

# The Clinical Characteristics of Intestinal Flora Imbalance in Stable Chronic Obstructive Pulmonary Disease (COPD) and Construction of Early Warning Model

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## Research

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# Abstract

**Background** There is still a lack of predictive models for early identification of intestinal flora imbalance in stable Chronic Obstructive Pulmonary Disease (COPD) patients. We assessed the risk factors related to intestinal flora imbalance in COPD patients, and established a simple predictive model and scoring rules suitable for clinical medical staff in the respiratory department.

**Methods** From January 1, 2018 to December 31, 2019, COPD patients (195 cases), who attended the Outpatient Department of Respiratory and Critical Care of Yixing Hospital of Jiangsu University, were collected for a cross-sectional study. The patients were divided into the experimental groups (41 cases) and the control group (154 cases) based on the results of stool examination. By single-factor analysis and logistic regression analysis, the baseline data of two groups were performed to obtain a new prediction model, and then simplified it.

**Results** The five predictive factors including body mass index(BMI), serum albumin(ALB), Charlson comorbidity index(CCI), gastrointestinal symptom score(GSRs), and Global Initiative for Chronic Obstructive Lung Disease(GOLD) classification entered the model. The area under the ROC curve of the model for predicting intestinal flora imbalance in patients with stable COPD is 0.953 [95%CI (0.924, 0.982)], further simplifying the scoring rules, and the area under the ROC curve is 0.767 [95%CI (0.676, 0.858)].

**Conclusions** In the study, the prediction model can scientifically and effectively predict the risk of intestinal flora imbalance in patients with stable COPD, and then implement early treatment to improve the prognosis. Furthermore, all indicators can be obtained easily and simply.

## Background

Chronic Obstructive Pulmonary Disease (COPD) is a common chronic pulmonary disease in the middle-aged and elderly people, and the prevalence of people over 60 years is more than 27%[1]. The vital characteristics of COPD include small airway injury remodeling, incomplete reversible and gradually aggravating dyspnea, strong destruction and high disability rate. Recently, its morbidity and mortality are increasing year by year, affect over 250 million people globally and may be the third leading cause of death in the world by the end of 2021[2]. Among them, Stable COPD patients account for about 210 million people worldwide and bring a huge burden to the economy[3]. In the United States and Asia, the cost of COPD hospitalization account for nearly half of the total medical expenses [4]. In recent years, with the development and improvement of the lung-intestine axis, more and more attention has been paid to the role of intestinal flora in COPD. The imbalance of intestinal flora can lead to an increase in potential pathogenic bacteria, which greatly increases the possibility of bacterial translocation. Further, the increase of G<sup>-</sup> bacilli aggravates intestinal endotoxin release, which can cause indirect damage to the lung and then accelerate the progression of COPD[5]. In addition, intestinal flora could metabolize choline and phosphatidylcholine to produce trimethylamine (TMA), and then oxidized into TMAO by liver after

being absorbed into blood; And TMAO was associated with long-term all-cause mortality in patients with COPD[6]. On the other hand, the increased metabolic demand of patients with COPD could lead to ischemia and hypoxia of intestinal mucosal, damage of the intestinal tract integrity, and then cause the imbalance of intestinal flora[7]. Therefore, even stable COPD patients should be alert to the occurrence of intestinal flora imbalance, and to the identification of vulnerable patients. In the study, an early warning model and specific scoring rules was established for intestinal flora disorders in patients with stable chronic obstructive pulmonary disease, so as to be more intuitive and convenient for clinicians to identify early patients, and then carry out intervention to improve their prognosis. Furthermore, the model was also used as a risk stratification basis for intestinal flora disorders in stable COPD patients.

## Methods

### Study subjects

COPD patients who attended the Outpatient Department of Respiratory and Critical Care of Yixing Hospital Affiliated to Jiangsu University from January 1, 2018 to December 31, 2019 as the research objects. The inclusion criteria were as follows: (1) all met the diagnostic criteria of the Global Initiative for Chronic Obstructive Pulmonary Disease (revised in 2019)[8]: lung function test (after inhaling bronchodilators): first second forced expiratory volume ( $FEV_1$ ) / forced vital capacity (FVC) < 0.7) and respiratory physicians helped to confirm that it was stable: there was no acute exacerbation in the past 8 weeks; there is no clear history of infection; sign the relevant informed consent form of this study; (2) the diagnostic criteria of intestinal flora disorders in the recommendations for diagnosis and treatment of intestinal flora disorders[9, 10]: intestinal flora disorders can be diagnosed by meeting any of the following laboratory tests. Fecal laboratory examination: the number of enterococci and bacilli was observed and counted under oil microscope, and it was found that the proportion of cocci was more than 40%; Bifidobacterium/Enterobacter < 1; abnormal type of  $G^-$  bacilli was 50%. Abnormal type of Gram-positive bacilli < 68%; (3) complete clinical data [including demographic characteristics, fecal flora smear or culture results, partial pressure of oxygen, lung function] and so on. Exclusion criteria: there were primary intestinal diseases causing intestinal flora imbalance; diseases such as pulmonary interstitial fibrosis and active tuberculosis; antibiotics, systemic intravenous hormones, probiotics and so on were used 8 weeks before admission; the data were incomplete or dropped out. A total of 213 patients with stable COPD were enrolled. 18 patients were deleted because of primary intestinal diseases, pulmonary interstitial fibrosis, use of antibiotics or probiotics in the past 8 weeks, incomplete data, midway withdrawal and so on. Finally, 195 patients were divided into experimental group (41 cases) and control group (154 cases) according to whether they were diagnosed as intestinal flora imbalance or not, as shown in Fig. 1.

## Research Tools

## Data collection

General clinical data of all subjects in the study group were collected, including sex, age, smoking status, drinking status, body mass index (BMI), course of disease, hospital admission for COPD in the 1 previous years, inhaled corticosteroids and drugs as required (leakage rate less than 20%), etc. Pulmonary function indicators, including ratio of forced expiratory volume in 1 second to predicted value ( $FEV_1\%pred$ ), ratio of forced expiratory volume to forced vital capacity ( $FEV_1/FVC$ ), and GOLD classification (divided into 4 groups according to the (GOLD) rating of the Global Initiative for Chronic Obstructive Lung Disease: GOLD I:  $FEV_1\%pred \geq 80\%$ , GOLD II:  $50\% \leq FEV_1\%pred < 80\%$ , GOLD III:  $30\% \leq FEV_1\%pred < 50\%$ , GOLD IV:  $FEV_1\%pred < 30\%$ ) and laboratory data, including arterial oxygen partial pressure ( $PaO_2$ ), serum albumin (ALB), triglyceride (TG), total cholesterol (TC), endogenous creatinine (Scr), B-type natriuretic peptide (BNP), pulmonary artery systolic pressure (PASP), hemoglobin (Hb), white blood cell count (WBC), eosinophil count, lymphocyte count, fasting blood glucose and so on.

## Chalson Comorbidity Index Questionnaire

Charlson Comorbidity Index (CCI) is a scale based on the risk of complications and death. In this study, the CCI score was calculated according to the basic condition, and the specific score scale was as follows: myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic lung disease, connective tissue disease, ulcer, mild liver disease, diabetes mellitus, hemiplegia, moderate/severe kidney disease, diabetes with organ damage, tumor, leukemia, lymphoma, etc. The patients with moderate/severe liver disease were given 3 points, metastatic tumor and acquired immunodeficiency syndrome were given 6 points. A total of 19 items were assigned 1, 2, 3 and 6 points respectively, with a total score of 0–36 points.

## Gastrointestinal Symptom Rating Scale (GsrS)

The Gastrointestinal Symptom Rating scale (GSRs) scores, GSRs scores include abdominal pain (abdominal pain, nausea, vomiting), reflux (heartburn, belching, acid reflux), diarrhea (fecal diarrhea, fecal incontinence, fecal urgency), dyspepsia (abdominal ringing, abdominal distension, increased exhaust) and constipation (infrequent defecation, stool tumor, incomplete defecation). The score for each symptom ranges from 0 to 3. The scores of asymptomatic, mild, moderate and severe symptoms were 0, 1, 2 and 3 respectively. The total score of symptoms was 0–15.

## Chronic Obstructive Pulmonary Disease Assessment Test Score (Cat)

CAT score is a comprehensive questionnaire on the health damage of COPD patients, including 8 items, which can be used to observe the effects of COPD on patients by evaluating cough, expectoration, chest tightness, sleep, energy, mood and activity ability. Patients score each item according to their own conditions (0–5), CAT scores range from 0–40). 0–10: "slight impact"; 11–20: "moderate impact"; 21–30: "serious impact"; 31–40: "very serious impact".

## Modified British Medical Research Council Questionnaire (Mmrc)

It is a questionnaire score scale used to evaluate the severity of dyspnea in patients with COPD. The details are as follows: it is divided into 0–4 grades, and the higher the grade, the more severe the dyspnea. Level 0: wheezing occurs only when exercising hard; level 1: difficulty breathing when walking on flat ground or climbing a small slope; level 2: walking on flat ground is slower than others of the same age and needs to stop and have a rest; level 3: need to stop and rest after walking on flat ground for about 100 meters or a few minutes; level 4: unable to leave home due to severe breathing difficulties, or difficulty breathing when getting dressed or undressing.

## Stool And Blood Specimen Collection

All subjects collected 10ml of fasting elbow venous blood and placed it in the 10ml anticoagulant tube. After collection, the blood vessels were turned upside down several times to fully mix. After being placed at room temperature for 1 hour, the plasma was separated by centrifugation at a rotational speed of 3500 rpm/min for 10 min. The supernatant was absorbed and packed in the Eppendorf tube and stored in the refrigerator at -80°C. At the same time, the fecal samples are collected in the special and clean urinal, and only the middle part of the feces is sampled to remove the surface part. In the process of sampling, avoid contact with the inner surface of the bottom of the container, ensure no urine and other pollution in the whole process, use a special aseptic spoon to move the feces around 2ml to the Eppendorf tube and immediately cover them tightly, mark them, and quickly store them in the refrigerator at -80°C. The blood and fecal samples were sent for examination within 4 hours, and the feces were examined and cultured under microscope.

## Statistical Analyses

According to the results of stool microscopy and culture, it was divided into two groups with stable COPD with intestinal flora imbalance and without intestinal flora imbalance. The general clinical data characteristics were analyzed. The data was entered using Excel 2016 and SPSS 22.0 statistical software was used for analysis. The measurement data conformed to the normal distribution, expressed as  $\bar{x} \pm s$ , the comparison between the two groups was expressed by the t-test, the non-normal distribution was expressed by M (P25, P75), and the comparison of the differences between the groups was expressed by

the Mann-Whitney U rank sum test. Count data is expressed in frequency (composition ratio). The comparison between the two groups is performed by  $\chi^2$  test, and statistically significant variables are included. ROC curve is drawn to obtain the best cut-off value and Youden index of the above variables, and the continuous variables in the variables according to the cut-off value transformed into a binary variable, a multi-factor logistic regression analysis was performed to establish a preliminary prediction model. The fit of the obtained model is tested by Hosmer & Lemeshow. In the result, the corresponding  $\beta$ -value is given as an integer to assign a score, and a simplified early warning model scoring rule is established. The ROC curve verification of the scoring model, the use of GraphPad Prism software to draw the ROC curve, and the ROC curve to verify the effectiveness of the early warning scoring model for intestinal dysbiosis in COPD.  $P < 0.05$  is considered as statistically significant.

## Results

### General clinical data of the subjects

In this study, the information of 195 subjects was collected, 41 cases (21.0%) had intestinal flora imbalance (case group) and 154 cases (79.0%) had no intestinal flora imbalance (control group). The median ages of the control group and the case group were 77 (71,82) and 76 (73,84), of which males accounted for 80.5% (124/154) and 85.4% (35/41). Former smokers in the case group accounted for 17.1% (7/41), the control group accounted for 12.3% (19/154), past drinkers accounted for 7.3% (3/41) in the case group, and 15.6% in the control group (24 /154), as shown in Table 1.

Table 1  
Comparison of general data between the two groups

Characteristics	control group(n = 154)	Experimental group (n = 41)	Statistics	P value
Age (years) <sup>a</sup>	77(71,82)	76(73,84)	-0.513	0.608
Gender <sup>c</sup>			0.505	0.477
male	124(80.5)	35(85.4)		
female	30(19.5)	6(14.6)		
BMI(kg/m <sup>2</sup> ) <sup>a</sup>	24.80(23.38,26.60)	22.30(21.10,23.85)	-5.312	<b>&lt; 0.001</b>
Smoking Status <sup>c</sup>			3.205	0.201
No smoker	88(57.1)	17(41.5)		
Former smoker	47(30.5)	17(41.5)		
Current smoker	19(12.3)	7(17.1)		
Drinking Status <sup>c</sup>			1.782	0.455
No drinker	100(64.9)	29(70.7)		
Former drinker	30(19.5)	9(22.0)		
Current drinker	24(15.6)	3(7.3)		
Hospital admission for COPD in the 1 previous years <sup>c</sup>			2.787	0.095
0 or 1	111(72.1)	24(58.5)		
≥ 2	43(27.9)	17(41.5)		
Course of disease (years) <sup>a</sup>	17(13,24)	17(13,23)	-0.543	0.587
FEV <sub>1</sub> /FVC(%) <sup>a</sup>	55.34(46.43,60.55)	54.50(44.66,58.38)	-1.034	0.301
FEV <sub>1</sub> %pred(%) <sup>a</sup>	48.90(41.05,62.18)	45.50(36.90,52.50)	-2.209	<b>0.027</b>
GOLD classification <sup>c</sup>			12.5	<b>0.005</b>
GOLD I	16(10.4)	/		

<sup>a</sup>M(P25,P75);<sup>b</sup> $\bar{x} \pm s$ ; <sup>c</sup>example(%); GOLD classification (according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) )classification into 4 groups: GOLD I: FEV<sub>1</sub>%pred ≥ 80%; GOLD II: 50% ≤ FEV<sub>1</sub>%pred < 80%; GOLD III: 30% ≤ FEV<sub>1</sub>%pred < 50%; GOLD IV: FEV<sub>1</sub>%pred < 30%.

Characteristics	control group(n = 154)	Experimental group (n = 41)	Statistics	P value
GOLD II	54(35.1)	9(22.0)		
GOLD III	74(48.1)	24(58.5)		
GOLD IV	10(6.5)	8(19.5)		
TG(mmol/L) <sup>a</sup>	1.09(0.76,1.52)	0.97(0.66,1.32)	-1.149	0.251
TC(mmol/L) <sup>a</sup>	4.04(3.56,4.78)	3.94(3.44,4.82)	-0.556	0.578
ALB(g/L) <sup>a</sup>	36(32,38)	31(28,35)	-4.732	<b>&lt; 0.001</b>
PaO <sub>2</sub> (mmHg) <sup>a</sup>	96(94,98)	94.0(92.5,96.5)	-2.437	<b>0.015</b>
WBC(*10 <sup>9</sup> /L) <sup>a</sup>	6.60(4.88,8.63)	6.30(4.65,9.05)	-0.016	0.988
Lymphocyte count(*10 <sup>9</sup> /L) <sup>a</sup>	1.30(0.85,1.71)	1.20(0.85,1.69)	-0.313	0.754
Eosinophil count(*10 <sup>9</sup> /L) <sup>a</sup>	0.04(0.00,0.10)	0.01(0.00,0.11)	-0.935	0.35
Scr(umol/L) <sup>a</sup>	70.9(56.9,83.10)	71.20(59.00,95.20)	-0.638	0.523
Fasting blood glucose(mmol/L) <sup>a</sup>	5.27(4.39,6.18)	5.24(4.46,6.57)	-0.441	0.659
BNP(pg/ml) <sup>a</sup>	45(35,63)	45(34,71)	-0.497	0.619
PASP(mmHg) <sup>a</sup>	34(28,37)	34(33,38)	-1.194	0.233
Hb(g/L) <sup>b</sup>	129.37 ± 19.53	126.44 ± 23.34	0.818	0.414
CAT score <sup>a</sup>	9(7,14)	14(9,19)	-3.701	<b>&lt; 0.001</b>
mMRC score <sup>a</sup>	1(0,2)	2(1,3)	-4.227	<b>&lt; 0.001</b>
CCI score <sup>a</sup>	1(0.75,2)	3(2,4)	-8.181	<b>&lt; 0.001</b>
GSRs score <sup>a</sup>	1(0,1)	2(1,4)	-6.818	<b>&lt; 0.001</b>
Inhaled corticosteroids (COPD controller) <sup>c</sup>			1.423	0.233

<sup>a</sup>M(P25,P75);<sup>b</sup>  $\bar{x} \pm s$ ; <sup>c</sup>example(%); GOLD classification (according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) )classification into 4 groups: GOLD I: FEV<sub>1</sub>%pred ≥ 80%; GOLD II: 50% ≤ FEV<sub>1</sub>%pred < 80%; GOLD III: 30% ≤ FEV<sub>1</sub>%pred < 50%; GOLD IV: FEV<sub>1</sub>%pred < 30%.

Characteristics	control group(n = 154)	Experimental group (n = 41)	Statistics	P value
Inhale	22(14.3)	9(22.0)		
Not inhaled	132(85.7)	32(78.0)		
Inhaled drugs as required (the leakage rate is less than 20%) <sup>c</sup>			0.807	0.369
as required	15(9.7)	6(14.6)		
Not as required	139(90.3)	35(85.4)		
<sup>a</sup> M(P25,P75); <sup>b</sup> $\bar{x} \pm s$ ; <sup>c</sup> example(%); GOLD classification (according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) )classification into 4 groups: GOLD I: FEV <sub>1</sub> %pred $\geq$ 80%; GOLD II: 50% $\leq$ FEV <sub>1</sub> %pred < 80%; GOLD III: 30% $\leq$ FEV <sub>1</sub> %pred < 50%; GOLD IV: FEV <sub>1</sub> %pred < 30%.				

### Comparative analysis of base data of intestinal flora imbalance in patients with COPD

Compared with the control group, the case group had lower body mass index, worse pulmonary function, lower partial pressure of arterial oxygen and lower albumin concentration(all P < 0.05). The median CAT scores of the case group and the control group were 14 (9,19) and 9 (7,14). The median mMRC scores were 2 (1,3) and 1 (0,2). The CCI scores of 3(2,4)and (0.75,2) GSRs scores were 2(1,4) and 1(0,1). Therefore, the case group had higher CAT, mMRC, CCI and GSRs scores than the control group (all P < 0.05), The other factors were not significantly different between the two groups (P > 0.05),as shown in Table 1.

### Establishment and simplification of a model for predicting intestinal flora disorders in patients with stable chronic obstructive pulmonary disease

#### Preliminarily screen out the variables to be entered into Logistic regression analysis

According to the results in Table 1, select meaningful BMI, FEV<sub>1</sub>%pred, PaO<sub>2</sub>, ALB, GOLD classification, CAT, mMRC, CCI, GSRs scores, and use SPSS software to obtain the best cut-off values of the above continuous variables and Youden Index in Table 2. According to the cut-off values,9 continuous variables were transformed into binary variables, among which, BMI  $\leq$  23.25kg/m<sup>2</sup> = 0,BMI>23.25kg/m<sup>2</sup> = 1; FEV<sub>1</sub>%pred  $\leq$  49.75%=0, FEV<sub>1</sub>%pred>49.75%=1; PaO<sub>2</sub>  $\leq$  97mmHg = 0, PaO<sub>2</sub>>97mmHg = 1;ALB  $\leq$  32.5g/L = 0,ALB > 32.5g/L = 1;CCI score  $\leq$  2 = 0, CCI score>2 = 1; mMRC score  $\leq$  2 = 0, mMRC score > 2 = 1;CAT score  $\leq$  9 = 0, CAT score > 9 = 1;GSRs score  $\leq$  2 = 0, GSRs score > 2 = 1.GOLD classification:I-II = 0,III-IV = 1,in Table 3.The above categorical variables were included in the univariate logistic regression analysis, using the entry method, the occurrence of intestinal flora imbalance was the patient outcome. The parameter estimation and test results are shown in Table 4.

Table 2  
The best cut-off value of predictor variables and Yoden index

<b>Covariates (potential predictors)</b>	<b>Best cutoff value</b>	<b>Yorden Index</b>	<b>Sensitivity</b>	<b>Specificity</b>
BMI	23.25	0.474	0.766	0.707
FEV <sub>1</sub> %pred	49.75	0.224	0.468	0.756
ALB	32.50	0.405	0.747	0.659
PaO <sub>2</sub>	96.50	0.237	0.481	0.244
CCI score	1.500	0.649	0.902	0.747
mMRC score	1.500	0.327	0.659	0.669
CAT score	8.500	0.290	0.829	0.461
GSRs score	1.500	0.451	0.659	0.792
GOLD classification	2.500	0.235	0.780	0.455

Table 3

The general situation of categorical predictors between the two groups

<b>Covariates (potential predictors)</b>	<b>control group (n = 154)</b>	<b>Experimental group (n = 41)</b>
BMI		
≤ 23.25kg/m <sup>2</sup>	36(23.4%)	29(70.7%)
> 23.25kg/m <sup>2</sup>	118(76.6%)	12(29.3%)
FEV <sub>1</sub> %pred		
≤ 49.75%	82(53.2%)	31(75.6)
> 49.75%	72(46.8%)	10(24.4)
ALB		
≤ 32.5g/L	39(25.3%)	27(65.9%)
> 32.5g/L	115(74.7%)	14(34.1%)
PaO <sub>2</sub>		
≤ 97mmHg	95(61.7%)	32(78.0%)
> 97mmHg	59(38.3%)	9(22.0%)
CCI score		
≤ 2	142(92.2%)	12(29.3%)
> 2	12(7.8%)	29(70.7%)
mMRC score		
≤ 2	139(90.3%)	29(70.7%)
> 2	15(9.7%)	12(29.3%)
CAT score		
≤ 9	85(55.2%)	11(26.8%)
> 9	69(44.8%)	30(73.2%)
GSRs score		
≤2	145(94.2%)	22(53.7%)
> 2	9(5.8%)	19(46.3%)
GOLD classification		

Covariates (potential predictors)	control group (n = 154)	Experimental group (n = 41)
GOLD I-II	70(45.5%)	8(19.5%)
GOLD III-IV	84(54.5%)	33(80.5%)

Table 4

Univariate and multivariate logistic regression analysis of intestinal flora imbalance in patients with stable COPD

Predictors	Univariate analysis				Multivariate analysis			
	$\beta$	OR	P value	95%CI	$\beta$	OR	P value	95%CI
BMI	-2.070	0.126	<b>&lt;0.001</b>	0.058–0.272	-2.406	0.090	<b>&lt;0.001</b>	0.026–0.317
FEV <sub>1</sub> %pred	-1.001	0.367	<b>0.012</b>	0.168–0.807	2.010	7.460	0.161	0.450-123.767
ALB	-1.738	0.176	<b>&lt;0.001</b>	0.084–0.369	-1.280	0.278	<b>0.040</b>	0.082–0.945
PaO <sub>2</sub>	-0.792	0.453	0.055	0.202–1.016				
CCI score	3.353	28.597	<b>&lt;0.001</b>	11.693–69.938	3.244	25.633	<b>&lt;0.001</b>	6.652–98.775
mMRC score	1.344	3.834	<b>0.002</b>	1.626–9.044	1.454	4.281	0.094	0.782–23.427
CAT score	1.212	3.360	<b>0.002</b>	1.571–7.186	0.093	1.097	0.894	0.280–4.302
GSRs score	2.633	13.914	<b>&lt;0.001</b>	5.595–34.605	2.840	17.113	<b>&lt;0.001</b>	4.012–72.997
GOLD classification	1.235	3.437	<b>0.004</b>	1.491–7.923	3.237	25.469	<b>0.034</b>	1.276-508.213
Constant					-4.332	0.013	0.009	
Equation and performance of the COPD prediction model, by using the aforementioned five independent variables, the logit model to determine PRE_1 was expressed as follows: $\text{Logit}(p) = -4.332 + (-2.406 \times \text{BMI}) + (-1.280 \times \text{ALB}) + (3.244 \times \text{CCI score}) + (2.840 \times \text{GSRs score}) + (3.237 \times \text{GOLD classification})$ ;								
$P = 1 / (1 + e^{-x})$ $x = -4.332 + (-2.406 \times \text{BMI}) + (-1.280 \times \text{ALB}) + (3.244 \times \text{CCI score}) + (2.840 \times \text{GSRs score}) + (3.237 \times \text{GOLD classification})$ .								

### Logistic regression analysis was used to establish a predictive model of intestinal flora imbalance in patients with COPD

The meaningful indicators screened out in the univariate logistic regression analysis (screening criteria  $P < 0.05$ ) were included in the multivariate Logistic regression. The results of parameter estimation and testing are shown in Table 4. The five independent variables (BMI, ALB, CCI score, GSRs score, GOLD classification) finally included in the model are all statistically significant ( $P < 0.05$ ), the sensitivity of the

model is 90.2%, the specificity is 87.7%, and the model was tested by Hosmer&Lemeshow goodness of fit test  $X^2 = 9.683$ ,  $P = 0.288$ . The available logistic regression equation is:

$\text{Logit}(p) = -4.332 + (-2.406 \times \text{BMI}) + (-1.280 \times \text{ALB}) + (3.244 \times \text{CCI score}) + (2.840 \times \text{GSRs score}) + (3.237 \times \text{GOLD classification})$ ;

The aforementioned equation was transformed as follows:

$P = 1 / (1 + e^{-x})$ ;  $x = -4.332 + (-2.406 \times \text{BMI}) + (-1.280 \times \text{ALB}) + (3.244 \times \text{CCI score}) + (2.840 \times \text{GSRs score}) + (3.237 \times \text{GOLD classification})$ .

### **The ROC curve of the model and its variables for predicting the occurrence of intestinal flora imbalance in patients**

The five variables of BMI, ALB, CCI score, GSRs score, and GOLD classification grading entered into the model (PRE\_1) and the new variable predicted probability of joint diagnosis when the Logistic regression model was established in this study were used to draw the ROC curve chart with GraphPad Prism software. The AUC = 0.782 [95%CI(0.709, 0.856)], 0.740 [95%CI(0.660, 0.820)], 0.898 [95%CI(0.845, 0.951)], 0.831 [95%CI(0.764, 0.897)], 0.657 [95%CI (0.567, 0.747)] 0.953 [95%CI (0.924, 0.982)]. It can be seen that the combined diagnosis model is superior to the test power of a single variable, as shown in Fig. 2,3 .

## **Simplify The Resulting Model**

After the statistically significant regression coefficients of BMI, ALB, CCI score, GSRs score, and GOLD classification in the model were taken as integers (that is, the values were 2, 1, 3, 3, 3), a simplified scoring model (PRE\_2) was established, where BMI  $\leq 23.25 \text{ kg/m}^2$  is assigned 2 points,  $> 23.25 \text{ kg/m}^2$  is assigned 0 points; ALB  $\leq 32.5 \text{ g/L}$  is assigned 1 point,  $> 32.5 \text{ g/L}$  is assigned 0 points; CCI score  $\leq 2$  points are assigned 0 points,  $> 2$  points are assigned 3 points; GSRs score  $\leq 2$  points are assigned 0 points,  $> 2$  points are assigned 3 points; GOLD classification: GOLD I-II are assigned 0 points, GOLD III-IV are assigned 3 points. The score of this simplified model is 0–12 points, as shown in Table 5. The AUC value of the simplified model is 0.767 [95%CI (0.676, 0.858)]. This model (PRE\_2) has a moderate predictive power for the risk of intestinal flora imbalance in COPD patients, as shown in Fig. 4. Use SPSS software to determine that the best critical value of the above model is score  $> 7$  (finally rounded according to clinical analysis), which is used as the basis for risk stratification of intestinal flora imbalance in patients with stable COPD, and patients can be divided For the occurrence of low-risk group ( $\leq 7$  points) and high-risk group ( $> 7$  points). The ROC curve is shown in Fig. 4.

Table 5  
Risk score sheet for intestinal flora imbalance in patients with stable COPD

Risk factors	Score points
BMI	
≤ 23.25kg/m <sup>2</sup>	2
> 23.25kg/m <sup>2</sup>	0
ALB	
≤ 32.5g/L	1
> 32.5g/L	0
CCI score	
≤ 2	0
> 2	3
GSRs score	
≤ 2	0
> 2	3
GOLD classification	
GOLD I-II	0
GOLD III-IV	3
	Total score 0–12

## Discussion

In this study, it was found that BMI, ALB, CCI score, GSRs score and GOLD classification were the risk factors of intestinal flora imbalance in patients with COPD and had good predictive value for the prognosis of COPD patients. Among them, CCI score > 2 and GSRs score > 2 increased the risk of intestinal flora disorders in stable COPD patients by 25.633 times and 17.113 times, respectively, whereas BMI > 23.25kg/m<sup>2</sup> and ALB > 32.5g/L might be protective factors, which decreased the risk of intestinal flora disorders in stable COPD patients by 0.090 times and 0.278 times, respectively. BMI is often regarded as indicator of the body's nutritional status and reports also demonstrated the association of low BMI with exacerbation in patients with COPD[11]. A 3-year South Korean study of follow-up BMI to predict the prognosis of COPD found that the decrease in BMI was independently related to the deterioration of COPD and the increase of mortality (HR = 3.167) [12]. COPD patients is often

accompanied with increased energy expenditure, when malnutrition occurs, the body's defense ability decreases, and then results in intestinal flora imbalance followed deterioration of COPD. In addition, serum albumin is another indicator of nutritional status, because it can well predict in-hospital mortality (ALB level is 30.5g/L) and readmission rate within 30 days (ALB level is 30.1g/L) in patients with COPD[13]. When hypoalbuminemia occurs, it not only leads to malnutrition and low immunity in patients with COPD, but also leads to decrease of colloidal osmotic pressure, edema of intestinal wall and structural disorder of intestinal mucosa, thus affecting the ecological stability of intestinal flora and resulting in the occurrence of flora imbalance[14, 15]. Furthermore, albumin / globulin ratio has more predictive value than the single index of serum albumin, and it can be used as a reference index to evaluate the condition of elderly patients with COPD[16]. Although albumin / globulin ratio could determine whether COPD patients were complicated with infection and predicted prognosis, but it was not found in this study. An Italian study showed that CCI score is an independent risk factor for acute exacerbation and death in patients with COPD; the death rate of patients with COPD increased by 4.8% when CCI > 1[17]. Both COPD and its complications can aggravate the weakness or disability progress of COPD patients[18], and further be more likely to lead to intestinal flora imbalance. The interaction between them accelerate the disease process and the number of acute exacerbation of COPD. In addition, some studies showed that the score of gastrointestinal symptoms in patients with COPD was higher than that in healthy individuals ( $2.12 \pm 1.96$ ), it showed that there was a negative correlation between gastrointestinal symptoms and mental health impairment ( $r=-0.49, p < 0.001$ ) [19]. COPD patients were more apt to psychological disorders, such as anxiety and depression, which could easily lead to the disappearance of intestinal dominant flora, the loss of intestinal wall barrier function and the release of intestinal endotoxin, which jointly promoted the occurrence of intestinal flora imbalance and led to the gastrointestinal symptoms[20]. Compared with non-COPD patients and COPD GOLD I patients, GOLD II-IV patients have significantly higher symptoms (CAT score) and lower lung function variables (including FEV<sub>1</sub>/FVC, FVC); Especially, COPD patients with GOLD III-IV are considered to have good clinical significance for judging the prognosis of COPD[21]. Furthermore, Patients with moderate to severe pulmonary insufficiency tend to have more clinical symptoms and a higher risk of acute exacerbation. According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD), this type of population is usually divided into GOLD D group. Compared with A group patients group A patients has a more higher risk of death (25 times)[22], which indicates that worse lung function can easily lead to acute exacerbation of COPD, and then aggravate intestinal flora imbalance. Moreover, age is also regarded as a risk factor for intestinal flora disorders in patients with stable COPD. The older the patients are, the more serious the decline of organ function and gastrointestinal function are; In addition, the decline of gastrointestinal function is prone to enterotoxin accumulation, and then leads to flora imbalance and accelerates COPD[23]. However, age is not found to be one of the risk factors attributed to the majority of elderly patients in this study ( $p>0.05$ ).

In this study, it was found that any single indicator, including BMI, ALB, CCI score, GSRs score, and GOLD classification, was not effective in predicting the occurrence of intestinal flora imbalance in stable COPD, with AUC of 0.782, 0.740, 0.898, 0.831 and 0.657, respectively. Therefore, in this study, we combined with

the 5 risk factors to construct a new prediction model:  $\text{Logit}(p) = -4.332 + (-2.406 \times \text{BMI}) + (-1.280 \times \text{ALB}) + (3.244 \times \text{CCI score}) + (2.840 \times \text{GSRs score}) + (3.237 \times \text{GOLD classification})$ .

The predictive value of the model for intestinal flora imbalance in patients with stable COPD is better than a single variable. The area under the ROC curve is 0.953, the sensitivity is 90.2%, and the specificity is 87.7%, which indicates that the predictive model has good clinical predictions. So it provides a basis for early screening and identification of prone patients. However, the calculation process of the prediction model is relatively cumbersome, and it is not suitable for rapid clinical judgment and early identification of prone patients. Therefore, we further simplified the model, and assigned the corresponding  $\beta$ -values of the 5 predictors to integer scores to obtain the stable period. Predictive scoring rules for intestinal flora imbalance in COPD patients is: BMI  $\leq 23.25 \text{ kg/m}^2$  (2 points), ALB  $\leq 32.5 \text{ g/L}$  (1 point), CCI score  $> 2$  (3 points), GSRs score  $> 2$  (3 points), GOLD classification III-IV (3 points). Fast screening of high-risk patients who are prone to intestinal flora imbalance by the scores has good predictive performance, with an AUC of up to 0.767. The simplified scoring model allows us to intervene in this group of people early, so as to extend the stable period of COPD and improve their prognosis.

## Limitation Of The Study

However, it is a single-center retrospective study and the number of observation samples is small; Besides, the factors, such as the use of antibiotics and the total dose of glucocorticoids, which has a certain impact on the authenticity of the conclusions of the study, are not included in the analysis. Therefore, it needs to be verified by further expanding the sample size, prolonging the follow-up time and conducting a multicenter prospective study. In this study, only the ROC receiver operating curve was used to evaluate the value of the simplified scoring model in predicting the occurrence of intestinal dysbiosis in patients with stable COPD. We will prospectively include stable COPD patients as verification in the next experiment. Cohort, to further verify the clinical test efficacy of the model, and to further optimize it for better clinical application.

## Conclusion

Finally, the simplified scoring model (PRE\_2) established by the five indicators of BMI, ALB, CCI score, GSRs score, and GOLD classification showed moderate predictive value for intestinal dysbiosis in patients with stable COPD. All the indicators are easy to detect and obtain quickly. The scoring model can identify high-risk groups that are prone to intestinal flora imbalance, and implement intervention measures, such as oral probiotics, as soon as possible. By adjusting the steady state of the intestinal flora, the goal of reducing the number of acute attacks of COPD patients and improving the prognosis is achieved.

## Abbreviations

COPD: Chronic Obstructive Pulmonary Disease; BMI: Body mass index; ALB: serum albumin; CCI:charlson comorbidity index; GSRs:gastrointestinal symptom score; CAT:Chronic Obstructive Pulmonary Disease assessment test score; mMRC: Modified British Medical Research Council questionnaire;GOLD:Global Initiative for Chronic Obstructive Lung Disease; TMA:trimethylamine; FEV1:first second forced expiratory volume;FVC:forced vital capacity; FEV1%pred:ratio of forced expiratory volume in 1 second to predicted value; PaO2:arterial oxygen partial pressure; TG:triglyceride; TC:total cholesterol; Scr: endogenous creatinine; BNP:B-type natriuretic peptide; PASP:pulmonary artery systolic pressure; Hb:hemoglobin;WBC:white blood cell count;ROC curve: receiver operating characteristic curve;AUC: area under the curve.

## **Declarations**

### **Acknowledgments**

Not applicable.

### **Authors' contributions**

Zeng Xuetao and Yang Hongfeng conceived and designed the study, analysed the data and drafted this manuscript. Ma Xiuqin and Zhu Taofeng contributed to the design of this study, analysis of the data, and revising of the manuscript. Yang Yan, Gu Xinnan, contributed to conception and design. All authors read and approved the final 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. The data are not publicly available due to privacy or ethical restrictions.

### **Ethics approval and consent to participate**

This study was approved by the Ethics Committee of the People's Hospital of Yixing City, Jiangsu Province (batch number: 2019-002), and all the subjects signed the informed consent form.

### **Consent for publication**

Not applicable.

### **Competing interests**

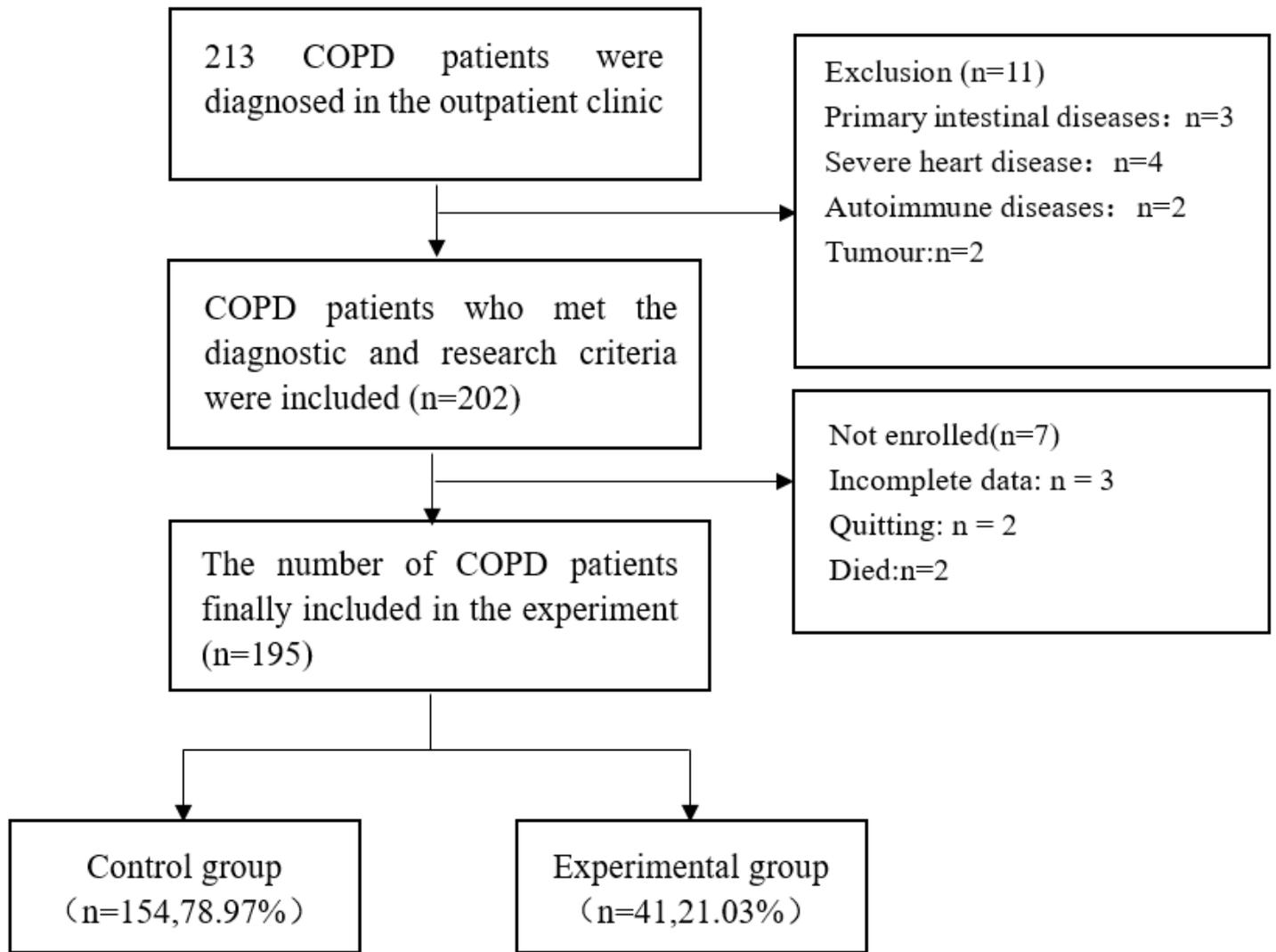
The authors report no conflicts of interest in this work.

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## Figures



**Figure 1**

Patient flow diagram

## ROC curve: 5 predictors predict the risk of intestinal flora imbalance in COPD

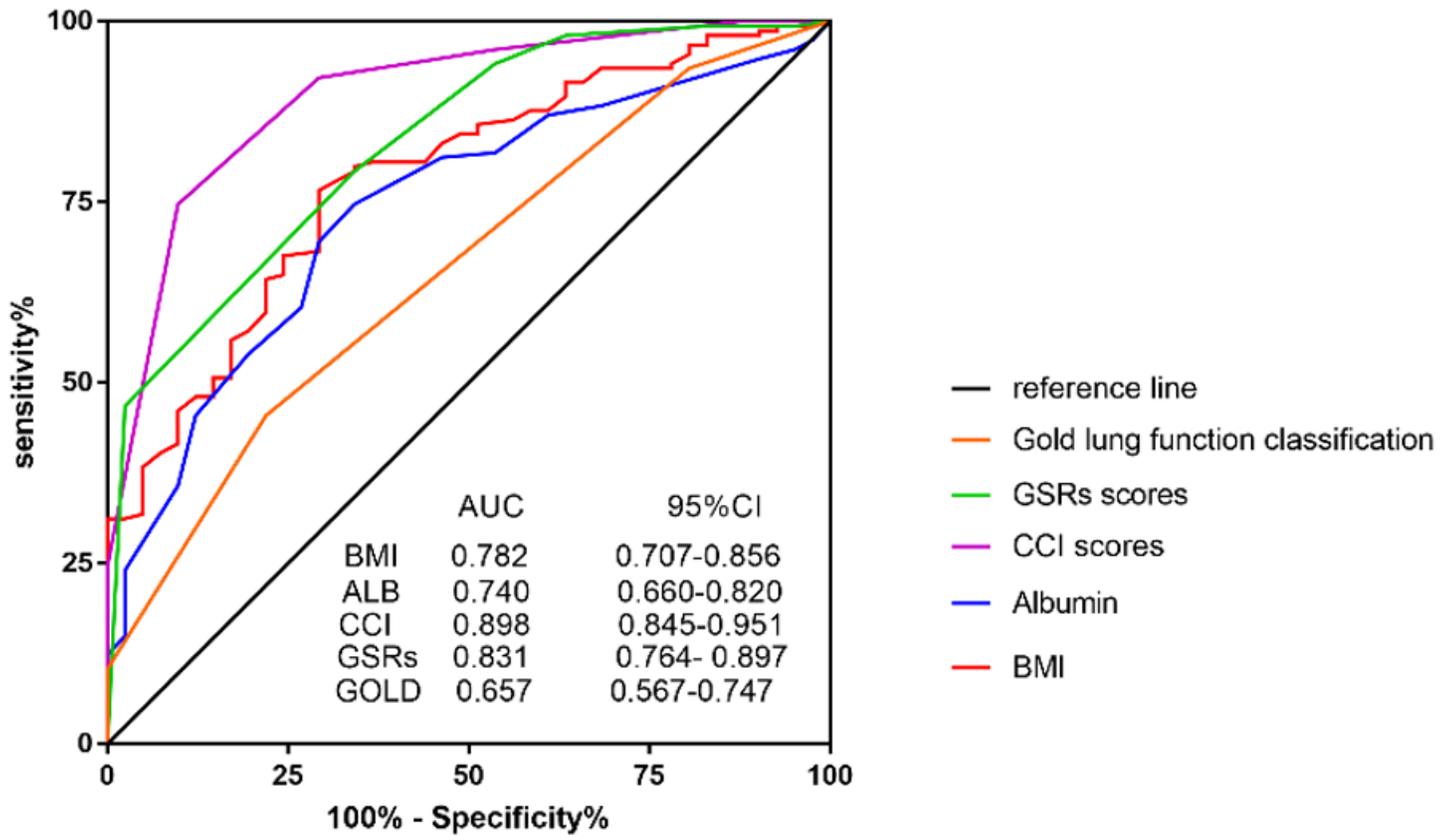
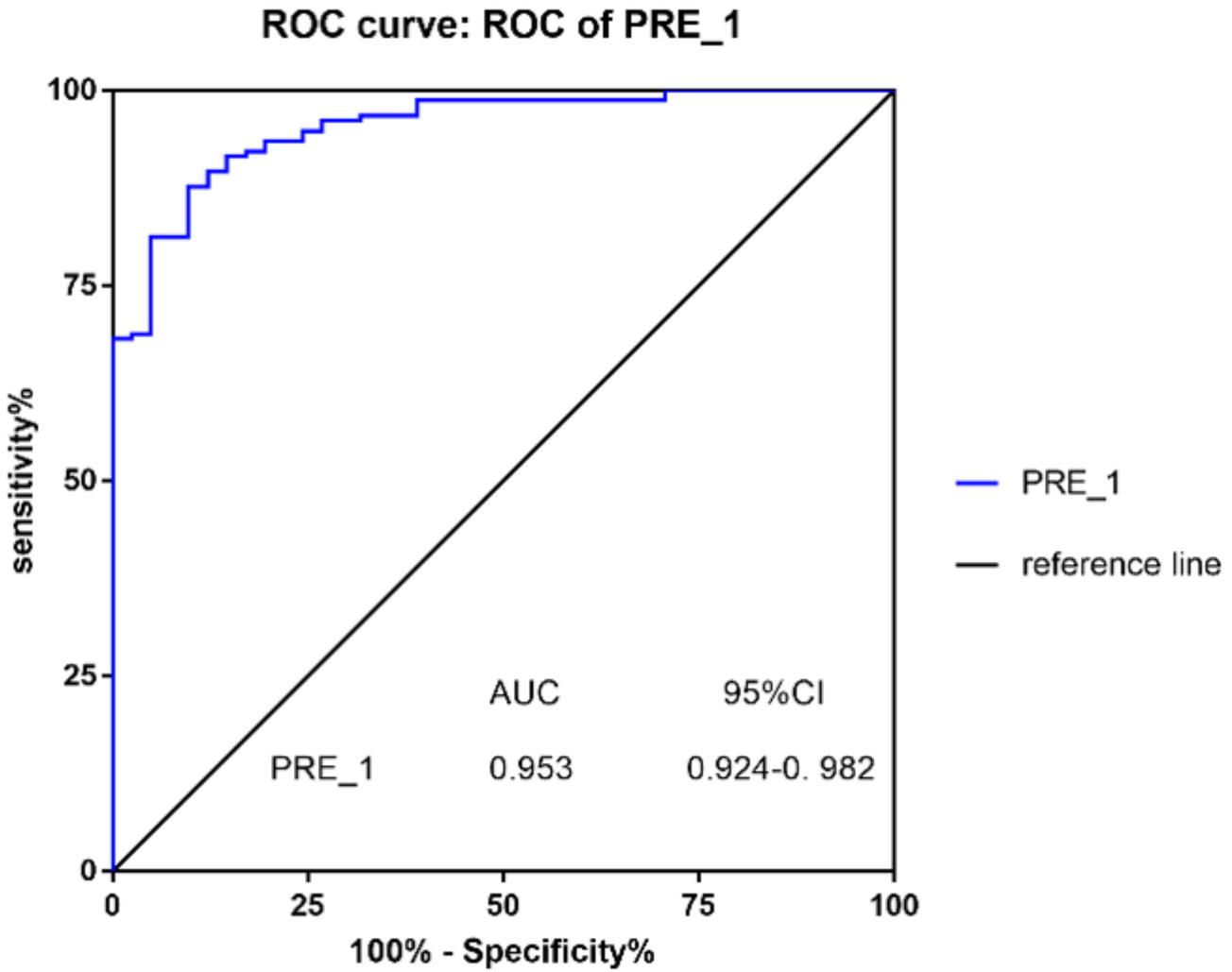


Figure 2

5 predictive indicators (BMI, ALB, CCI, GSRs, GOLD classification) to predict the ROC curve of intestinal flora imbalance in patients with stable COPD



**Figure 3**

ROC curve of PRE\_1 for predicting intestinal flora imbalance in patients with stable COPD

ROC curve: ROC of PRE\_2

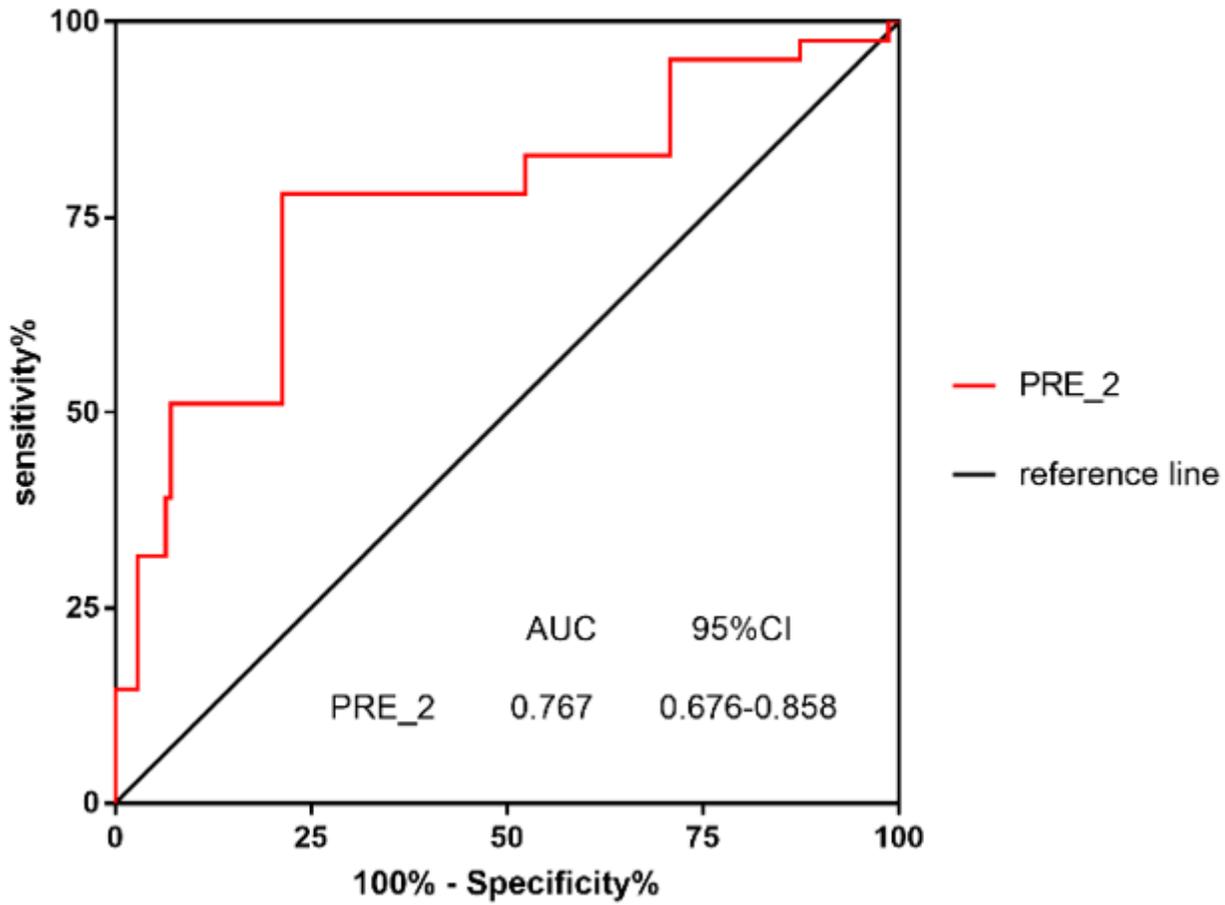


Figure 4

ROC curve of simplified scoring rules(PRE\_2) for risk prediction of intestinal flora imbalance in patients with stable COPD