A Canonical Correlation Analysis of Factors that Influence Quality of Life Among Patients with Chronic Obstructive Pulmonary Disease Based on QLICD-COPD (V2.0)

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Abstract

Background

Chronic obstructive pulmonary disease (COPD) is one of the diseases with the highest morbidity and mortality globally. The Quality of Life Instrument for Chronic Diseases(QLICD)-COPD(V2.0) was designed to assess the health condition of patients with COPD. The objective of this study was to evaluate the quality of life (QOL) of patients, the influential clinical factors, and the relationships between QOL and clinical objective indicators.

Methods

Two hundred and sixty-one in-patients with COPD in the acute exacerbation stage were evaluated using the QLICD-COPD(V2.0) and data on clinical objective indicators were collected. The relationships between QOL and the clinical objective indicators were determined using canonical correlation analysis.

Results

The standardized scores for the patients in four domains, namely physical function, psychological function, social function, and a disease-specific module, were 49.00 ± 12.91, 59.89 ± 13.51, 68.59 ± 11.94, and 51.84 ± 13.58, respectively. The total score for the QOL of patients was 57.17 ± 10.26. Two pairs of canonical correlation variables were statistically significant ($P < 0.05$), with coefficients of 0.35 and 0.26. These variables respectively accounted for 45.8% and 33.8% of the variance. The levels of total protein, albumin, serum sodium, and alkaline phosphatase and the percentages of neutrophils and lymphocytes were correlated with the QOL.

Conclusion

During COPD treatment, clinicians should pay close attention to the levels of total protein, albumin, serum sodium, and alkaline phosphatase and the percentages of neutrophils and lymphocytes to improve the QOL of patients.

Background

Chronic obstructive pulmonary disease (COPD) is a chronic bronchitis and/or emphysema characterized by airflow obstruction, which can develop further into a common chronic disease, such as pulmonary heart disease or respiratory failure. COPD has become a serious public health problem because of the large number of patients, high fatality rate, and heavy social and economic burden. As a chronic respiratory disease, COPD has a long disease course and a high recurrence rate. Most of the available treatments are temporary interventions and both the cure rate and the therapeutic effects of treatment are

difficult to establish. Clinical objective indicators such as blood gas and pulmonary function data are used to determine the health condition of patients with COPD.

In recent years, methods to assess the quality of life (QOL) of patients with COPD have attracted significant attention globally. Many drugs, clinical treatments, and rehabilitation techniques have been evaluated to improve the QOL of patients and many studies have been conducted to explore the factors that affect QOL [1–3]. The tools commonly used to evaluate the status of patients with COPD are the Short Form 36 (SF-36), Chronic Respiratory Questionnaire (CRQ), and St George's Respiratory Questionnaire (SGRQ). Most studies only focus on the patient's lung function and the results of blood gas analysis. In order to obtain data in line with China's national standards and a more comprehensive understanding of the objective indicators that affect QOL, the COPD measurement scale known as the Quality of Life Instrument for Chronic Diseases (QLICD)-COPD (V2.0) was used to evaluate patients in this study. Blood routine, urine routine, blood biochemistry, and blood gas analyses were performed as well as pulmonary function tests.

Using a combination of the QOL scores and the above objective clinical indicators, we aimed to investigate QOL and its clinical influencing factors in patients with COPD as well as to obtain valid evidence regarding methods to improve QOL. The patient-reported outcomes (PROs) and QOL assessments were based on the patient's subjective feelings and were measured using a scale for individual health. These two variables are manifestations of the same measurements in different stages of development. Therefore, PROs and QOL are difficult to separate and can be measured simultaneously using the PRO/QOL tool library [4, 5]. The general view is that QOL can be used instead of PROs when evaluating clinical outcomes in patients [6]. In this study, these two concepts were considered to be equivalent.

Methods

Participants

In this study, 261 patients with COPD with acute exacerbation treated at the First Affiliated Hospital of Kunming Medical University, Dalang Hospital of Dongguan City, Shilong PokOi Hospital of Dongguan City, and the Affiliated Hospital of Guangdong Medical University of Respiratory Medicine were selected as study participants.

The inclusion criteria were as follows: (1) patients met the COPD diagnostic criteria [7], (2) had the ability to read and write, and had a primary school educational level or above, and (3) participated in the evaluation voluntarily. The exclusion criteria were: (1) illiteracy or poor language ability; (2) inability to clearly express their feelings.

QLICD-COPD(V2.0)

Description: The QOL scale for COPD used in this study, the QLICD-COPD(V2.0), is a QOL measurement tool for patients with chronic diseases [8]. The scale consists of two modules: a generic module for QOL (QLICD-GM) and a disease-specific module for the relatively important and unique characteristics of patients. QLICD-GM consists of 9 items on physical functions (PHD), 11 items on psychological functions (PSD), and 8 items on social functions (SOD), for a total of three domains, nine facets, and 28 items. The specific module consists of four facets, namely cough and expectoration, dyspnea, pulmonary encephalopathy, and special psychological impact. The entire scale contains four domains with 37 items and each item is separated into five levels.

Scoring: The scale consists of positive and negative items, which are scored using the five-point equidistant method. Higher scores for positive items indicate better QOL whereas higher scores for negative items indicate poorer QOL. During the summation process, the total score for negative items is converted into a positive value. The standardized total score for each field and domain is calculated and presented as a range: standardized score = (raw score - minimum score) * 100/ (maximum score-minimum score) [9].

Evaluation: The scale was evaluated by Yang et al. [10] and the results showed that the α value for the coefficient of internal consistency, the r value for the test-retest reliability, and the split-half value for the scale were 0.93,0.94, and 0.84, respectively. Most of the α and r values for each domain were above 0.6. Additionally, most of the correlation coefficients between each item and the associated domain were above 0.5. Most patients completed the questionnaire in 15–20 minutes. The results demonstrated that the QLICD-COPD V2.0 is a valid, reliable, sensitive, and feasible tool in clinical settings, and that the tool can be used in China to assess QOL for patients with COPD.

Data collection

The investigator distributed the questionnaire to each patient and provided a brief description of the purpose and significance of the survey. The patients completed the questionnaire on the first day or on the second day of admission according to their condition. The investigator reviewed the questionnaires completed in order to ensure its integrality. If missing values were found, the questionnaire would be returned to the patients to fill in the missing item. The clinical objective indicators for the patients were also assessed with blood routine, urine routine, blood biochemistry, blood gas, and pulmonary function tests.

Statistical analysis

SPSS13.0 software and SAS8.0 software were used to process the data. Descriptive analysis was used to assess the overall QOL in all domains and correlations between the clinical objective indicators and QOL scores were analyzed using simple correlation analysis. Simple correlation analysis is used to determine the correlation between a single X and Y variable without taking into account the correlation between individual variables within the X and Y sets of variables.

Canonical correlation analysis is an approach that involves the application of structure coefficients as indices for the identification of important indicators. As a generalization of simple and multiple correlations, canonical correlation analysis is a statistical analysis method used to determine the correlation between two sets of variables. The pair of linear combinations with the largest correlation coefficients is identified and the correlation coefficients for both sets of variables are combined to obtain the correlation for the combined set, which serves as a more comprehensive and representative indicator [11]. In canonical correlation analysis, a linear equation is applied separately to the observed predictor and dependent variables to create one unobserved variable for each set. These two equations are generated because they yield the largest possible correlation between the two unobserved variables. The first canonical correlation is the highest possible correlation between any synthetic predictor variable and synthetic outcome variable and is the most suitable candidate for interpretation. The criteria for important variables in each canonical function are the structure coefficient and the bivariate correlation between an observed variable and a synthetic variable [12]. The clinical objective indicators which were found to be correlated through simple correlation analysis were defined as canonical variables V. The four domain scores for the QLICD-COPD (V2.0) scale were defined as canonical variables U and canonical correlation analysis was performed.

Results

Socio-demographic characteristics of the participants

A total of 261 participants were included in this study. The patients met the COPD diagnostic criteria and were in the acute exacerbation stage. There were 199 men (76.2%) and 62 women (23.8%) and the average age was 70.9 ± 9.6 years. Other individual socio-demographic characteristics are presented in Table 1.

Table 1
Socio-demographic characteristics of the participants (N = 261)

Characteristics	N (%)	Characteristics	N (%)
Age		**Occupation**	
< 50	5 (1.9)	Worker	114 (43.7)
50 ~ 59	25 (9.6)	Farmer	96 (36.8)
60 ~ 69	83 (31.8)	Teacher	36 (13.8)
70 ~ 79	98 (37.5)	Cadre	9 (3.4)
80 ~ 89	47 (18.0)	Individual household	6 (2.3)
≥ 90~	3 (1.2)	Other	114 (43.7)
Gender		**Ethnic groups**	
Male	199 (76.2)	Han	253 (96.9)
Female	62 (23.8)	Others	8 (3.1)
Marriage		**Income#**	
Unmarried	4 (1.5)	Poor	54 (20.7)
Married	220 (84.3)	Fair	182 (69.7)
Divorced	3 (1.2)	High	25 (9.6)
Widowed	34 (13.0)		
Education		**Medical insurance**	
Primary school	114 (43.7)	Self-paid	27 (10.3)
Junior middle school	96 (36.8)	Social medical insurance	195 (74.7)
High school	36 (13.8)	Commercial insurance	4 (1.6)
Junior college	9 (3.4)	Rural cooperative public medical Service	35 (13.4)
Bachelor or above	6 (2.3)		
Note: **#** *is evaluated by patients himself/herself according to their perceptions.*			

The total scores for QLICD-COPD(V2.0) in COPD patients

The score for each domain was calculated according to the rules for QLICD-COPD (V2.0). The raw, standardized, and total scores for all domains are presented in Table 2.

Table 2
The domains and total scores for QLICD-COPD(V2.0) in COPD patients ($\pm s$)

Domain	Items	Range	Raw score	Standardized score
PHD	9	9–45	26.64 ± 4.65	49.00 ± 12.91
PSD	11	11–55	37.35 ± 5.95	59.89 ± 13.51
SOD	8	8–40	29.95 ± 3.82	68.59 ± 11.94
SPD	16	16–80	27.66 ± 4.89	51.84 ± 13.58
TOT	44	44–220	121.61 ± 15.19	57.17 ± 10.26

Values of clinical objective indicators in COPD patients

The clinical objective indicators collected in this study were relatively diverse. Correlation analysis showed that the detection ranges and average values of the clinical objective indicators were related to the QOL of patients with COPD (Table 3).

Table 3
Values of clinical objective indicators in COPD patients

Clinical objective indicator	Number	Range	Average (*± s*)	Minimum	Maximum
TP (g/L)	260	60–82	64.61 ± 7.07	49.00	112.60
ALB (g/L)	260	35–54	35.65 ± 4.93	20.70	49.20
TBA (umol/L)	194	0–15	7.45 ± 10.09	0.10	70.0
ALP (IU/L)	196	31–115	75.00 ± 24.87	38.70	176.00
Scr (umol/L)	260	42–97	77.89 ± 30.00	33.00	272.00
K (mmol/L)	260	3.5–5.5	4.03 ± 0.48	2.68	5.55
Na (umol/L)	260	136–146	140.00 ± 3.84	118.50	151.60
NEUT%	259	50–70	72.02 ± 12.76	40.00	96.70
LYMPH%	259	20–40	18.34 ± 10.14	0.90	48.40
MONO%	258	3–10	7.90 ± 4.03	0.10	27.60
LYMPH (10^9/L)	259	0.8-4	1.36 ± 0.75	0.23	4.91
MONO (10^9/L)	258	0.12-1	0.64 ± 10.57	0.00	3.91
HCT (%)	257	33.5–45	41.01 ± 7.98	17.40	68.30
PCO_2 (mm Hg)	205	35–45	52.28 ± 11.33	24.00	89.00
FVC (L)	153	2.31–3.18	2.26 ± 0.46	1.06	3.66
FEV1 (L)	152	2.31–3.18	1.23 ± 0.37	0.43	2.71
CRP (mg/l)	212	≤ 10	33.24 ± 26.77	1.25	128.00

Note: TP = total protein, ALB = albumin, TBA = total bile acid, ALP = alkaline phosphatase, Scr = creatinine, K = serum potassium, Na = serum sodium, NEUT%=percentage of neutrophils, LYMPH%=percentage of lymphocyte, MONO%=percentage of monocytes, LYMPH = lymphocyte, MONO = monocyte, HCT = hematocrit, PCO_2 = partial pressure of carbon dioxide, FVC = forced vital capacity, FEV1 = 1 second forced expiratory volume, CRP = C-reactive protein.

Simple correlation analysis

Simple correlation analysis was performed for the clinical objective indicators with complete data, the scores for each QLICD-COPD (V2.0) domain, and the total scores (Table 4).

The results showed that the level of total protein (TP), albumin(ALB), total bile acid(TBA), alkaline phosphatase(ALP), creatinine(Scr), serum potassium(K), serum sodium(Na) for the blood biochemistry, the percentage of neutrophils(NEUT%), percentage of lymphocytes (LYMPH%), percentage of monocytes(MONO%), lymphocytes (LYMPH), monocytes (MONO), and hematocrit(HCT) in blood routine

tests, the partial pressure of carbon dioxide (PCO_2) in blood gas analysis, forced vital capacity (FVC), 1-second forced expiratory volume (FEV1) in the pulmonary function test, and C-reactive protein (CRP) were related to QOL.

Table 4
Simple correlation analysis for the scores of each domain and the total score of QLICD-COPD(V2.0) with values of clinical objective indicators in COPD patients

Clinical objective indicator	Number	PHD	PSD	SOD	SPD	TOT
TP	260	0.150*	0.199**	0.125*	0.165**	0.209**
ALB	260	—	—	—	0.174**	—
TBA	194	-0.176*	—	—	—	—
ALP	196	—	-0.176*	-0.174*	—	—
Scr	260	0.122*	—	—	—	—
K	260	—	—	-0.133*	—	—
Na	260	—	—	—	-0.132*	-0.127*
NEUT%	259	-0.132*	-0.147*	-0.161**	—	-0.160**
LYMPH%	259	—	0.149*	0.145*	—	0.143*
MONO%	258	0.183**	—	—	—	0.128*
LYMPH	259	—	—	0.133*	—	—
MONO	258	0.163**	—	—	—	—
HCT	257	-0.217*				
PCO_2	205	-0.268**	—	—	—	-0.148*
FVC	153	0.161*	—	—	—	0.164*
FEV1	152	0.217**		0.163*		—
CRP	212	-0.136*	—	—	—	-0.144*

*Note: the value in table represents the correlation coefficient, -means that the clinical objective indicators are not related to the domain score, * means that the correlation is significant when the confidence level(two-side) is 0.05, * * means that the correlation is significant when the confidence level (two-side) is 0.01*

Canonical correlation analysis

Because the FVC and FEV1 values were too small, they were not included in canonical correlation analysis. Fifteen clinical objective indicators were identified by screening the simple correlations, with correlation as the X value and the four domains of QLICD-COPD (V2.0) as the Y values. X_1 was TP, X_2 was ALB, X_3 was TBA, X_4 was ALP, X_5 was Scr, X_6 was K, X_7 was Na, X_8 was NEUT%, X_9 was LYMPH%, X_{10} was MONO%, X_{11} was LYMPH, X_{12} was MONO, X_{13} was HCT, X_{14} was PCO_2, X_{15} was CRP, Y_1 was PHD, Y_2 was PSD, Y_3 was SOD, and Y_4 was SPD. Using canonical correlation analysis for the X and Y variables, four common variables were obtained (Table 5).

The results showed that within the four pairs of canonical variables, two pairs of canonical variables were statistically significant ($r_1 = 0.35$, $P < 0.0001$; $r_2 = 0.26$, $P < 0.05$), demonstrating that there was a correlation between QOL and the clinical objective indicators. The first pair of canonical variables contained 45.8% of the information and the second pair contained 33.8%.

Table 5
Canonical correlation analysis for the scores of each domain of QLICD-COPD(V2.0) with values of clinical objective indicators in COPD patients

Number	Correlation coefficient	Proportion	Approximate F value	*df*	*P*
1	0.35	0.458	3.03	60	< 0.0001
2	0.26	0.338	2.37	42	< 0.0001
3	0.08	0.105	1.48	26	0.0625
4	0.08	0.100	1.57	12	0.1022

Table 6 shows that in the first pair of canonical variables, the levels of TP(X_1), ALB(X_2), and LYMPH% (X_9) were positively correlated with PSD (Y_2) and SPD (Y_4) while NEUT%(X_8) were negatively correlated. In other words, the higher the levels of TP, ALB, and LYMPH% in the blood biochemistry and the lower the levels of NEUT% in blood routine results were, the higher the PSD and SPD scores and the better the QOL for patients with COPD would be. In the second pair of canonical variables, the level of Na (X_7) was positively correlated with SOD (Y_3) while ALP(X_4) was negatively correlated. In other words, the higher the level of Na in blood biochemistry and the lower the level of ALP were, the higher the SOD scores and the better the QOL of patients with COPD would be.

Table 6 The correlation coefficients between the canonical variables of clinical objective indicators and the scores of each domain of QLICD-COPD(V2.0) in COPD patients

Clinical objective indicator	Variable	V_1	V_2	Variable	U_2	U_2
TP	X_1	0.592	0.277	Y_1	0.423	0.167
ALB	X_2	0.451	-0.008	Y_2	0.898	0.324
TBA	X_3	-0.129	-0.002	Y_3	0.391	0.847
ALP	X_4	-0.175	-0.435	Y_4	0.780	-0.326
Scr	X_5	-0.007	0.120	—	—	—
K	X_6	0.032	-0.371	—	—	—
Na	X_7	0.063	0.675	—	—	—
NEUT%	X_8	-0.450	-0.302	—	—	—
LYMPH%	X_9	0.456	0.300	—	—	—
MONO%	X_{10}	0.187	0.146	—	—	—
LYMPH	X_{11}	-0.341	0.396	—	—	—
MONO	X_{12}	-0.051	0.005	—	—	—
HCT	X_{13}	-0.076	0.354	—	—	—
PCO_2	X_{14}	0.060	0.252	—	—	—
CRP	X_{15}	-0.247	-0.120	—	—	—

Redundancy analysis showed that among the first pair of canonical variables, V_1 could explain 8.27% of the total variation in the X variable set and 2.16% in the Y variable set, while U_1 could explain 43.64% of the total variation in the Y variable set and 11.38% in the X variable set. In the second pair of canonical variables, V_2 could explain 9.57% of the total variation in the X variable set and 1.98% in the Y variable set, while U_2 could explain 23.92% of the total variation in the Y variable set and 4.94% in the X variable set 4.94% (Table 7).

Table 7
Redundancy analysis

	The proportion of X variable set that V can explain (%)	The proportion of Y variable set that V can explain (%)	The proportion of Y variable set that U can explain (%)	The proportion of X variable set that U can explain (%)
First pair	8.27	2.16	43.64	11.38
Second Pair	9.57	1.98	23.92	4.94

Discussion

At present, the number of deaths from respiratory diseases accounts for a quarter of all deaths, with COPD accounting for a large proportion [13]. QOL is an important indicator of the effectiveness of disease prevention and treatment measures [14] as well as a sensory representation of a person's physical, mental, and social abilities.

Comparison of the average scores for patients with COPD in various domains after standardization revealed that social function had the highest score, followed by psychological function and the score for the disease-special module, which reflects COPD symptoms. Physiological function had the lowest score. Physiological function mainly reflects a patient's appetite, sleep, defecation, and other basic physiological functions, as well as pain and fatigue. The average score for this domain was low, possibly because COPD is a lung disease that limits airflow, which can seriously damage the human body, mostly due to chronic bronchitis and bronchial asthma complicated by emphysema. There is no method to cure COPD currently and the only available options are for controlling infection and treating symptoms using anti-spasmodic and anti-asthmatic drugs. COPD has many complications, such as chronic pulmonary heart disease, diabetes mellitus, cardiovascular and cerebrovascular diseases, cerebrovascular diseases, dyslipidemia, and peptic ulcer, and severe disease will directly result in the death of the patient. The etiology of the disease is related to hypoxia, high blood viscosity, capillary spasms, and long-term use of glucocorticoids and other drugs [15]. The average age of the participants in this study was 71 years. Most of the current research results suggest that there is a correlation between the age of patients and their QOL. Some researchers think age is an independent determinant of QOL for patients with COPD. With the increase of age, elderly patients with COPD develop more complications, which may affect their QOL [16].

The psychological function mainly reflects the mental status of patients, including the three aspects of cognition, emotion, will and personality. Chronic diseases tend to increase an individual's susceptibility to emotional depression and produce a long-lasting decrease in mood [17]. Depression, anxiety, and other emotional disorders have an important impact on the QOL of patients. The incidence of depression among patients with COPD is higher than that in healthy individuals. Prigatano et al. found that depression and anxiety were highly correlated with QOL among patients with COPD using the Profile of

Mood States [18]. Individuals with COPD also experience significant changes in their mental health when they suffer from long-term physical pain; hence, they tend to have a low psychological function score.

Because the factors that affect QOL in patients with COPD vary, including social and demographic factors, physical factors, psychological factors, and clinical factors [19], we used canonical correlation analysis to comprehensively assess the group relationships for multiple factors. Canonical correlation analysis showed that in the first pair of canonical variables, lower levels of TP, ALB, and LYMPH% in blood biochemistry and higher levels of NEUT% in blood routine tests were correlated to lower scores for psychological function and the disease-specific module as well as worse QOL. TP consists of ALB and globulin and low levels of TP and ALB usually indicate a poor nutritional status. Patients should eat a light diet with a rich and reasonable nutrition, drink adequate amounts of water, rest sufficiently, perform physical exercise, reduce excess fat, burn excess calories, etc. [20]. Both LYMPH and NEUT are white blood cells. Researchers have found that many lung diseases are characterized by excessive NEUT, which leads to inflammatory responses such as pneumonia and bronchiectasis. A large number of NEUT can be found in the airway walls and lung tissues of patients with COPD and the concentration and infiltration of NEUT in the airways and lung tissues are related to the severity and progression of lung infection [21, 22]. In the second pair of canonical variables, lower levels of serum Nain blood biochemistry and higher levels of ALP were correlated with lower social function scores and worse QOL. Acid-base disturbances and electrolyte disturbances are common among patients with obstructive pulmonary disease because of decreased lung function and ventilatory disturbances. Low serum Na in the body leads to hyponatremia, which can cause nausea, vomiting, bloating, and drowsiness and makes the patient believe the condition is more serious. This leads to a negative state of mind, influences the life and interpersonal relationships of patients, and seriously affects their quality of life. Shi [23] also reached the same conclusion. Bruno et al. [24] described the pathophysiological mechanisms of acid-base disorders and their impact on patient mortality, and noted that paying attention to the serum Na in the blood is helpful for correct diagnosis and targeted therapy. ALP is an indicator of liver function and is widely distributed in various organs of the body. Mosqueira et al. [25] found that ALP in tissues is involved in lipid metabolism and related gene expression. Hepatobiliary disease or bone disease can lead to elevated ALP, which affects the patient's position in their family and at work, reduces the amount of contact with other people, and affects their QOL.

In this study, the lack of some clinical objective indicators is a serious limitation. Because these objective indicators were not included in the relevant statistical analysis, the final results were affected. In subsequent questionnaire collection processes, the accuracy and integrity of the data will be guaranteed as much as possible and priority will be given to patients who are not within the normal range for the indicators. Redundancy analysis results showed that two pairs of canonical variables related to the QOL had a low explanatory value. The selected clinical objective indicators only reflect a small portion of the factors that influence QOL and many other factors can affect the QOL of patients with COPD. Therefore, social demographic factors, physical and psychological factors, clinical factors, and other factors should be taken into account for the development and application of scales and records of clinical objective indicators.

To summarize, among the clinical objective indicators evaluated for patients with COPD, the levels of TP, ALB, NEUT%, LYMPH%, serum Na, and ALP can partly reflect the patient's QOL. In the course of treatment, clinicians should pay close attention to increases in ALP and NEUT% as well as decreases in TP, ALB, LYMPH%, and serum Na. While using drugs and other therapeutic means to ease the patient's pain, health education and psychological treatment should also be provided. Physicians need to consider the various factors that affect the patient and take appropriate steps to improve their QOL.

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of the Affiliated Hospital of Guangdong Medical University (REC: PJ2015050KT) and informed consent was provided by all patients.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Yuxi Liu and Jinghao Ruan contributed to the study design, data analysis, and manuscript writing. Chonghua Wan contributed to study design and revision of the manuscript. Jianfeng Tan and Bin Wu contributed to the study design and made comments on the paper. Zhihua Zhao contributed to preparation of materials and data collection. All authors read and approved the final manuscript.

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