	0.121
MCHC (g/dL)	33.6 ± 0.9	33.1 ± 1.1	0.017
NLR	2.2 ± 1.2	2.0 ± 1.5	0.623
LMR	5.2 ± 1.8	5.4 ± 2.5	0.595
PLR	124.8 ± 49.9	136.3 ± 71.5	0.381
ESR (ml/min/1.73m^2)	14.0 ± 16.0	15.2 ± 15.0	0.738
CRP (mg/L)	2.9 ± 5.6	2.5 ± 8.3	0.769
BUN (mg/dL)	22.4 ± 12.5	19.4 ± 5.5	0.159
Creatinine (mg/dL)	0.9 ± 0.4	0.8 ± 0.2	0.160
eGFR (ml/min/1.73m^2)	70.7 ± 18.9	74.2 ± 15.2	0.354
Total protein (g/dL)	7.0 ± 0.4	6.9 ± 0.6	0.293
Albumin (g/dL)	4.4 ± 0.3	4.5 ± 0.3	0.100

Data are presented as mean ± standard deviation. MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; NLR, Neutrophil to Lymphocyte Ratio; LMR, Lymphocyte to Monocyte Ratio; PLR, Platelet to Lymphocyte Ratio; ESR, Erythrocyte Sedimentation Rate; CRP, C-Reactive Protein; BUN, Blood Urea Nitrogen; eGFR, Estimated Glomerular Filtration Rate.

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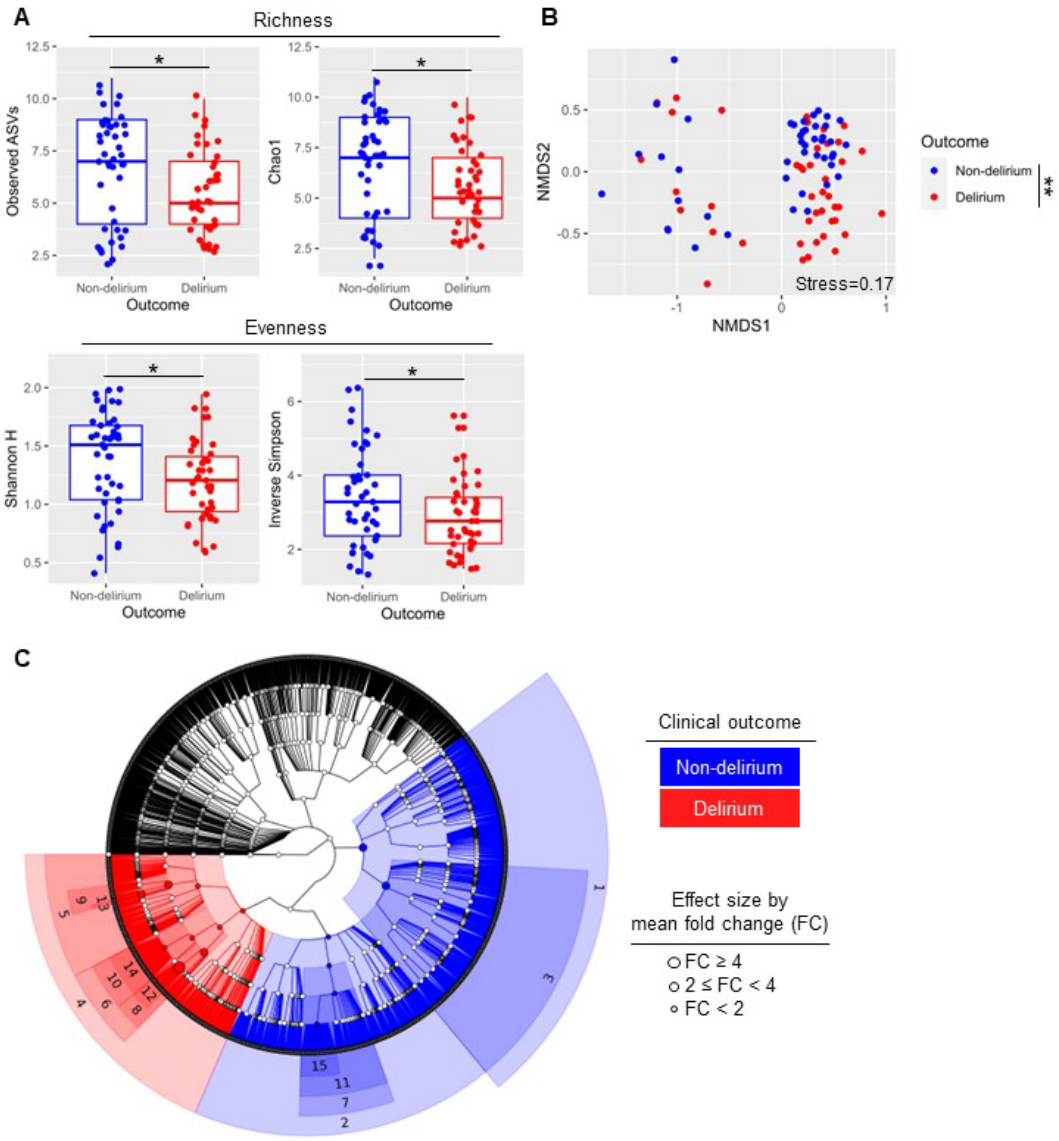

Clinical outcome	Bacterial lineages detected in systemic bacterial extracellular vesicles
Non-delirium	[1]Firmicutes|[3]Bacilli
	[2]Alphaproteobacteria|[7]Sphingomonadales|[11]Sphingomonadaceae|[15]Sphingomonas
Delirium	[4]Gammaproteobacteria|[6]Pseudomonadales|[8]Moraxellaceae|[12]Acinetobacter
	[4]Gammaproteobacteria|[6]Pseudomonadales|[10]Pseudomonadaceae|[14]Pseudomonas
	[4]Gammaproteobacteria|[5]Burkholderiales|[9]Oxalobacteraceae|[13]Herbaspirillum

Figure 2 systemic BEVs diversity was illustrated with individual (α-diversity) (A)

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and community based (β-diversity) (B) analysis.

(A) The α-diversity was measured by observed number of amplicon sequence variants and Chao1 index for richness, and by Shannon H and inverse Simpson for evenness showing a lower alpha diversity in patients with delirium than in non-delirium. Significance between the groups was performed by t-test. Data were expressed with median with interquartile range. (B) Non-metric dimensional scaling (NMDS) ordinations based upon Bray-Curtis dissimilarity using all amplicon sequence variants were used to understand β-diversity. Significance between the groups was performed by analysis of similarity (ANOSIM). (C) Significant bacterial taxa were organized on cladogram based upon median fold changes to understand differential bacterial lineages. ASV, Amplicon Sequence Variants; BEV, bacteria-derived extracellular vesicle; * $P<0.05$; ** $P<0.01$

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3. The systemic BEVs do not mirror the gut microbial communities.

To link the results of BEVs with gut microbiota, we analyzed gut environment including gut microbes and functional pathways under the hypothesis that gut microbial taxa is similar to the bacterial taxa rescued from systemic BEVs. For the analysis of microbial diversity, diversity within group (Figure 3A) as well as between groups (Figure 3B) failed to reach statistical significance. In addition, few gut bacterial taxa were associated with clinical outcomes; merely two taxa, *Peptococcales* and *Peptococcaceae* were detected more in patients with delirium than non-delirium (Figure 3C). Through the PICRUSt analysis, 16 functional pathways were inferred to be significantly enriched in gut environment of patients with non-delirium, and mainly composed of TCA cycle and nucleotide-related pathways (Figure 3D). To understand the possible causality, the strength and direction of association between significant factors from blood and stool were measured using Spearman correlation (Figure 4). Unexpectedly, there were no strong correlation between signature BEVs, gut microbes and functional pathways.

4. Random Forest model with significant BEVs for the prediction of the postoperative delirium

Machine learning algorithm has been applied to predict clinical outcomes before intervention[56], resulting in great contribution to the patient management. To establish prediction model for the postoperative delirium status, random forest classifier was used with the significant factors; EVs from *Sphingomonadales, Pseudomonadaceae and Peptococcales* were not considered for the prediction model because of redundancy. Compared to that with the factors of clinical laboratory tests (Figure A3A) or gut microbiome (Figure A3B), the random forest classifier with BEVs showed the lowest prediction error rate, 21.59%, measured by Out-of-bag (OOB) error with 100 trees and 9 variables (Figure 5A). To understand what features are most important for the prediction

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model, a mean decrease in accuracy across all trees was reported; of the13 significant factors, *Moraxellaceae* and *Acinetobactor* were the top 2 important features across the 100-decision tress (Figure 5B). To apply the prediction model described above into clinical settings, it is important to validate the model with independent data sets. To this end, the prediction model was validated with external data set, composed of 40 patients; 13 patients showed delirium and 27 patients did not after surgery (Table 3). The prediction model correctly classified 32, but misclassified 8 out of 40 patients, resulting in 80.00% accuracy, 20.00% error rate, 81.48% sensitivity, 76.92% specificity, 88.00% positive predictive value (PPV) and 66.67% negative predictive value (NPV) (Figure 6A).

To understand how the relative abundance of BEVs impacts the prediction of random forest, partial dependence plots were constructed with each significant bacterial taxon (Figure 6B and C). There are two relationship patterns; the alteration of relative abundance of BEVs did not change prediction of clinical outcomes (Figure 6B), and prediction of the outcomes was shifted in the change of relative abundance of BEVs (Figure 6C). The EVs derived from *Bacilli, Pseudomonas, Gammaproteobacteria, Burkholderiales* and *Oxalobacteriaceae* were used to consistently predict patients with non-delirium or delirium regardless of their relative abundance (Figure 6B). However, the predictions of random forest with the other BEVs were shifted, dependent upon the relative abundance. The BEVs derived from *Pseudomonadales* (≲ 57%), *Herbaspirillum* (≲ 5.35%), *Sphingomonadaceae* (≲ 41%) and *Sphingomonas* (≲ 30%) were used to predict mainly patients with non-delirium. However, interestingly, the high abundance of the BEVs was used to predict patients with delirium (Figure 6C). Even though the low abundance were used to predict patients with delirium, patients with non-delirium were mainly predicted by the high abundance of the EVs derived from Alphaproteobacteria (≳ 8.35%) and Firmicutes (≳ 5.5%) (Figure 6C). In contrast, the high abundance of the EVs from Moraxellaceae (≳ 6.5%) and Acinetobacter (≳ 2.6%) were used to predict mainly patients with delirium (Figure 6C). Of the significant factors, two BEVs showed the highest impact on prediction of clinical outcomes in spite of their low relative abundance; patients with ≳ 5.8%

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Acinetobacter or ≧ 8.3% Moraxellaceae EVs is more likely to be delirious after surgery with a probability of 66 and 65%, respectively (Figure 6C).

Taken together, random forest classifier with significant BEVs was validated with independent data set, resulting in 80% accuracy. Additionally, EVs released from Acinetobacter and Moraxellaceae were estimated to have the most impact on the probability of clinical outcomes after surgery.

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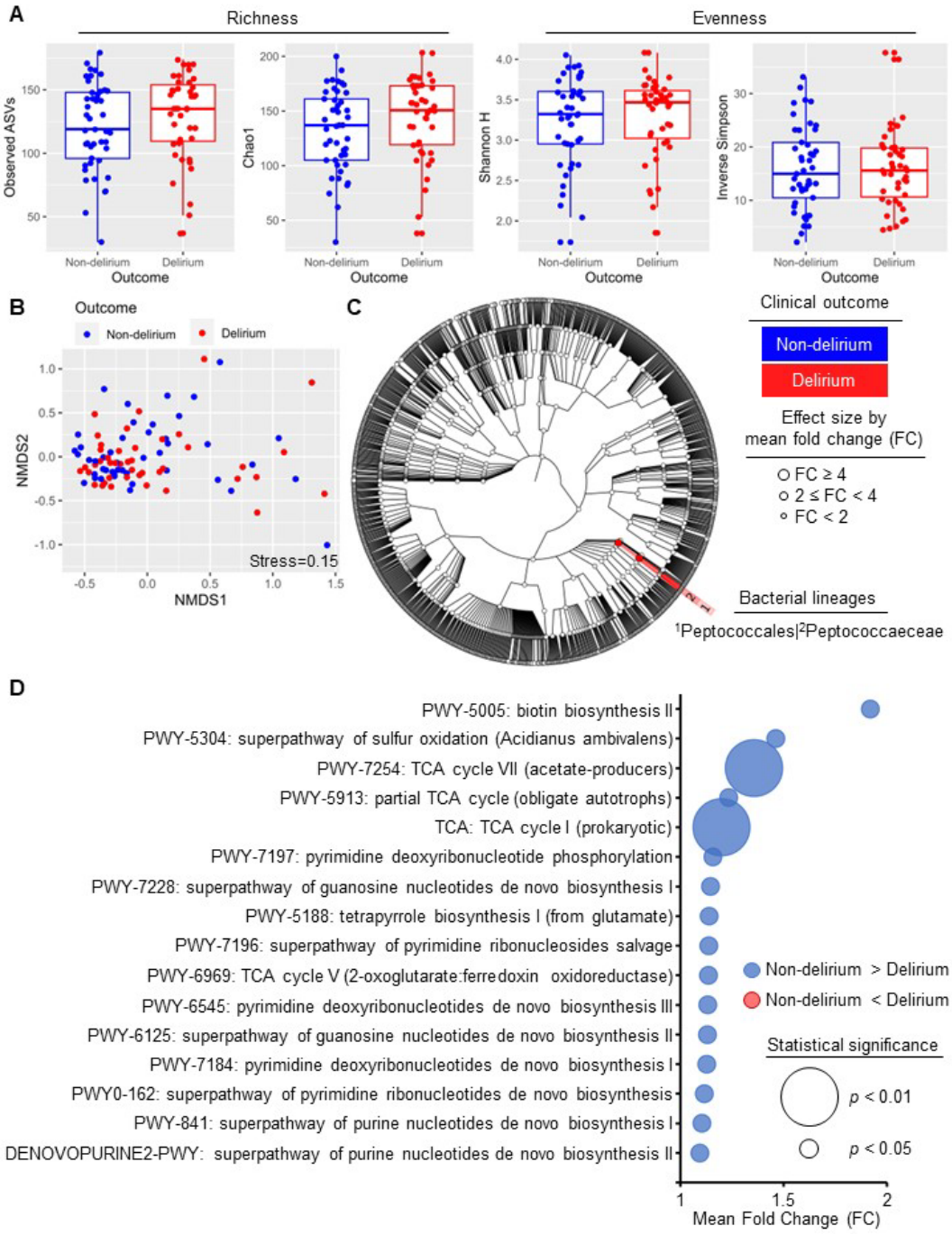

Figure 3. Comparison of alpha and beta diversity index in gut environment

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(A) The α-diversity was measured by observed number of amplicon sequence variants and Chao1 index for richness, and by Shannon H and inverse Simpson index for evenness. (B) Non-metric dimensional scaling (NMDS) ordinations based upon Bray-Curtis dissimilarity using all amplicon sequence variants were used to understand β-diversity. Significance between the groups was performed by analysis of similarity (ANOSIM). (C) Significant bacterial taxa were organized on cladogram based upon median fold changes to understand differential bacterial lineages. (D) The changed pathways of two groups were predicted by PICRUSt2. ASV, Amplicon Sequence Variants

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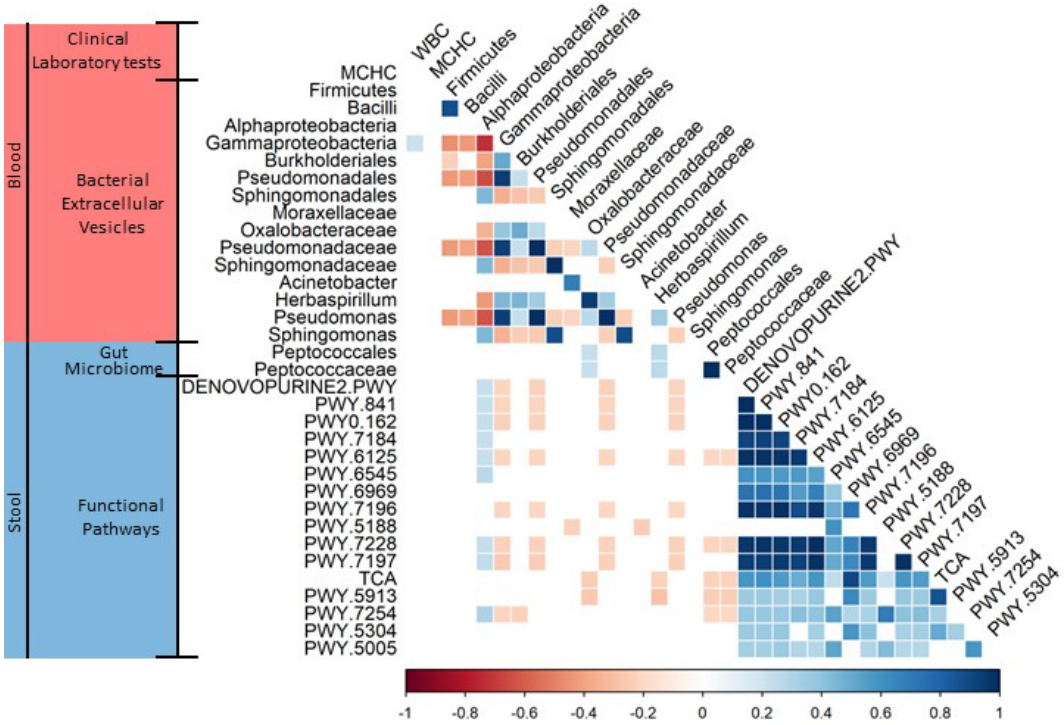

Figure 4. Heatmap of correlation analysis among the variables and functional pathways

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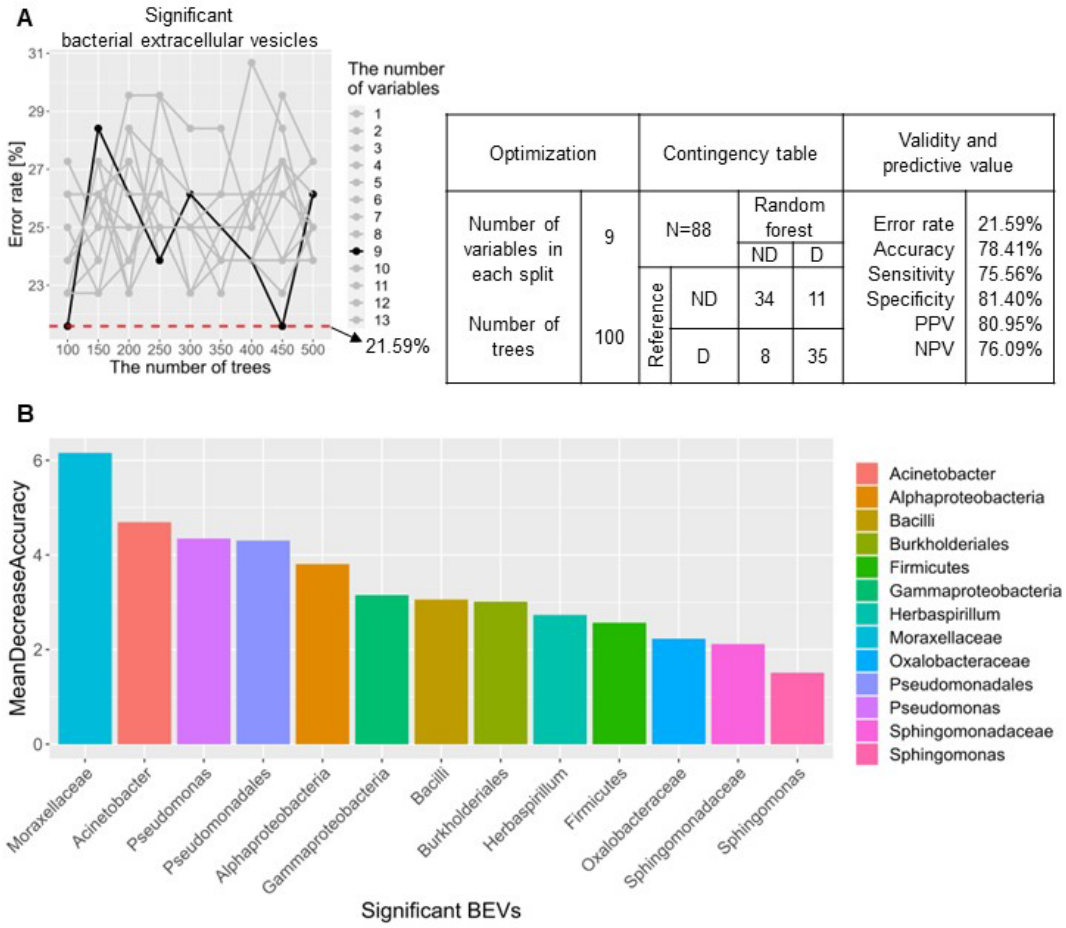

Optimization		Contingency table			Validity and predictive value	
Number of variables in each split	9	N=88	Random forest ND	Random forest D	Error rate	21.59%
					Accuracy	78.41%
					Sensitivity	75.56%
Number of trees	100	Reference ND	34	11	Specificity	81.40%
					PPV	80.95%
		Reference D	8	35	NPV	76.09%

Figure 5. Validity and variable importance in random forest model

(A) Plot of tree numbers versus error rate. This analysis indicated that 9 variables and 100 of trees were optimal. (B) The variable importance based on mean decrease in accuracy from the final random forest prediction model. The most important 13 variables are listed in the plot.

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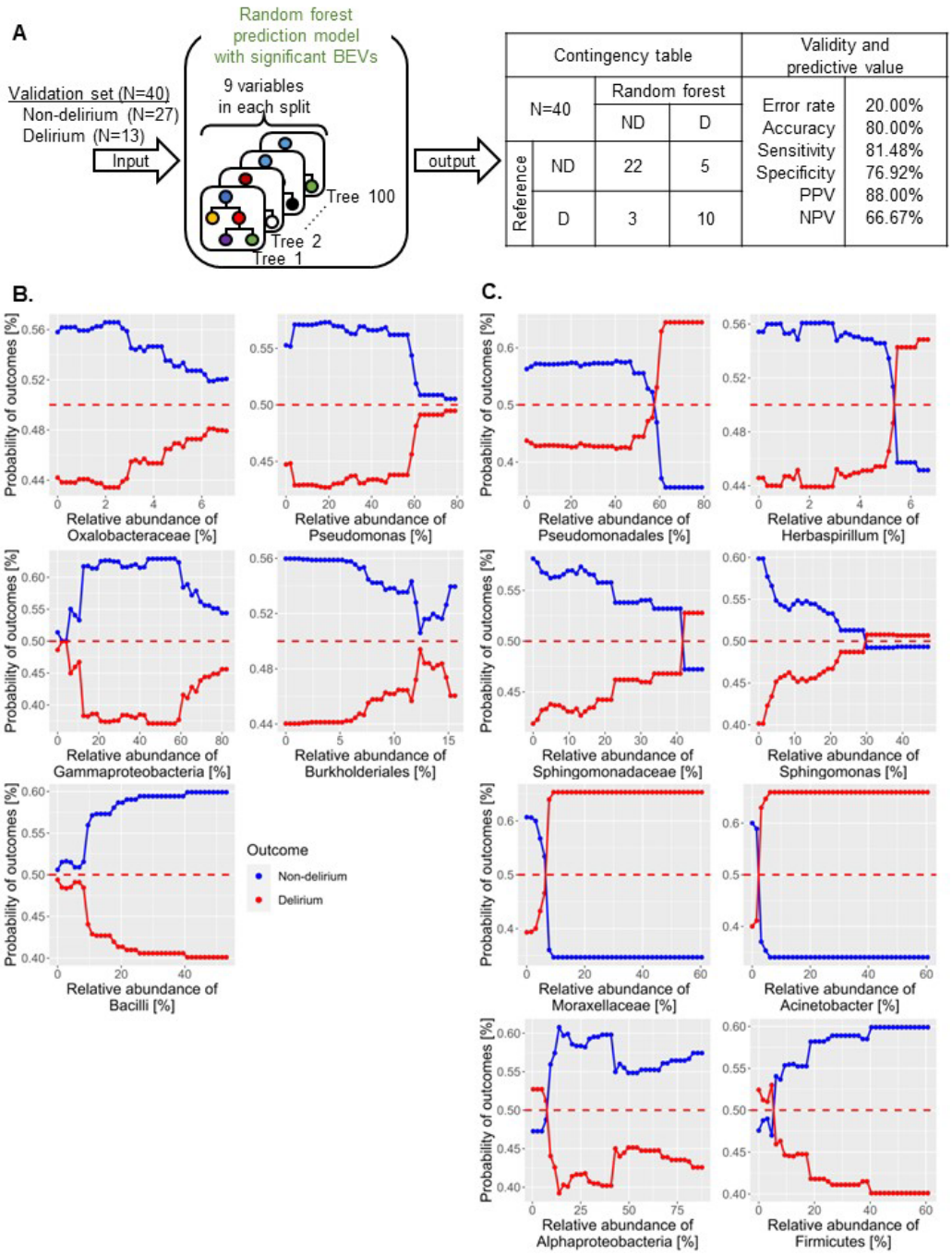

Contingency table				Validity and predictive value	
N=40		Random forest			
		ND	D		
Reference	ND	22	5	Error rate	20.00%
				Accuracy	80.00%
				Sensitivity	81.48%
				Specificity	76.92%
	D	3	10	PPV	88.00%
				NPV	66.67%

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Figure 6. Performance of random forest model

ND, non-delirium ; D, delirium ; PPV, positive predictive value ; NPV, negative predictive value

(A) Validity and predictive value of random forest prediction model in validation set (B) Consistent prediction in the change of relative abundance of extracellular vesicles (C) Prediction shift of outcomes depending on the relative abundance

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Table 3. Prediction of clinical outcomes by random forest classification using validation set

Patient ID	Reference	Random forest prediction	Vote [%]	Reference = Prediction
1	Delirium	Delirium	79	Y
2	Non-delirium	Non-delirium	90	Y
3	Delirium	Delirium	69	Y
4	Delirium	Non-delirium	64	N
5	Delirium	Delirium	58	Y
6	Delirium	Delirium	67	Y
7	Delirium	Non-delirium	60	N
8	Delirium	Non-delirium	80	N
9	Delirium	Delirium	96	Y
10	Delirium	Delirium	58	Y
11	Delirium	Delirium	68	Y
12	Delirium	Delirium	50	Y
13	Delirium	Delirium	97	Y
14	Non-delirium	Non-delirium	76	Y
15	Non-delirium	Delirium	59	N
16	Non-delirium	Non-delirium	72	Y
17	Non-delirium	Delirium	77	N
18	Non-delirium	Delirium	68	N
19	Non-delirium	Delirium	94	N
20	Non-delirium	Non-delirium	80	Y
21	Non-delirium	Delirium	84	N
22	Non-delirium	Non-delirium	63	Y
23	Non-delirium	Non-delirium	74	Y

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24	Non-delirium	Non-delirium	75	Y
25	Non-delirium	Non-delirium	73	Y
26	Non-delirium	Non-delirium	54	Y
27	Non-delirium	Non-delirium	72	Y
28	Non-delirium	Non-delirium	73	Y
29	Non-delirium	Non-delirium	74	Y
30	Non-delirium	Non-delirium	84	Y
31	Non-delirium	Non-delirium	78	Y
32	Non-delirium	Non-delirium	74	Y
33	Non-delirium	Non-delirium	77	Y
34	Non-delirium	Non-delirium	51	Y
35	Non-delirium	Non-delirium	98	Y
36	Non-delirium	Non-delirium	69	Y
37	Non-delirium	Non-delirium	75	Y
38	Non-delirium	Non-delirium	52	Y
39	Non-delirium	Non-delirium	93	Y
40	Delirium	Delirium	89	Y

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IV. DISCUSSION

We carried out microbiome profiling by examining stool and blood samples to discover diagnostic biomarkers for delirium. In systemic BEV in blood, Bacilli and Sphingomonas increased in the non-delirium group, while Acinetobacter, Pseudomonas, and Herbaspirillum increased in the delirium group. In the gut microbiome, Peptococcales and Peptococcaceae increased in the delirium group. Machine learning methods like random forest are frequently applied to evaluate the association between the microbiome and various disease conditions. In this study, systemic BEVs among a lot of variables were pointed out as the most powerful tool, and we develop a prediction model using a random forest for predicting delirium. In the model, 13 variables, including Moraxellaceae, Acinetobacter, Pseudomonas, Alphaproteobacteria, and Gammaproteobacteria, were identified as high-priority taxonomies. Out of these 13 important taxonomies, the model was constructed by creating 100 trees using 9 of them. In the external validation, the predictive model accurately categorized 32 patients, while inaccurately classifying 8 out of 40 patients, leading to an accuracy of 80.00% and an error rate of 20.00%.

Delirium poses lots of burden in the elderly population after surgery, with increased healthcare costs, long-term cognitive dysfunction, poor quality of life and even elevated mortality[57]. Delirium is a disease that is particularly easier to prevent than to treat, so it is important to classify risky patients for whom delirium should be actively prevented. Until now, predicting delirium has been challenging[58,59]. Prediction model in this study has good performance and the advantage of this model is that delirium can be predicted solely through a blood test without considering other confounding factors. Subsequently, through the development of a kit product in the future, it will be possible to easily predict delirium before surgery, which enables postoperative care planning and the execution of specific preventive measures on delirium.

According to the alpha-diversity analysis, the diversity of species in delirium group was less than that in non-delirium group. It is generally thought that a rich diversity of bacteria provides beneficial effects on metabolism and immune system regulation.

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In our findings, Genus *Acinetobacter, Herbaspirillum,* and *Pseudomonas* belonging to *Gammaproteobacteria* class were impactful variables in the model. This class encompasses a range of bacteria, including the prototypical bacterium *Escherichia coli*, well-known pathogens *Salmonella, Yersinia, Vibrio, Acinetobacter, and Pseudomonas*. Within the *Gammaproteobacteria* class, the genera *Pseudomonas*, *Herbaspirillum*, and *Acinetobacter* were detected at higher frequencies in the delirium group than in the non-delirium group. In Jie Z et al's research, *Gammaproteobacteria* class was detected higher in gut microbiota of mice with delirium-like behavior[13]. Similarly, patients who experienced cognitive impairment after a stroke also demonstrated an increased abundance of this bacterial class[60]. Importantly, Ling Y and his colleagues pointed out a negative correlation between the presence of *Gammaproteobacteria* and the MoCA score, suggesting that an increased presence of this bacterial class could be associated with poorer cognitive function. Some studies suggested that higher abundance of *Gammaproteobacteria* were significantly related to autism spectrum disorder and major depressive disorder. Pseudomonas aeruginosa, a member of the Gammaproteobacteria class, is linked to the progression of Alzheimer's disease and is believed to contribute to the buildup of amyloid-β, a phenomenon known as amyloidosis[61,62].

Gammaproteobacteria can utilize the byproducts of inflammation for its survival. Specifically, they can use reactive nitrogen species - substances produced by numerous inflammatory cells - to foster their growth under inflammatory conditions. This, in turn, could stimulate ongoing or chronic inflammation[63]. Postoperative delirium is probably associated to systemic inflammation and the disruption of the blood-brain barrier[64]. Surgery and other related physical traumas trigger an inherent immune response, leading to the release of proinflammatory cytokines like TNF and interleukins[65,66]. but several previously known inflammation markers such as CRP, ESR, and neutrophil-lymphocyte ratio were not helpful in predicting delirium as in this study.

We also observed that genus level of *sphingomonas* was significantly reduced in delirium group. A prominent feature exhibited by members of the *Sphingomonadaceae* family is the

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absence of lipopolysaccharides in their outer membrane, which is replaced by glycosylceramides[67]. Several studies indicate that heat-killed *Sphingomonadaceae* spp. bacteria elicit a response from invariant natural killer T cells acting as a antigen. To be more precise, research demonstrated that these glycosphingolipids trigger the proliferation and secretion of cytokines in iNKT cells, with their effects relying on CD1d[68,69]. These activated iNKT cells produce anti-inflammatory cytokines.

The PICRUSt analysis further validated that the synthesis of biotin and tricarboxylic acid (TCA) cycle, along with other related functions, were crucial roles played by the microecology of plasma BEVs in delirium. The TCA cycle is a key component of cellular metabolism and is crucial for the biosynthesis of macromolecules[70]. It is a series of chemical reactions used by all aerobic organisms to generate energy through the oxidation of acetyl-CoA derived from carbohydrates, fats, and proteins into carbon dioxide and chemical energy in the form of adenosine triphosphate (ATP). Hypoxia after dysfunction of the TCA cycle promotes the pathogenesis of Alzheimer's disease by promoting tau hyperphosphorylation, Aβ accumulation and dysfunction of the blood-brain barrier. Also, in Guo et al.'s study[71], the levels of products of tricarboxylic acid cycle such as citric acid were significantly lower in the delirium group. They showed that energy metabolism and oxidative stress, and interactions between hypoxia and mitochondrial dysfunction were altered in the postoperative delirium group. These studies suggest that the metabolism associated with the tricarboxylic acid (TCA) cycle plays a significant role in the development of delirium.

The family *Peptococcaceae* encompasses strictly anaerobic Gram-positive cocci and includes the genera *Peptococcus, Peptostreptococcus, Ruminococcus*, and *Sarcina.* However, only the *Peptococcus* and *Peptostreptococcus* genera are commonly detected in human samples. According to a. Houlden et al.'s research, Peptococcaceae after brain injury was increased[72]. Moreover, the proinflammatory cytokine, CCL5 have correlation with Peptococaceae level. In this study, high Peptococcaceae detection in preoperative patients' stool implicated high risk for delirium.

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The microbiota profile assessed from plasma EVs might reflect a gut microbiota. However, the microbiota profile from plasma EVs and gut microbiota did not match perfectly. Both present significant differences from one another, implying that the gut barrier and host immunity may function as a filter[73]. Rather, analysis of BEVs rather than gut microbiota helped improve the predictive power for delirium in this study.

Several important limitations should be acknowledged to provide a comprehensive understanding of the constraints in our study. Firstly, the influence of various factors on both gut microbiota and BEVs was not fully explored. Our study did not investigate the potential impact of probiotics, a variable that could significantly affect the composition of gut microbiota. Additionally, all patients received preoperative antibiotics for preventing postoperative infection, which may have influenced both gut microbiota and BEVs. Furthermore, the relationship between delirium and brain inflammation has been established in literature[64]. It might be worth investigating whether NSAIDs or steroid are being taken. In this study, relative abundance of BEVs at the level of ASVs appeared to distinctively cluster our patient cohort into two groups: group 1 and 2, clustered left and right, respectively (Figure 2B and A4A). Even though they did not reach statistical significance, respiratory, bone and joint diseases, and hyperlipidemia tended to be more observed in group 1 and 2, respectively (Figure A4B). Considering oxidative stress as an inducer of BEVs[74,75], the effect of analgesics, the first-line pharmacotherapy for spinal stenosis, on BEV-induction should be included in further studies, because some analgesics were shown to induce oxidative stress[76,77]. The investigation will firmly establish the link between medication and BEVs, and will contribute to health care decision for postoperative delirium.

Secondly, the sample size in our study was relatively small, which may limit the generalizability of our findings to the broader population of delirious patients, when considering the number of microorganisms in the intestine. The small sample size also raises the possibility of not capturing the full spectrum of variations in gut microbiota and

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BEVs among individuals who develop postoperative delirium. Larger, multicenter studies are warranted to validate and extend our findings to diverse patient populations.

Thirdly, our investigation focused solely on establishing an association between delirium and the composition of gut microbiota and BEVs. While this association is valuable, the specific underlying mechanisms were not examined in detail. Further studies should delve into the molecular and cellular pathways to unravel the intricate connections among BEVs, gut microbiota, and the development of postoperative delirium.

Lastly, as an observational study, our research design does not permit the establishment of causality. While we observed associations between alterations in BEVs and the occurrence of delirium, these findings do not imply a cause-and-effect relationship. Experimental studies with controlled interventions are necessary to explore the causal connections and elucidate the pathways through which differences in BEVs may contribute to the development of postoperative delirium.

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V. CONCLUSION

Although delirium is often overlooked in diagnosis or optimal treatment by attending physicians, effective prediction could greatly enhance the prevention or treatment of postoperative delirium. Our study provides compelling evidence for the significance of bacteria-derived extracellular vesicles in postoperative delirium. Moreover, we have successfully developed clinically pertinent predictive models based on selected taxa that exhibit a strong correlation in bacteria-derived extracellular vesicles with postoperative delirium.

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APPENDICES

Table A1. Data on Comprehensive Geriatric Assessment, intraoperative information, and laboratory test results on discharge ; Continuous variables are expressed as median (IQR) and were compared using the independent t-test or rank sum test depending on the data distribution. Categorical variables are expressed as numbers (%) and were compared using the chi-square test.

		delirium	Non-delirium	P value
Education level (0-3year / 4-6 year / 7-12 year / 13 year ~)		4 / 15 / 20 / 4	4 / 12 / 24 / 5	0.900
Instrumental activities of daily living		10.00 (10.00, 12.75)	10.00 (10.00, 12.00)	0.400
Frailty index		2.0 (1.0, 3.0]	2.0 (1.0, 2.0)	0.698
Mini nutritional assessment		13.00 (12.00, 14.00)	13.00 (12.00, 14.00)	0.300
Surgery type	Cervical, N (%)	2 (4.7%)	3 (6.7%)	
	Lumbar, N (%)	38 (88.4%)	41 (91.1%)	0.530
	Others, N (%)	3 (7.0%)	1 (2.2%)	
Anesthesia duration (minutes)		229 (175, 268)	235 (190, 280)	0.900
Estimated intraoperative blood loss (mL),		515 (310, 768)	415 (200, 720)	0.400
Operation level		2.1 ± 1.3	1.6 ± 0.8	0.070
Duration of postoperative hospital stay (day)		9.00 (7.25, 10.00)	8.00 (7.00, 9.00)	0.500
* laboratory test on discharge				
White blood cell count (10^3/ μL)		6.29 (5.48, 8.03)	6.26 (5.36, 7.14)	0.300
Hemoglobin (g/dL)		9.20 (8.80, 9.95)	9.60 (8.88, 10.33)	0.800
Platelet (10^3/μL)		279 (190, 320)	225 (198, 280)	0.400
Erythrocyte sedimentation rate (mm/hr)		49 (26, 62)	37 (25, 58)	0.600
C-reactive protein (mg/L)		24 (14, 68)	32 (16, 49)	0.300
Estimated glomerular filtration rate (ml/min/1.73m^2)		89 (75, 94)	89 (84, 93)	0.088
Total protein (g/dL)		5.60 (5.30, 6.05)	5.60 (5.30, 5.90)	0.300
Albumin (g/dL)		3.50 (3.30, 3.75)	3.55 (3.40, 3.80)	0.700
Neutrophil Lymphocyte Ratio		3.5 (2.2, 4.2)	2.9 (2.0, 3.5)	0.090
Lymphocyte Monocyte Ratio		2.6 (1.8, 3.8]	2.7 (2.1, 3.7]	0.316
Platelet Lymphocyte Ratio		196.8 (151.5, 286.2)	165.2 (139.7, 215.4)	0.130

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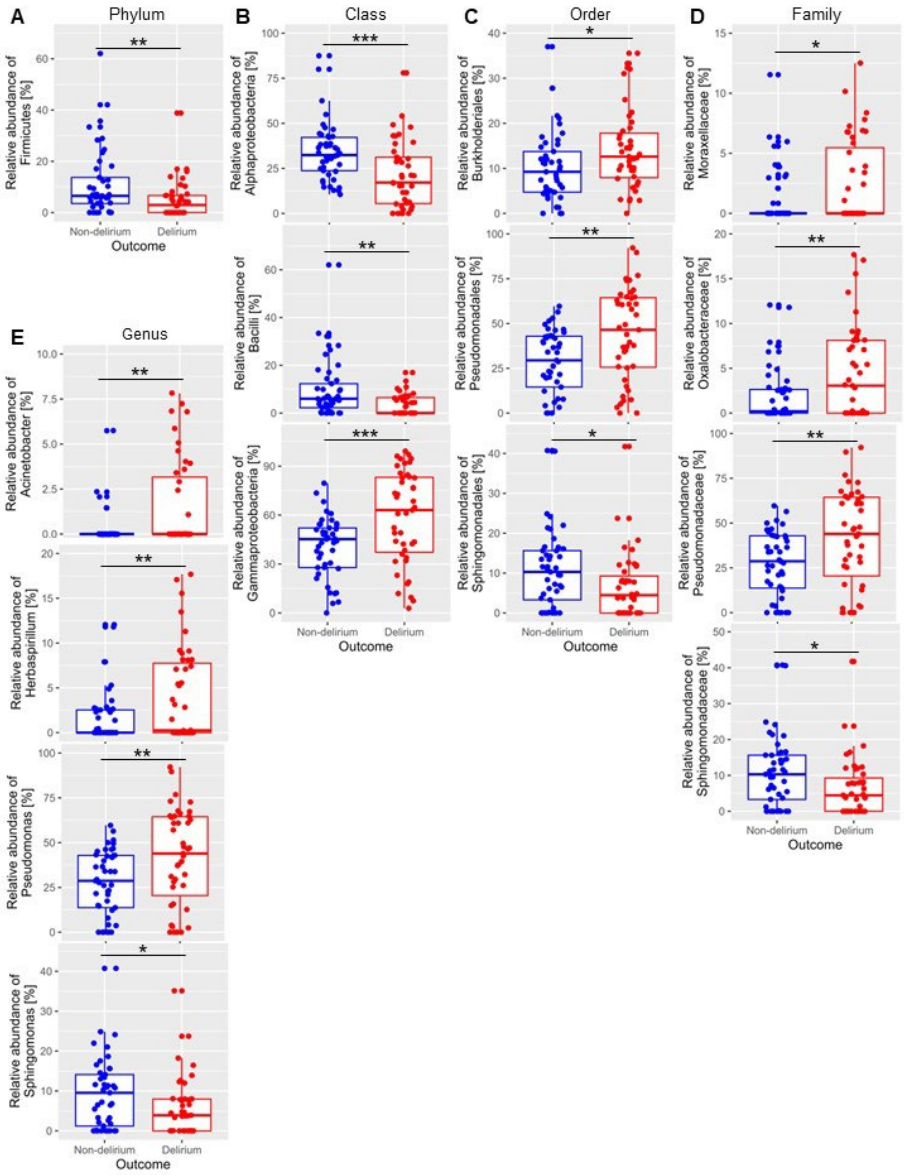

Figure A1. Analysis results of microbial differences of systemic bacteria-derived extracellular vesicles in blood in the non-delirium and delirium group at the level of the (A) Phylum, (B) Class, (C) Order, (D) Family, and (E) Genus.

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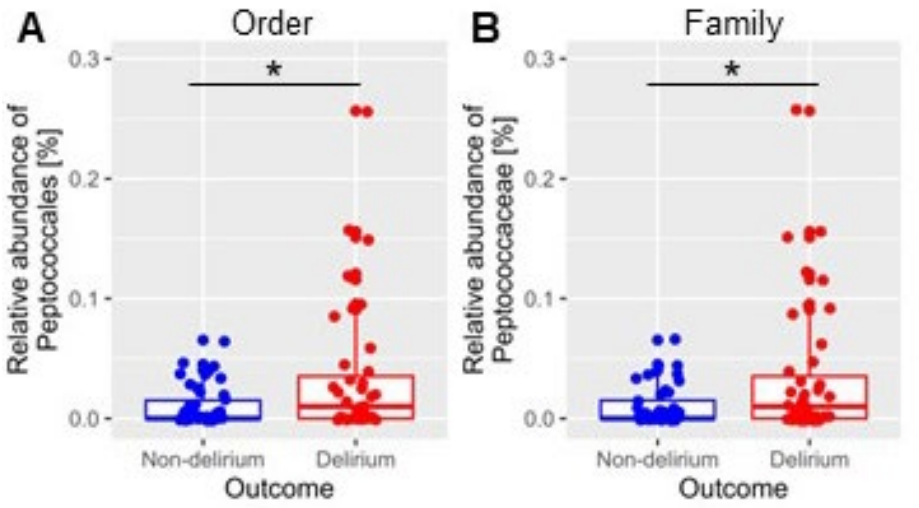

Figure A2. Analysis results of microbial differences of gut microbiome in blood in the non-delirium and delirium group at the level of (A) order and (B) family.

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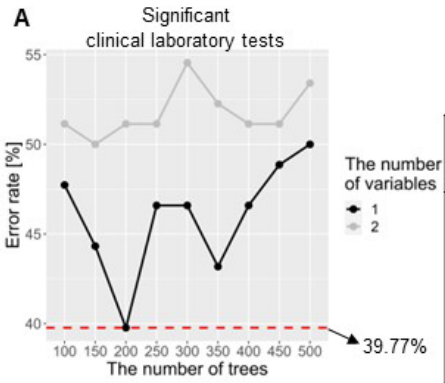
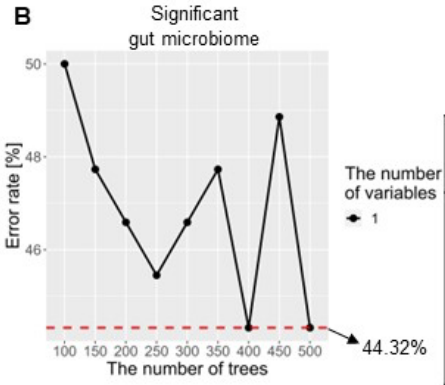


Optimization		Contingency table				Validity and predictive value	
Number of variables in each split	1	N=88		Random forest		Error rate	39.77%
				ND	D	Accuracy	60.23%
		Reference	ND	30	15	Sensitivity	66.67%
Number of trees	200					Specificity	53.49%
			D	20	23	PPV	60.00%
						NPV	60.53%



Optimization		Contingency table				Validity and predictive value	
Number of variables in each split	1	N=88		Random forest		Error rate	44.32%
				ND	D	Accuracy	55.68%
		Reference	ND	36	9	Sensitivity	80.00%
Number of trees	400					Specificity	30.23%
			D	30	13	PPV	54.55%
						NPV	59.09%

Figure A3. Error rate of random forest model with clinical laboratory tests or gut microbiome. ND, non-delirium ; D, delirium ; PPV, positive predictive value ; NPV, negative predictive value

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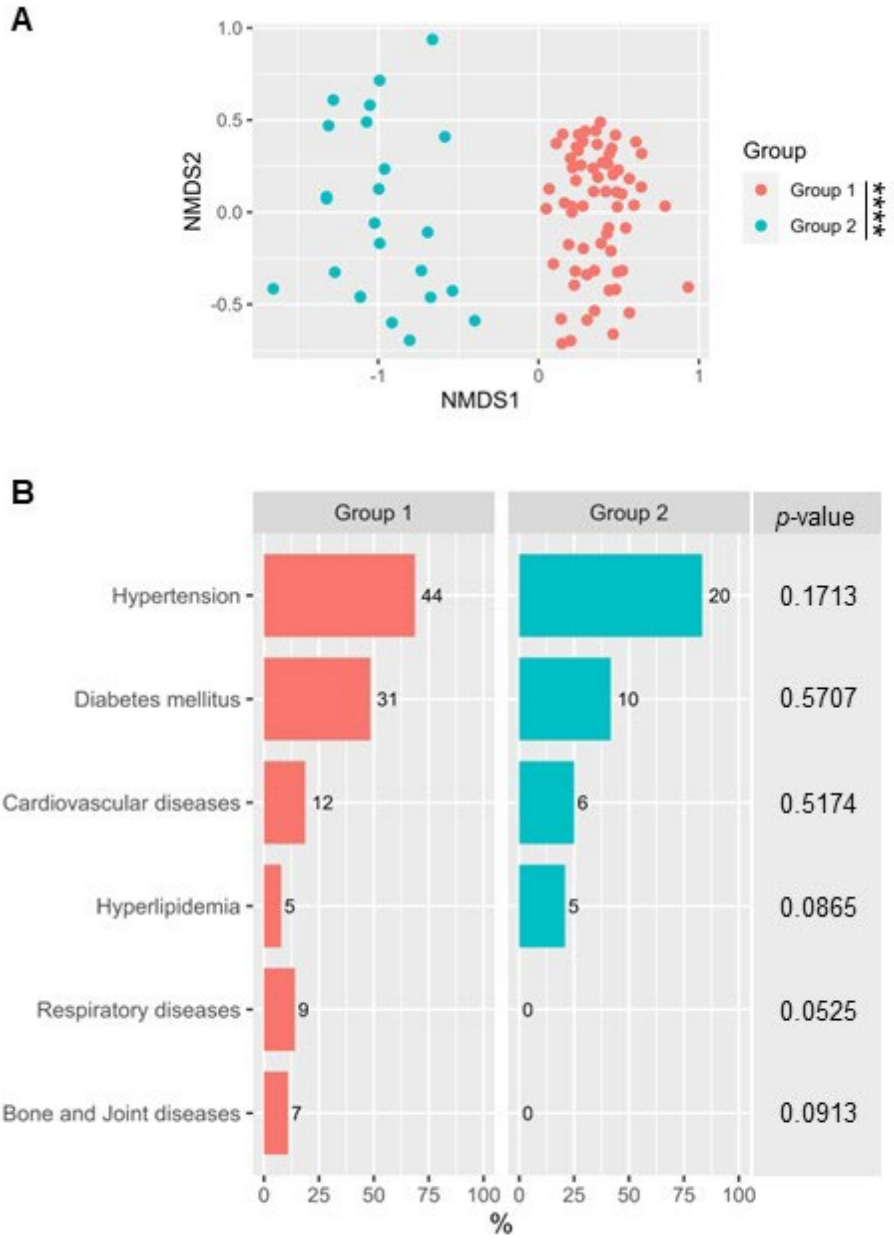

- Figure A4. (A) Non-metric dimensional scaling (NMDS) ordinations based upon Bray-Curtis dissimilarity using all amplicon sequence variants were used to understand β-diversity. Two distinctive pattern of patients were defined with NMDS 1 axis; group 1 and 2 with positive and negative values of NMDS 1, respectively. Significance between the groups was performed by

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analysis of similarity (ANOSIM). (B) Disease or disorder diagnosed at least 10% of either group were further analyzed to understand comorbidity. The numbers beside columns indicate the frequencies of each disease or disorder. ASV, Amplicon Sequence Variants; BEV, bacteria-derived extracellular vesicle; ****, P<0.0001.

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ABSTRACT(IN KOREAN)

수술 후 섬망 예측을 위한 바이오마커로써의 세균 유래 세포외 소포

<지도교수 구본녀>

연세대학교 대학원 의학과

성 명

박수정

섬망은 급성 인지 장애의 일종으로 노인 환자들 사이에서 수술 후 흔히 발생한다. 섬망 환자들은 예후와 경과가 다양한 것이 특징이며, 인식, 지남력, 기억 및 행동에 장애를 초래한다. 60세 이상 환자 중 약 20-25%에게 섬망이 나타나는 것으로 알려져 있으며, 섬망을 겪고 난 환자들의 경우 입원 기간이 증가되고, 사망률 또한 증가하는 것으로 알려져 있어 사회적인 관심이 높다. 섬망에 대한 예방 조치가 취해진다면, 섬망에 취약한 노인환자들의 위험을 경감시킬 수 있다. 높은 유병률에도 불구하고 불명확한 병태생리와 기전의 복잡성으로 인해 진단, 치료 및 연구가 잘 진행되지 못하고 있다.

장-뇌 축은 장 내 미생물 군과 뇌 간의 통신 체계로, 소화기 기능뿐만 아니라 다른 영역에도 영향을 미친다. 장 내 미생물 군의 불균형 또는 이상 발생은 다양한 신경 및 기분 장애와 관련이 있다. 이전의 다른 연구에서 인간 미생물 군집이 수술 후 섬망과도 관련이 있다는 사실이 확인되었다. 또한, 세포외 소포들은 세포 간 상호작용에서 중요한 역할을 하며, 질병 예측과 신속한 평가를 위한 잠재적인 생체 표지로 제안되었다. 또한 혈뇌 장벽을 통과할 수 있는 박테리아 유래의 세포외 소포는 면역 체계 조절에 중요한 역할을 할 수 있다. 본 연구는 섬망 환자에서 장 내 미생물 군집과 혈액 내의 박테리아 유래의 세포외 소포 (BEVs)의 조성을 탐구하고 섬망에 대한 예측 모델을 구축하는 것을 목표로 했다.

우리는 척추 수술이 예정되어 있는 70세 이상 환자 128명의 혈액과 대변을 분석했다. 수술 전 대변 및 혈청 샘플을 얻은 후, 수술 후 입원 기간 동안 섬망을 최소한 두 번 이상 평가하여 섬망군, 비섬망군으로 분류하였다.

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우리는 수술 전 대변 샘플과 수술 전 혈청 박테리아 유래의 세포 간 소포에서 차세대 염기서열 분석을 수행했다. 섬망 및 비섬망 그룹 간의 세포 간 소포와 장 내 미생물 군의 조성 및 기능을 비교했다. 이러한 변수를 사용하여 랜덤 포레스트 방법을 이용하여 섬망에 대한 예측 모델을 구축했다.
88명의 환자의 기준 특성은 양쪽 그룹 모두에서 비슷하였다. 세포외 소포의 분석에서, 섬망 그룹은 비섬망 그룹보다 그룹 내 다양성이 감소한 것으로 나타났다. 게다가, 섬망 그룹은 유전적 동질성이 크게 감소한 Inverse Simpson 지수를 가지고 있었다. 섬망을 구별하기 위해 13가지 세포외 소포 세균 분류군이 확인되었다.
장 내 미생물 군집 분석에서는 양쪽 그룹 모두에서 비슷한 다양성과 동질성이 나타났다. 강(order) 수준에서, *Peptococcales*은 섬망 그룹에서 더 풍부하게 나타났으며 과(family) 수준에서 *Peptococcaceae*가 비섬망 그룹보다 더 자주 감지되었다. PICRUSt 분석을 통해 시크르산 회로와 대사에 관련된 기능 경로 16개를 발견했다. 논문에서 제시한 모델은 섬망에 대한 탁월한 예측 능력을 가지고 있었다. 따라서 본 연구는 수술 후 섬망 환자에서 박테리아 유래의 세포 간 소포의 중요성을 밝혀주었으며, 섬망에 대한 가치 있는 예측 모델을 제시했다.

핵심되는 말 : 섬망 ; 세포외 소포 ; 장내 미생물 군집 ; 정밀의학