Dysbiosis of intestinal microbiota to predict in-hospital mortality in critically ill patients: results of a prospective observational cohort study

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Ru Wei
Guangzhou Women and Children's Medical Center

Xu Chen
The Second School of Clinical Medicine, Southern Medical University

Linhui Hu
Maoming People's Hospital

Zhimei He
Guangdong Provincial People's Hospital

Xin Ouyang
Guangdong Provincial People's Hospital

Silin Liang
Guangdong Provincial People's Hospital

Shixue Dai
Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, South China University of Technology

Weihong Sha
Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, South China University of Technology

Chunbo Chen
Guangdong Cardiovascular Institute, Guangdong General Hospital and Guangdong Academy of Medical Sciences, National Clinical Research Center for Kidney Disease, State Key Laboratory of Organ Failure Research, Nanfang Hospital, Southern Medical University

✉ gghccm@163.comCorresponding Author
ORCiD: https://orcid.org/0000-0001-5662-497X

Abstract

Background: Despite the essential functions of the intestinal microbiota in human physiology, little research has been reported on the gut microbiota alteration in intensive care patients. This investigation aimed to explore the dysbacteriosis of intestinal flora in critically ill patients, and evaluate the prognostic performance of this dysbiosis to predict in-hospital mortality. Methods: A prospective cohort of patients were consecutively recruited at Intensive Care Units (ICUs) in Guangdong Provincial People's Hospital from March 2017 through October 2017. Acute Physiology and Chronic Health Evaluation (APACHE) II score and Sequential Organ Failure Assessment (SOFA) score were assessed, and fecal samples were taken for examination within 24 hours of ICU admission. The taxonomic composition of intestinal microbiome was determined using 16S rDNA gene sequencing. Patients were divided into survival and death group based on the outcomes in hospital. The two groups were statistically compared using the independent samples t test and Metastats analysis. Genera of bacteria showing significantly different abundance between groups were assessed for predictors of in-hospital death. The prognostic value of bacterial abundance alone and in combination with APACHE II or SOFA score were evaluated using the area under the receiver operating characteristic curve (AUROC). Results: Among the 61 patients that were examined, a total of 12 patients (19.7%) died during hospital stay. *Bifidobacterium* differed significantly in abundance between survival and death group (P =0.031). The AUROC of *Bifidobacterium* abundance identifying in-hospital death at a cut-off probability of 0.0041 was 0.718 (95% confidence interval [CI], 0.588-0.826). The panel of *Bifidobacterium* abundance plus SOFA (AUROC, 0.882; 95% CI, 0.774-0.950) outperformed SOFA (AUROC, 0.649; 95% CI, 0.516-0.767; P =0.012) and *Bifidobacterium* abundance alone (P =0.007). The panel of *Bifidobacterium* abundance plus APACHE II (AUROC, 0.876; 95% CI, 0.766-0.946) outperformed APACHE II (AUROC, 0.724; 95% CI, 0.595-0.831; P =0.035) and *Bifidobacterium* abundance alone (P =0.012). Conclusions: Dysbiosis of intestinal microbiota with variable degree of reduction in *Bifidobacterium* abundance exhibits promising performance in predicting in-hospital mortality, and provides incremental prognostic value to existing scoring systems in the adult intensive care unit (ICU) setting.

Background

Intestinal microbiota diversity plays a vital role in maintaining intestinal homeostasis, human nutrition and health [1]. Nevertheless, this diversity is highly vulnerable to diseases, especially to the critical illnesses with high mortality rates [2, 3]. Critical illnesses could frequently cause gastrointestinal motility disorder, intestinal mucosal ischemia, and impaired intestinal barrier function [4, 5], leading to dramatically altered intestinal microbiota [3, 6]. Dysbiosis of gut microbiota has been widely viewed as a potential marker for many diseases [7], hence may also have the potential to be a new mortality predictor for critically ill patients [5, 7]. A previous analysis by Xu et al. indicated that the gut microbiota dysbiosis was associated with patients' 180-day mortality in the settings of neurological intensive care unit [6]. However, the prognostic ability of this potential marker in predicting in-hospital mortality in intensive care unit patients remains to be determined.

This prospective observational cohort study sought to investigate the intestinal dysbiosis in crticially ill patients at admission, detect some bacterial genus whose altered abundance were associated with in-hospital mortality. Further assessment of the prognostic performance of this dysbiosis along with existing scoring systems (APACHE II / SOFA score) had also been conducted in an effort to obtain an optimal clinical model to predict in-hospital mortality.

Methods

Study Design and Population

A prospective cohort of patients were consecutively recruited at Intensive Care Units (ICUs) in Guangdong Provincial People's Hospital from March 2017 through October 2017. Patients were admitted from general or emergency wards. Participants were excluded if any of the following criteria are met: (1) the participant was under age 18 years; (2) the participant received systematic antibiotic treatment within 48 hours before ICU admission; (3) the participant did not defecate within 24 hours of ICU admission; (4) the participant was admitted to ICU for end-stage chronic diseases; (5) the participant died within 24 hours of ICU admission; (6) the participant was discharged from hospital with discontinued follow-up. The study was approved by the local ethics committee. All patients provided informed consent form before participating in the study.

Sample size consideration

The sample size was calculated according to the one in ten rule, a rule of thumb recommended by Peduzzi et al. [8] and Harrell et al. [9], namely, events per variable being 10 or greater in the setting of a multivariate logistic regression model. Our prediction model covers two variables - *Bifidobacterium* abundance and APACHE II or SOFA score. This would have required a minimum sample size of 20 participants who had events (dying in hospital) to predict the outcome of in-hospital death.

Data Collection

At admission to the ICU, demographic, laboratory and clinical variables were recorded. SOFA score and APACHE II score were assessed twice within 24 hours of ICU admission with the worst value chosen. Stool samples were collected at ICU admission and immediately frozen at -80°C. Taxonomic compositions of the intestinal microbiome were determined using the 16S rDNA gene sequencing of the stool sample.

Intestinal microbiota analysis

Intestinal flora analysis was performed by the 16S rDNA amplicon sequencing method, the most suitable tool for bacterial phylogeny and classification identification [10-12]. The genomic DNA of the sample was extracted by the sodium dodecyl sulphate (SDS) method. The purity and concentration of the DNA were detected on 1% agarose gel electrophoresis. Dilute the DNA to 1 ng/ml with sterile water depending on the concentration. Specific primer (e.g. 16S V4: 515F-806R, 18S V4: 528F-706R, 18S V9: 1380F-1510R, et. al) with the barcode was used to amplify the 16S rDNA gene in different regions (16S V4/16S V3/16S V3-V4/16S V4-V5, 18S V4/18S V9, ITS1/ITS2, Arc V4). All PCR reactions were performed using Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA). The same volume of 1X loading buffer (containing SYB green) was mixed with the PCR product and electrophoresed on a 2% agarose gel. Samples with a bright master band between 400-450 base paris were selected for further experiments. The mixture PCR product was then purified using a Qiagen Gel Extraction Kit (Qiagen, Duesseldorf, Germany). Following the manufacturer's

recommendations, TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA) was used to generate sequencing libraries and index codes were added. Qubit 2.0 fluorometer (Thermo Scientific, Waltham, MA, USA) and Agilent Bioanalyzer 2100 system (Agilent Technologies, Beijing, China) were used to evaluate the library quality [13]. The library was sequenced by an IlluminaHiSeq2500 (Illumina, San Diego, CA, USA) platform and 250 base pair paired-end reads were generated [14].

Sequences analysis was performed with Uparse software (Uparse v7.0.1001, http://drive5.com/uparse/) [15]. By default, the sequence was clustered into operational taxonomic units (OTUs) with 97% identity. Meanwhile, the representative sequence of OTUs was selected. According to the algorithm principle, the sequence with the highest frequency in OTUs was selected as the representative of OTUs. The Mothur method (with a cut-off value of 0.8) and Silva database (version 128, https://www.arb-silva.de) were used to annotate OTUS representative sequences and analyze species annotations to obtain classification information of each classification level: kingdom, phylum, taxa, order, family, genus and species [15-17]. Fast multi-sequence alignments were performed using MUSCLE (Version 3.8.31, http://www.drive5.com/muscle/) software to obtain phylogenetic relationships for all OTUs representative sequences [17]. At last, the data of each sample was homogenized, and the sample was homogenized according to the minimum amount of data in the sample.

Data analysis

Patients participating in the study were categorized into two groups according to their survival status in hospital: survival and death groups. All baseline data between groups were assessed. Based on the OTUs clustering results, the abundance analysis of OTUs was carried out to explore the differences in community structure between groups. The difference between the beta diversity index groups was then analyzed. Metastats analysis was performed at each classification level (Phylum, Class, Order, Family, Genus, Species). *P* values were obtained by performing permutation tests between groups, and abundance distribution boxes of different species among groups were plotted.

The prognosis prediction models for *Bifidobacterium* abundance, APACHE II score, SOFA score were

established with univariate logistic regression. The prognosis prediction models for APACHE II plus

Bifidobacterium abundance, and SOFA score plus *Bifidobacterium* abundance were established with

multivariate logistic regression. All prediction models were evaluated by the area under the receiver

operating characteristic curves (AUROC). To avoid overfitting effect due to relative small sample size,

The bootstrap method was used with 1000 resamples, and the bootstrap-corrected AUROC and 95%

CI were reported. The Youden index [18] was used to determine the optimal cut-off points, at which

sensitivity and specificity were calculated. Comparisons between groups of AUROCs were performed

using the DeLong method [19]. using bootstrapping methods [19].

  Continuous variables are presented as mean ± standard deviation (SD), and were compared using

the independent sample Student's *t* test. Categorical variables were presented as number

(percentage) and were compared using the Pearson X^2 test or Fisher's exact test, as appropriate. The

significance level was set at $P < 0.05$. The analysis was performed with SPSS 24.0 software (SPSS,

Chicago, IL, USA) and MedCalc 12.5.0 software (MedCalc Software, Ostend, Belgium) , and R 3.3.1

software (R Foundation for Statistical Computing, Vienna, Austria) using RStudio v1.0.136 (RStudio

Inc, Boston, MA, USA).

Results

A total of 61 critically ill patients with a mean age 60.2 years were included in this study. Forty-nine

patients (80.3%) survived, and twelve patients (19.7%) died in hospital. The patients' clinical and

laboratory characteristics were shown in Table 1. Notably, the score of SOFA and APACHE II were

markedly higher in the death group than in the survival group (P = 0.001).

Table 1

Clinical and demographic characteristics of patients

Variables	Total (n = 61)	Death (n = 12)	Survival (n = 49)	P value
Age, y	60.2 ± 19.0	61.1 ± 20.2	60.0 ± 18.9	0.871
Male	42 (68.8)	10 (83.3)	32 (65.3)	0.186
Primary diagnosis				0.541
Neurologic	25 (41.0)	3 (25.0)	22 (44.9)	
Respiratory	9 (14.8)	2 (16.7)	7 (14.3)	
Digestive	10 (16.4)	3 (25.0)	7 (14.3)	
Others	17 (27.8)	4 (33.3)	13 (26.5)	
CRP, mg/L	67.3 ± 65.6	97.8 ± 81.9	59.4 ± 59.2	0.149
PCT, ng/L	40.5 ± 14.6	40.3 ± 66.1	8.3 ± 29.0	0.127
ICU stay, days	26.6 ± 43.7	14.7 ± 11.9	29.5 ± 48.1	0.057
SOFA score	9.8 ± 3.7	13.4 ± 3.4	8.9 ± 3.1	0.001
APACHE II score	20.4 ± 7.1	27.5 ± 6.6	18.7 ± 6.0	0.001

Continuous variables are presented as mean ± SD and categorical variables as number (percentage). APACHE II, Acute Physiology and Chronic Health Evaluation II; CRP, C reactive protein; ICU, intensive care unit; PCT, procalcitonin; SD, standard deviation; SOFA, sequential organ failure assessment.

After paired-end read merging and error correction of 16S rDNA sequencing, a total of 4592443 effective tags were obtained from 61 stool samples with a mean of 75286 tags. Based on 97% sequence identity, amplicons were clustered into 4954 OTUs. 837 genera were identified in gut microbiomes in this study. OUT richness from intestinal microbiota showed no significant difference between groups. According to the results of species annotation, at the genus level, the abundance of ten genera was at a higher level in the two groups, accounting for more than 50% of the total. The top 10 genera with the highest abundance at the Genus classification level were enrolled to generate a columnar accumulation plot of relative abundance. The top 10 genera were Enterococcus, Clostridiuminnocuum, Subdoligranulum, Leuconostoc, Stenotrophomonas, Escherichia-Shigella, Streptococcus, Collinsella, Akkermansia, and Bifidobacterium. Figure 1 depicted their composition in each sample, and Fig. 2 described their relative abundance between groups. In order to find different species among the 10 genera, we performed a t test to analyze the intestinal flora and identify the species with a significant difference (P < 0.05). As demonstrated in Table 2, among the 10 genera only the Bifidobacterium abundance presented significant difference between groups with a larger reduction in the death group than in the survival group (P = 0.031). The abundance distribution of different species between groups were also mapped (Fig. 3).

Table 2

Mean abundance of gut microbiome taxa in patients

Taxa	Death		Survival	P value
Enterococcus	0.2846 ± 0.3221	0.3070 ± 0.2989		0.830
Clostridium innocuum	0.0888 ± 0.1989	0.0190 ± 0.0553		0.253
Subdoligranulum	0.0570 ± 0.1794	0.0055 ± 0.0088		0.342
Leuconostoc	0.0195 ± 0.0600	0.0213 ± 0.0895		0.933
Escherichia-Shigella	0.0243 ± 0.0428	0.0614 ± 0.0115		0.072
Streptococcus	0.0294 ± 0.0665	0.0241 ± 0.0723		0.810
Collinsella	0.0007 ± 0.0010	0.0122 ± 0.0681		0.242
Akkermansia	0.0115 ± 0.0304	0.0269 ± 0.0744		0.268
Stenotrophomonas	0.0014 ± 0.0021	0.0120 ± 0.0650		0.261
Bifidobacterium	0.0019 ± 0.0024	0.0308 ± 0.0912		0.031

Abundance of gut microbiome are presented as mean ± SD. Differences in taxa between death and survival group are calculated using independent sample t test.

AUROC analysis was used to assess the predictive accuracy of APACHE II, SOFA, and Bifidobacterium abundance for the prognosis of hospital death in critically ill patients. The AUROC analysis of the three univariate models (APACHE II, SOFA, and Bifidobacterium abundance) indicated that APACHE II (AUROC, 0.724; 95% CI, 0.595–0.831) had a slightly better discrimination than SOFA (AUROC, 0.649; 95% CI, 0.516–0.767) and Bifidobacterium abundance (AUROC, 0.718; 95% CI, 0.588–0.826) (Fig. 4a and Table 3). However, the pairwise comparative analysis showed no significant difference (Table 4).

Table 3

Predictive characteristics of admission indicator and their combinations for ICU mortality

Logistic regression model	AUROC (95% CI)	Youden index	Cut-off	Sensitivity (%)	Specificity (%)
Univariate models					
APACHE II	0.724 (0.595–0.831)	0.5714	27.00	100.00	57.14
SOFA	0.649 (0.516–0.767)	0.3452	10.00	91.67	42.86
Bifidobacterium	0.718 (0.588–0.826)	0.4677	0.0041	91.67	55.10
Multivariate models					
APACHE II plus Bifidobacterium	0.876 (0.766–0.946)	0.6514	1.82	91.67	73.47
SOFA plus Bifidobacterium	0.882 (0.774–0.950)	0.6071	1.07	75.00	85.71

Optimal cut-off value are determined by Youden's index. Cut-off points of the multivariate indicator models were the predicted probability generated by the multiple logistic regression model. AUROC, area under the receiver operating characteristic curve.

Table 4

Comparisons of AUROCs among prediction models

Comparison pairs	P value
AUROC comparison among univariate models	
APACHE II vs SOFA	0.419
APACHE II vs Bifidobacterium	0.927
SOFA vs Bifidobacterium	0.463
AUROC comparison between multivariate and univariate model	
APACHE II plus Bifidobacterium vs APACHE II	0.035
APACHE II plus Bifidobacterium vs Bifidobacterium	0.012
SOFA plus Bifidobacterium vs SOFA	0.012
SOFA plus Bifidobacterium vs Bifidobacterium	0.007
APACHE II, Acute Physiology and Chronic Health Evaluation II; AUROC, area under receiver operating characteristic curve; CI, Confidence Interval; SOFA, sequential organ failure assessment.	

In order to assess the performance of combinations of these predictors, we performed logistic regression using two panels of predictors (APACHE II plus Bifidobacterium abundance and SOFA scores plus Bifidobacterium abundance). The ROC curve analysis showed that the panel of Bifidobacterium abundance and APACHE II had the largest AUROC of 0.876 (95% CI 0.766–0.946) (Fig. 4b and Table 3) with no significant difference (P = 0.9274). By further comparison of AUROCs between individual and combined predictors, APACHE II plus Bifidobacterium abundance significantly outperformed Bifidobacterium abundance (P = 0.018) and APACHE II score (P = 0.035) (Table 3). The AUROC of SOFA was 0.649 (95% CI, 0.516–0.767), and the AUROC of SOFA plus Bifidobacterium abundance was 0.882 (95% CI, 0.774–0.950). The combination of Bifidobacterium abundance and SOFA had higher specificity (85.71%) but lower sensitivity (75%) (Fig. 4c). We compared the AUROC of SOFA versus SOFA plus Bifidobacterium abundance, and Bifidobacterium abundance versus SOFA plus Bifidobacterium abundance, both showed significant differences with P values of 0.007 and 0.012, respectively (Table 3).

Discussion

The results of our study suggest that dysbiosis of intestinal microbiota with variable degree of reduction in Bifidobacterium abundance is associated with critically ill patients' risk of death in hospital. The abundance of intestinal Bifidobacterium distributed markedly lower in the death group than in the survival group, hence exhibits good prognostic values for in-hospital mortality. It also provides incremental prognostic value to existing scoring systems in intensive care unit (ICU). Bifidobacterium is a genus of gram-positive, nonmotile, often branched anaerobic bacteria, first

discovered and isolated by Tissier et al in 1899 using breastfeeding infant feces [20]. Bifidobacteria represent one of the major genera of bacteria that make up the gut flora in humans, whose physiological functions include promoting nutrient absorption [21], maintaining intestinal micro-ecological balance [22], immune regulation [23], and anti-tumor [24–26]. However, bifidobacteria are susceptible to some adverse conditions like aging and disease [27], hence stand a good chance of reducing in intensive care patients [3, 28]. The pathophysiological effects of critical illness (i.e. decreased oral intake, intestinal dysmotility, gut hypoperfusion, reperfusion injury, or impaired mucosal integrity) and the clinical interventions of intensive care (i.e. supine positioning, gastric-acid suppression, sedatives, opiates and neuromuscular blockade, or systemic antibiotics) causing ecological imbalance, have substantially altered the gut microbiome.

The advent of culture-independent microbiology and high-throughput sequencing method enable researchers to identify specific features of the microbiome that promote and disrupt homeostasis in critically ill patients. In this study, we used 16S rDNA amplicon sequencing for bacterial phylogeny and classification identification. Interestingly, in our study we found that Bifidobacterium abundance was associated the severity of illness. Namely, the patients with less Bifidobacterium content in gut have higher risks for death in hospital. Subsequent statistical analysis of logistical regression determined Bifidobacterium abundance as an predictor for in-hospital mortality with an AUROC of 0.718. This findings were consistent with previous findings that the reduction of Bifidobacterium abundance was a sign of disorders [12, 28, 29].

Notably, the results in our study conflicts with that in a previous study by Xu et al. [6] who investigated the dysbiosis of gut microbiota in patients in a setting of neurocritical care unit. Rather than Bifidobacterium, Xu et al. discovered the gut genera with significantly decreased abundance over time with followed longitudinally were Ruminococcaceae and Lachnospiraceae, using the same 16S rRNA gene sequence analysis. This inconsistence can be attributed to several reasons. First, participates in our study are consisted of critically ill patients in general ICU rather than in neurosurgical ICU. Second, in contrast to healthy volunteers as control cohort in previous study, our control cohort is composed of patients who survived in hospital. Additionally, we focused on the

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outcome of in-hospital death as a binomial variable for logistic regression analysis rather than the 180-day death as a time-dependent covariate for COX regression analysis. Despite these differences in designs and results, the two studies effectively enhance our common understanding that intestinal microbiota dysbiosis are associated with patients' adverse outcomes and have the potential to act as a predictor for death risk for critically ill patients.

As new diagnostic, therapeutic and prognostic techniques become available and ICU populations change, the scoring systems for use in ICU may not be very closely relevant to your patient population. This evolving situation may calls for fresh biological or clinical markers to reflect current practice patterns and treatment for critical illness. Interestingly, we found that the Bifidobacterium abundance as a promising prognosis factor for in-hospital mortality was able to add the prognostic value of existing scoring systems including SOFA and APACHE II score. In comparison with SOFA and APACHE II score, Bifidobacterium abundance present a prognostic value slightly lower than APACHE II but higher than SOFA for in-hospital death in critically ill patients. When combined with Bifidobacterium abundance, both APACHE II score and SOFA score performed better in critically ill patients' prognosis prediction. The reason for this result may be that indicators with different characteristics have different sensitivities and specificities, and a reasonable combination between them can improve the diagnostic performance. This shared similar mechanism with Deng's research [30], in which the investigator combined a panel of a functional marker (sCysC) and a tubular injury marker (uNAG) to predict outcomes in patients with severe AKI, demonstrating superior discriminating performance compared to sCysC and uNAG alone.

To the best of our knowledge, there exists few reports in the literature on the gut microbiota dysbiosis in ICU patients. We have validated that the reduction of gut Bifidobacterium content was an independent risk factor for critically ill patients' prognosis. Some Bifidobacterium strains [31] are considered as important probiotics showing promise in treating ulcerative colitis [32] and reducing infections including ventilator-associated pneumonia (VAP) which is common in critical illness [33]. Therefore, we propose that some pharmaceutical probiotic products made of Bifidobacterium may facilitate maintaining or restoring the homeostatic balance of infected intestine, and improving the

survival prognosis of patients in ICU at last. Further prospective clinical trials are required to confirm this proposal.

However, our study has some limitations. First, the relative small sample size as a result of implementing rigorous inclusion criteria and avoiding spending too many resources (e.g. subjects, time and financial costs), might cause overestimation of the predictability of Bifidobacterium abundance. Nevertheless, we used bootstrapping methods with 1000 resamples to avoid overfitting effect in logistic regression, and reported the bootstrap-corrected AUROC and 95% CI. In addition, our findings are limited to a single-center study, and center-specific effects cannot be excluded. Further multicenter studies with larger sample sizes are therefore required to verify the present results. Moreover, the PCR method and 16S rRNA amplicon sequencing method, both based on DNA or RNA extraction, have high accuracy but are cumbersome and time-consuming, and cannot quickly quantify the intestinal flora [10, 34, 35]. Therefore, it is especially important to develop a method that can quickly and accurately quantify the abundance of intestinal flora.

Conclusion

Dysbiosis of intestinal microbiota with variable degree of reduction in Bifidobacterium abundance exhibits promising performance in predicting in-hospital mortality, and provides incremental prognostic value to existing scoring systems in the intensive care unit (ICU) setting.

Abbreviations

APACHE-II: Acute Physiology and Chronic Health Evaluation-II; AUROC: area under receiver operating characteristic curve; CI: Confidence interval; ICU: Intensive care unit; IQR: Interquartile range; OTU: Operational taxonomic unit; PCR: polymerase chain reaction; ROC: receiver operating characteristic curve; SD: Standard deviation; SOFA: Sequential Organ Failure Assessment

Declarations

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Author Contributions

RW, XC, LHH and CBC, WHS designed the study, analyzed the data, and wrote the manuscript. RW,

XC, LHH, ZMH, XOY, SLL, SXD, and CBC, WHS contributed to the study concept. CBC and WHS

contributed to study coordination. WR, LHH, ZMH, and XOY contributed to data collection. RW, XC,

and SXD did the statistical analysis. All authors contributed to data interpretation and approved the

manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding

author on reasonable request.

Ethical approval and informed consent

All procedures performed in the trial were in accordance with the ethical standards of the ethics

committee of all the participating centers and with the 1964 Helsinki Declaration and its later

amendments or comparable ethical standards. Informed consent was obtained from all individual

participants included in the study.

Consent for publication

Not applicable.

Conflicts of interest

The authors declare that they have no competing interests.

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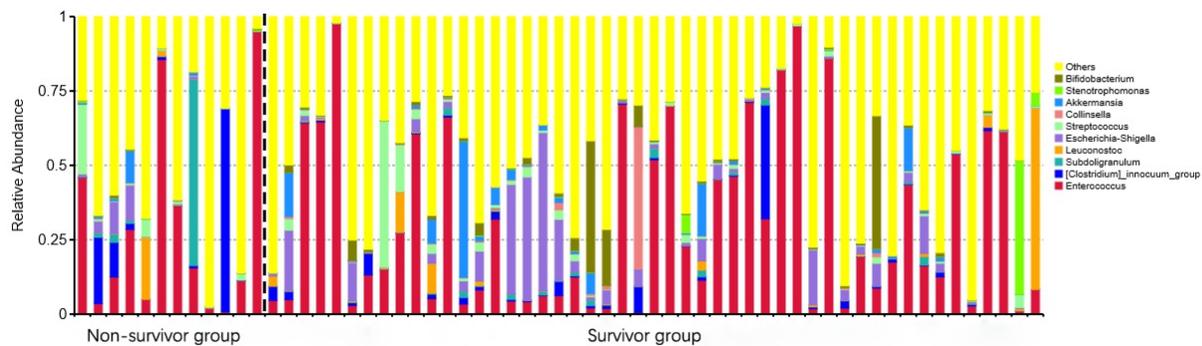
Figures

Figure 1

TOP 10 species relative abundance histogram at the genus level of the personal sample. The abscissa is the sample name; the ordinate is the relative abundance; the others represented the sum of the relative abundances of all the other genus except the 10 genera.
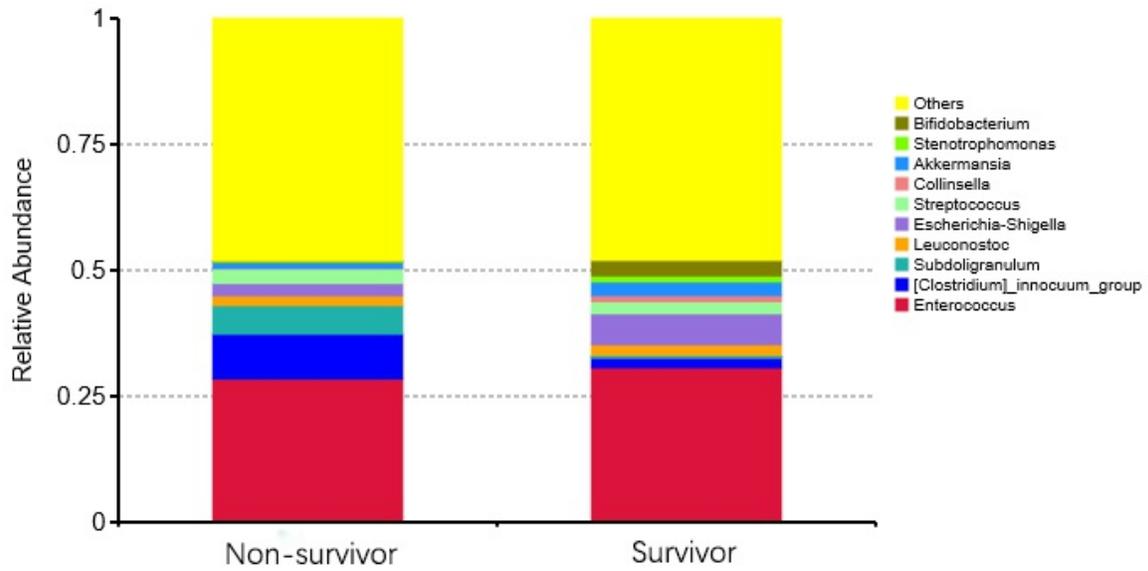
Figure 2

TOP 10 species relative abundance histogram at the genus level of the groups. The abscissa

is the group name; the ordinate is the relative abundance; the others represents the sum of

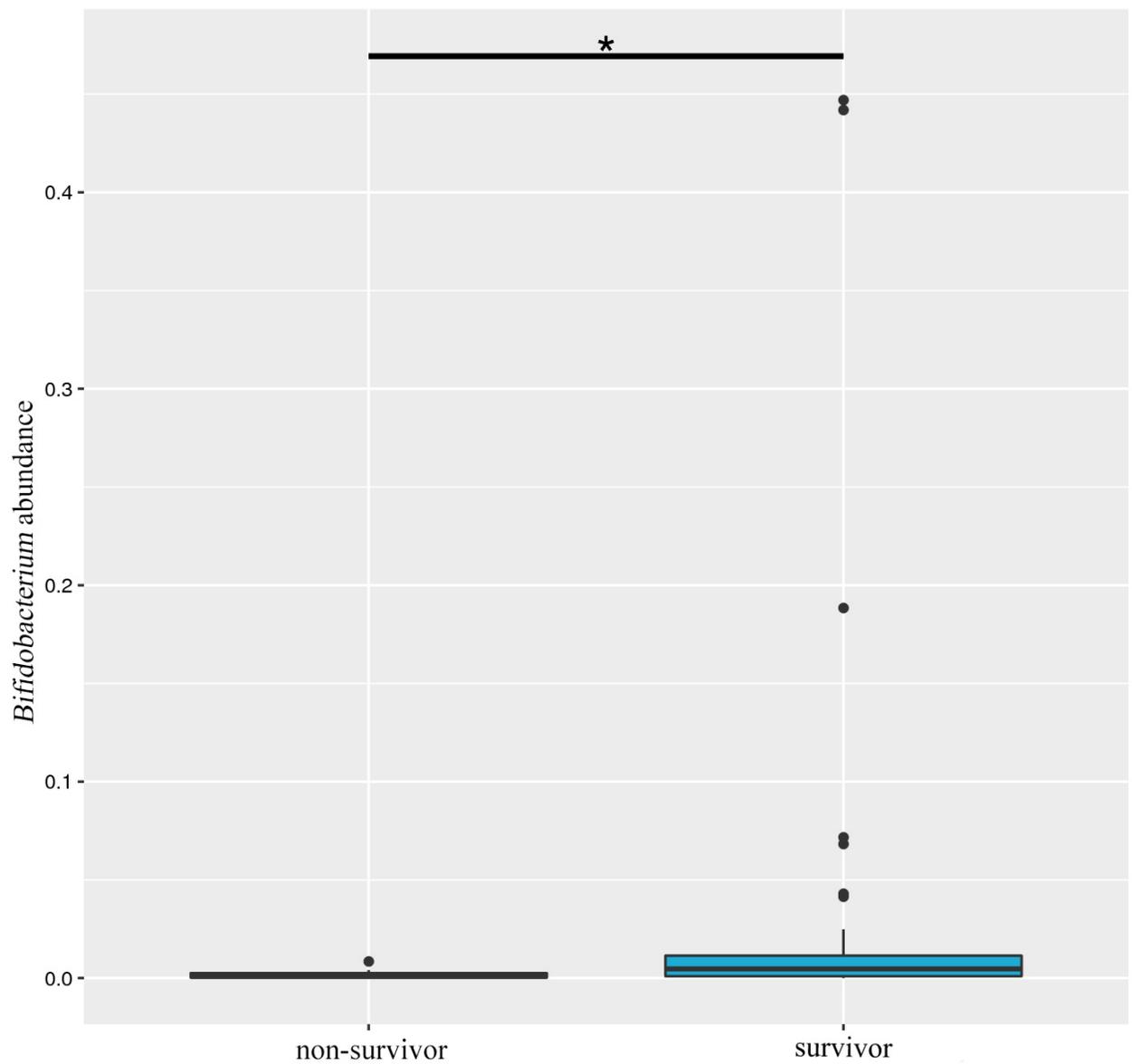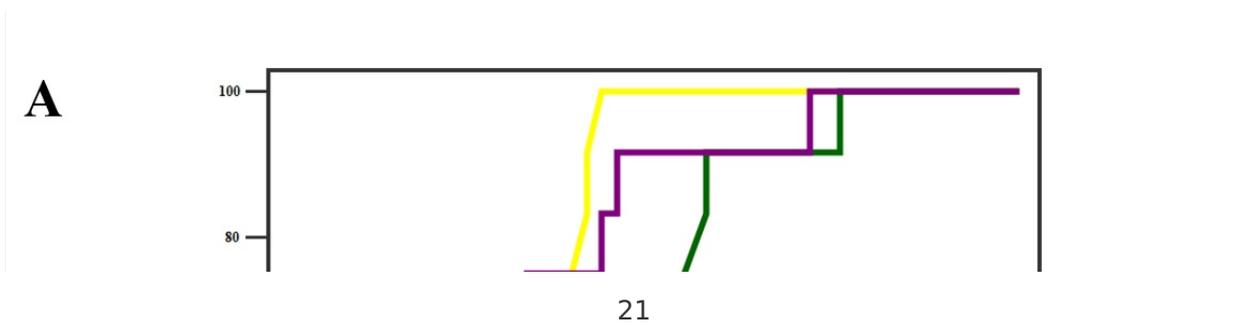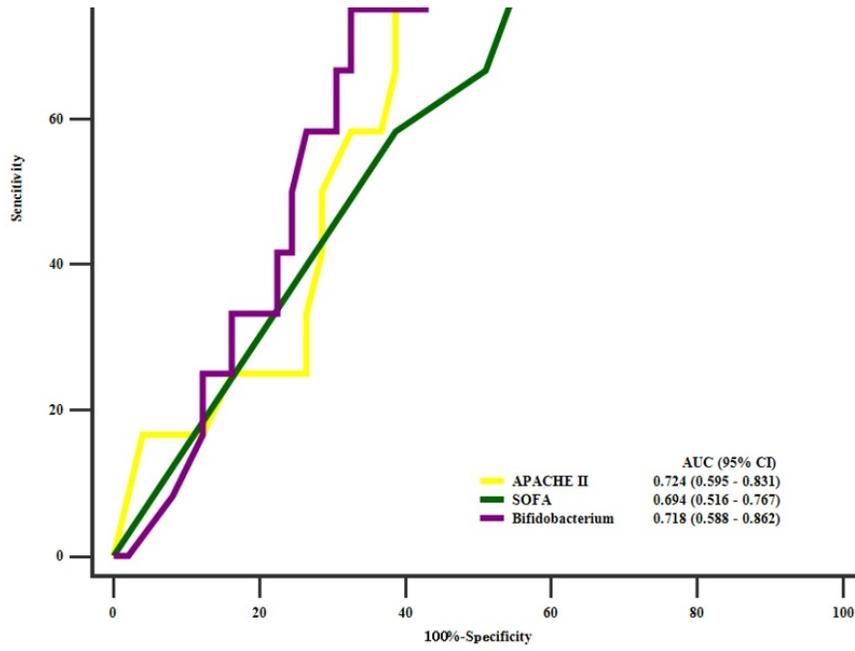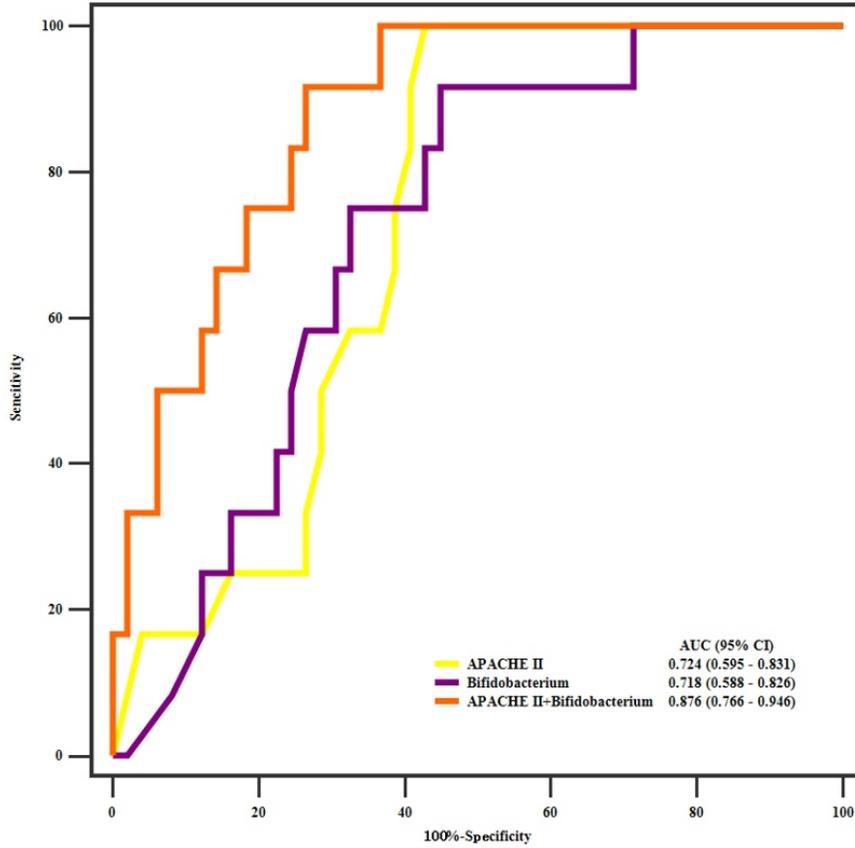the relative abundances of all the other genus except the 10 genera.

Figure 3

Box analysis of the abundance distribution of Bifidobacterium between groups. The horizontal axis is the sample grouping; the vertical direction is the relative abundance of Bifidobacterium; the horizontal lines represent two groups with significant differences. *P < 0.05.
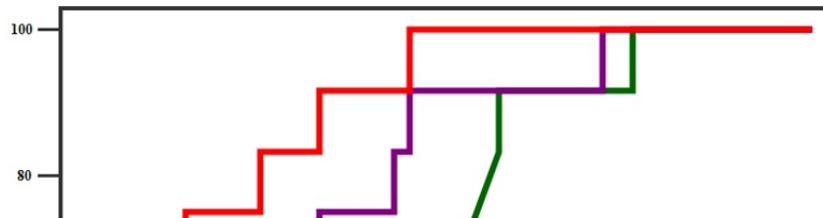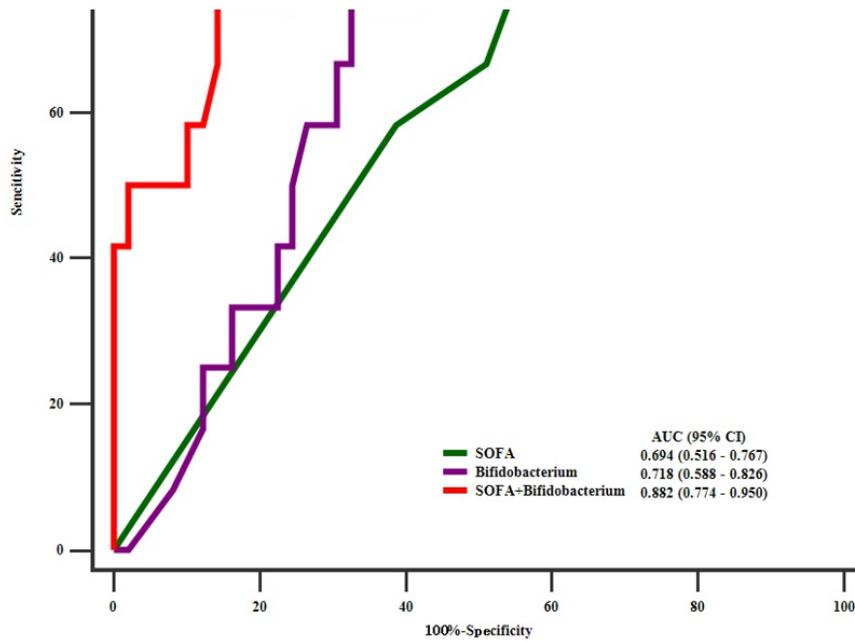
A

21

B

C

Figure 4

ROC curves for Univariate models and Multivariate models. The abscissa is the specificity, and the ordinate is the sensitivity. (a) There is no significant difference among APACHE II, SOFA, and Bifidobacterium. (b) The AUROC of APACHE II plus Bifidobacterium is larger than that of APACHE II and Bifidobacterium. (c) The AUROC of SOFA plus Bifidobacterium is significantly larger than that of SOFA and Bifidobacterium.