Original Article

Airway bacterial colonization in patients with non-small cell lung cancer and the alterations during the perioperative period

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Background: To observe the alterations in airway bacterial colonization during the perioperative period in patients with non-small cell lung cancer (NSCLC) and evaluate their clinical implications.

Methods: Patients with resectable primary NSCLC were enrolled from October 2011 to April 2012. Airway secretions were harvested for microbiological study after admission, immediately after surgery, and before endotracheal extubation. Spontaneous sputum was collected when patients presented with signs of postoperative pneumonia (POP). Detailed data on the isolated pathogens were carefully recorded. Risk factors for airway colonization and POP were analyzed.

Results: A total of 78 consecutive patients were enrolled. Fourteen patients (17.9%) had airway colonization at admission, including four cases of fungi and ten cases of Gram-negative bacilli (GNB). Five patients (6.4%) had colonized pathogens at the end of surgery, including three cases of GNB and two cases of Gram-positive cocci. Nine (11.5%) patients had positive culture of airway secretions collected before extubation, including seven cases of GNB and two cases of fungi. Eighteen patients (23.1%) had POP, of whom one suffered from bronchopleural fistula and one died of POP. Pathogens of POP were confirmed in 11 patients, including nine cases of GNB and two cases of fungi. Three patients had the same pathogens as preoperative colonization. The proportion of more antibiotic-resistant strains increased gradually. Advanced age [odds ratio (OR), 2.263; 95% confidence interval (95% CI), 1.030-4.970] and smoking (OR, 2.163; 95% CI, 1.059-4.429) were risk factors for airway colonization. Decreased diffusion capacity of the lung for carbon monoxide (OR, 5.838; 95% CI, 1.318-25.854), prolonged operation time (OR, 6.366; 95% CI, 1.349-30.033), and preoperative airway colonization (OR, 9.448; 95% CI, 2.206-40.465) were risk factors of POP.

Conclusions: Airway colonized pathogens altered and more antibiotic-resistant GNB emerged during the perioperative period. These pathogens played an important role in the presence of POP.

Keywords: Airway bacterial colonization; non-small cell lung cancer (NSCLC); operation; pneumonia

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Introduction

Postoperative pneumonia (POP) is one of the most common complications of lung surgery. The incidence of POP is as high as 22% to 25% in patients who underwent major lung resections (1-4). It causes most of the in-hospital deaths after lung surgery (5). In addition, patients who have suffered from POP may have poor prognosis (6). Versatile risk factors, including comorbidity of chronic obstructive pulmonary disease (COPD) (2,3,6), prolonged operative time and postoperative intensive care unit (ICU) admission (2), gender and extent of resection (3), low predicted postoperative forced expiratory volume in

1 second (FEV_1) (7), positive smoking history (8), advanced age and pathologic stage (8,9), and decreased diffusion capacity of the lung for carbon monoxide (DL_{CO}) (10-12), are predictors of patients at high risk of postoperative pulmonary complications. Besides, airway bacterial colonization is also an independent risk factor of POP (13). In contrast to healthy nonsmoking individuals, airway bacterial colonization is frequently seen in patients with chronic pulmonary diseases such as COPD, bronchiectasis, or non-small cell lung cancer (NSCLC) (14). However, little is known on the alterations in the colonization during the perioperative period. This prompted us to perform a pilot prospective observational study, investigating the alterations in the airway flora during the perioperative period in patients with resectable NSCLC, as well as the relationship between the flora and the pathogens of POP. We also investigated the risk factors of airway bacterial colonization and POP.

Materials and methods

Patients

From October 2011 to April 2012, consecutive patients admitted to West China Hospital, Sichuan University for major lung resection were included in this study according to the following criteria: (I) pathologically or clinically diagnosed as NSCLC before surgery; and (II) suitability for surgical treatment using TNM classification and cardiopulmonary assessment. Patients were excluded if they did not fulfill these criteria or if they: (I) suffered from signs of acute infection, including purulent sputum, hyperpyrexia, or leukocytosis within 2 weeks before surgery; (II) had a history of antibiotic medication within 4 weeks prior to surgery; (III) received neoadjuvant chemotherapy or radiotherapy before surgery; (IV) refused to participate in the study; or (V) were finally diagnosed with benign lesions. This study was approved by the Institutional Review Board of our hospital. Informed consent was obtained from each patient.

Data collection

We designed a standard questionnaire for data collection. All data concerning the patient's characteristics, preoperative assessments, surgical procedures, post-operative management, results of microbiological studies, pathological diagnosis, and in-hospital outcome were collected. The questionnaire was divided into three sections, namely, the patient's characteristics and preoperative assessments, operation room events, and post-operative management. These three sections were filled in by the attending doctor, the operation room staff, and the ICU staff, respectively.

Microbiological sampling

All the patients underwent microbiological sampling in the morning of the first three consecutive days after admission. Secretions in the distal airway were brought out by deep cough after gargling thrice with physiological saline and were collected for microbiological study. Surgical procedures were performed under general anesthesia with double-lumen endotracheal intubation. Immediately after the operation, airway secretions were harvested through the endotracheal intubation in the operation room using a sterile suction tube and a sputum container. All the patients were transferred to the ICU with endotracheal intubation after surgery. The intubation was removed once the patient had recovered well from anesthesia. Before removal of the intubation, secretions of the distal airway were also collected with the same method as in the operation room. Postoperatively, repeated spontaneous sputum or bronchial aspirates were collected for microbiological study when patients presented at least one symptom that defined POP. According to the guidelines of the American Thoracic Society, POP is defined by the presence of clinical infectious signs, such as new onset of fever, purulent sputum, leukocytosis, decline in oxygenation, and positive culture of airway secretions, combined with or without new or progressive infiltrative shadows on chest X-ray (15).

Microbiological processing

All samples were delivered to the Clinical Laboratory of Microorganism of our hospital for microbiological study within 10 min after sampling. Antimicrobial susceptibility test was performed for the identified bacterial strains. Anaerobic culture was not routinely performed for these patients. Sputum samples were considered suitable for culture when less than 10 squamous cells and more than 25 leucocytes per low-power magnification field were seen. If contaminated by upper airway secretions, the sample was discarded. Samples were cultured quantitatively in accordance with standard laboratory procedures. Antimicrobial susceptibility test of the isolated Gram-negative bacilli (GNB) was carried out using different representative antibiotics of the selected categorizations, including penicillins, cephalosporins, carbapenems,

Table 1 Patients' characteristics and details of surgery (N=78)

Parameter	Value (%)
Male (n)	57 (73.1)
Age [years]	61.5 [33-78]*
Body mass index (kg/m^2)	23.6±3.2
Body mass index ≥25 (n)	23 (29.5)
Positive smoking history (n)	43 (55.1)
Smoking index ≥400 cigarette-years (n)	35 (44.9)
COPD (n)	17 (21.8)
Hypertension (n)	21 (26.9)
Diabetes mellitus (n)	7 (9.0)
Preoperative laboratory test	
Hemoglobin (g/L)	131.2±14.6
White blood cell count (×10^9/L)	6.2±1.4
Albumin (g/L)	41.2±4.8
Pulmonary function test	
% pre, FEV_1	84.8±17.2
≥80% pre (n)	45 (57.7)
70-80% pre (n)	16 (20.5)
<70% pre (n)	17 (21.8)
% pre, MVV	93.5±23.3
% pre, DLco	90.7±23.3
≥70% pre (n)	62 (79.5)
<70% pre (n)	16 (20.5)
Surgical procedure	
Open lobectomy (n)	57 (73.1)
Duration of operation (min)	192.6±52.5
Duration of operation ≤3 hrs (n)	39 (50.0)
Volume of blood loss (mL)	100 [20-1,500]*
Volume of blood loss ≤300 mL (n)	70 (89.7)
ICU	
Mechanical ventilation (hours)	3.7±2.4
PaO_2 (mmHg)	143.0±56.8
$PaCO_2$ (mmHg)	41.3±5.4
Histology	
Squamous cell carcinoma (n)	27 (34.6)
Adenocarcinoma (n)	34 (43.6)
Adenosquamous carcinoma (n)	13 (16.7)
Others (n)	4 (5.1)

Data are presented as mean ± SD or n (%) unless otherwise stated. *, these data are described with median and range; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in one second; MVV, maximum ventilatory volume; DLco, diffusion capacity of the lung for carbon monoxide; ICU, intensive care unit.

aminoglycosides, and quinolinones. When Gram-positive cocci or *Haemophilus influenzae* were isolated, Penicillins, Quinolinones, Tetracyclines, Macrolides, Clindamycin, Rifampicin and Vancomycin were selected for the antimicrobial susceptibility test.

Strategies of prophylactic antibiotics

The most preferred prophylactic antibiotics for lung surgery in China are first- or second-generation cephalosporins, as recommended by the Surgical Branch of the Chinese Medical Association. With the agreement of our Hospital Nosocomial Infectious Surveillance Committee, we chose cefmetazole as prophylactic medication during the perioperative period. Clindamycin was used as an alternative if there were contraindications for cefmetazole. Prophylactic antibiotics for patients with preoperative airway bacterial colonization were changed according to the antimicrobial susceptibility test. Antibiotic prophylaxis was administered during the induction of anesthesia. Another dose of antibiotics was added if the surgical procedure exceeded 3 hours. Postoperative antibiotic administration was ceased within 24 hours after surgery and may be prolonged in patients presented with clinical signs of POP, which was decided according to the expectoration, body temperature, pulmonary examination, and white blood cell count.

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Results are expressed as percentage, mean ± standard deviation (SD) or median and range. Categorical variables were analyzed using χ^2-test, Fisher's exact test, or univariate logistic regression when needed. The quantitative continuous variables were compared using the Student's t-test or Mann-Whitney U test. All were two-tailed tests with the level of significance set at 0.05. Logistic regression was performed to evaluate risk factors associated with airway bacterial colonization and POP. Variables with $P \leq 0.1$ were entered into the multivariate regression analysis.

Results

A total of 78 patients with primary NSCLC were included in this study. Characteristics and preoperative assessments of these patients, details of surgery, and diagnosis are summarized in *Table 1*.

Microbiological study

Fourteen patients (17.9%) were identified with airway bacterial colonization after admission, including four cases of fungi (three cases of *Candida*, one case of *Aspergillus*) and ten cases (12.8%) of GNB. *Klebsiella pneumoniae* was the most frequently involved microorganism (*Table 2*). Samples collected immediately after surgery were isolated with pathogens in five patients (6.4%). There were three strains of GNB and two strains of Gram-positive cocci. Nine (11.5%) patients had a positive culture of airway secretions harvested before removal of the endotracheal intubation, including seven cases of GNB and two cases of *Candida albicans*.

Repeated spontaneous sputum was collected for microbiological study from 32 patients (41.0%) who presented at least one sign indicating POP. Eighteen (23.1%) of these patients were diagnosed with POP, of whom one suffered from bronchopleural fistula and one died of pneumonia. Pathogens of POP were detected in 11 patients, including nine cases of bacteria and two cases of fungi. A total of 22 strains were isolated, including 16 strains of GNB, 2 strains of Gram-positive cocci, and 4 strains of *Candida albicans*. Samples collected immediately after surgery showed that airway colonized pathogens remained unchanged in two patients. Samples collected before removal of endotracheal intubation revealed the same pathogens as preoperative airway colonization in three patients. Postoperatively, five patients had more than one kind of pathogens isolated from the sputum. Pathogens of POP were the same as those of preoperative colonization in three patients. Airway colonization identified during the perioperative period is listed in *Table 2*.

Only 6 (15.0%) of the 40 isolated strains were gram-positive cocci. Antimicrobial susceptibility test was conducted for all of the isolated bacteria strains. Resistance to Penicillins was observed in 30 (75%) of these strains. The proportion of more antibiotic-resistant bacteria strains increased among the four sequential samplings (*Table 3*). At the time of admission, 2 of the 10 isolated strains (2/10) were multi-drug resistant bacteria (MDR). However, bronchial secretions collected after surgery revealed 4 strains (4/5) of MDR, and 3 strains (3/7) of MDR at extubation. Postoperatively, nearly half (8/18) of the isolated strains were MDR.

Risk factors for airway bacterial colonization

Potentially predictive variables of airway bacterial colonization included the following: gender (male or female), age (in years: <60, 60 to 70, or ≥70), body mass index (BMI) (<25 or ≥25), smoking index (in cigarette-years: 0, 0 to 400, or ≥400), comorbidity of COPD, FEV_1 (≥80%, 70% to 80%, or <70%), maximum ventilatory volume (MVV) (≥70% or <70%), and DL_{CO} (≥70% or <70%). After univariate analysis, advanced age and smoking index proved to be statistically significant. Furthermore, the logistic regression model showed that advanced age [odds ratio (OR), 2.263; 95% confidence interval (95% CI), 1.030-4.970] and smoking (OR, 2.163; 95% CI, 1.059-4.429) were independent predictors of airway bacterial colonization in NSCLC patients (*Table 4*).

Risk factors for POP

Several variables were analyzed as potential predictors of POP, including: gender (male or female), age (in years: <60, 60 to 70, or ≥70), BMI (<25 or ≥25), smoking index (in cigarette-years: 0, 0 to 400, or ≥400), FEV_1 (≥80%, 70% to 80%, or <70%), MVV (≥70% or <70%), DL_{CO} (≥70% or <70%), duration of the operation (≤3 or >3 h), blood loss (≤400 or >400 mL), surgical approaches (VATS or open), and preoperative airway colonization. BMI, DL_{CO}, duration of the operation, and preoperative airway colonization were proven to be statistically significant by univariate analysis. The logistic regression model showed that decreased DL_{CO} (OR, 5.838; 95% CI, 1.318-25.854), prolonged operation time (OR, 6.366; 95% CI, 1.349-30.033), and the presence of preoperative airway colonization (OR, 9.448; 95% CI, 2.206-40.465) were risk factors of POP (*Table 5*).

Discussion

The incidence of airway bacterial colonization in lung cancer patients undergoing surgery ranged between 10.5% and 83%, and colonization of potential pathogenic microorganisms ranged between 10.5% and 41% (3,6,16-20). Samples for microbiological study were collected at different time points using various methods in these studies, such as bronchoalveolar lavage (BAL) of resected specimen (16), bilateral bronchoscopic aspirate after endotracheal intubation (3,17,19), preoperative spontaneous sputum (6,18), and preoperative bronchoscopic brushing or BAL (6,20). Present data showed that the incidence of preoperative airway colonization was 17.9% in our patients. In addition to differences of the population studied and heterogeneity of the methodology, versatile

Table 2 Pathogens isolated during the perioperative period

No.	Age	Gender	Smoking	Admission	Completion of surgery	Endotracheal extubation	Post-operation	POP
4	57	M	Yes	*Candida glabrata*	–	–	NS	No
7	63	M	Yes	*Aspergillus*	–	–	*B/h. acinetobacter, Enterobacter Cloacae*	Yes
8	67	M	No	–	–	*Acid producing Klebsiella*	*Candida albicans, B/h. acinetobacter*	Yes
10	65	M	No	*K. pneumoniae*	*K. pneumoniae*	*K. pneumoniae*	*K. pneumoniae*	Yes
11	70	M	Yes	*K. pneumoniae*	–	–	NS	No
15	36	F	No	–	–	–	–	BPF
18	70	M	Yes	*B/h. acinetobacter*	*Enterobacter Cloacae*	*B/h. acinetobacter*	*B/h. acinetobacter, Enterobacter Cloacae*	Yes
20	77	M	Yes	*K. pneumoniae*	–	–	*K. pneumoniae*	Yes
21	62	M	Yes	–	–	*B/h. acinetobacter*	–	No
27	57	M	Yes	*P. aeruginosa*	–	–	NS	No
29	42	F	No	–	–	–	*B/h. acinetobacter*	Yes
30	78	M	Yes	*Acid producing Klebsiella*	*Acid producing Klebsiella*	–	*Candida albicans*	Yes
31	71	M	No	*P. aeruginosa*	–	*P. aeruginosa*	NS	No
42	74	M	Yes	*Candida albicans*	–	–	*P. aeruginosa*	Yes
43	63	F	Yes	*Candida albicans*	–	–	NS	No
52	43	M	Yes	–	*S. pneumoniae*	*S. pneumoniae*	–	Yes
61	59	F	No	–	*Staph. aureus*	–	NS	No
64	75	M	Yes	–	–	*Candida albicans*	*Candida albicans, B/h. acinetobacter*	Death
70	61	M	Yes	*Lwoffii Acinetobacter*	–	*S. pneumoniae*	*S. pneumoniae, H. influenzae*	Yes
73	50	M	Yes	*K. pneumoniae*	–	*Candida albicans*	*Candida albicans*	Yes
78	57	M	No	*K. pneumoniae*	–	–	NS	No

B/h., *acinetobacter*, Bowman/hemolytic acinetobacter; BPF, bronchopleural fistula; F, female; *H. influenzae*, haemophilus influenzae; *K. pneumoniae*, klebsiella pneumoniae; M, male; NS, not sampled; *P. aeruginosa*, pseudomonas aeruginosa; POP, postoperative pneumonia; *S. pneumoniae*, streptococcus pneumoniae; *Staph. aureus*, staphylococcus aureu.

Table 3 Alterations of the number of resistant bacteria strains isolated during the four sequential microbiological studies

Time of sampling	Antibiotic resistance (No. of resistant strains)						Total
	S	R1	R2	R3	R4	R5	
Admission	0	7	1	1	0	1	10
Completion of surgery	0	1	0	1	1	2	5
Endotracheal extubation	0	3	1	1	2	0	7
Post-operation	2	2	6	3	2	3	18

S, sensitive to all categories of the tested antibiotics; R1-5, resistant to 1 category to 5 categories of the tested antibiotics.

Table 4 Risk factors for airway bacterial colonization

Variables	OR	95% CI	P value
Univariate			
Gender (M/F)	0.169	0.021-1.384	0.131
Age (<60, 60-70, ≥70)	2.175	1.017-4.650	0.045
BMI (<25, ≥25)	1.420	0.419-4.816	0.810
Smoking index (0, 0-400, ≥400 cigarette-years)	2.097	1.043-4.215	0.038
Comorbidity of COPD	2.407	0.682-8.492	0.301
FEV_1 (≥80%, 70-80%, <70%)	1.289	0.651-2.554	0.466
MVV (≥70%, <70%)	2.127	0.247-18.310	0.795
DLco (≥70%, <70%)	2.677	0.751-9.546	0.234
Multivariate			
Age (<60, 60-70, ≥70)	2.263	1.030-4.970	0.042
Smoking index (0, 0-400, ≥400 cigarette-years)	2.163	1.059-4.429	0.034

BMI, body mass index; CI, confidence interval; COPD, chronic obstructive pulmonary disease; DLco, diffusing capacity of the lung for carbon monoxide; F, female; FEV_1, forced expiratory volume in one second; M, male; MVV, maximum ventilatory volume; OR, odds ratio.

diagnostic standards used in different labs could be an important reason for the huge variability of incidence.

The pathogens classically reported for early hospital-acquired pneumonia, such as *Haemophilus influenza*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*, also played an important role in the occurrence of POP (3,6,16-20). Previous studies have shown that these pathogens were also the most commonly identified pathogenic bacteria strains in the distal airways of patients with resectable NSCLC (3,6,16-20). However, the pathogens isolated from preoperative airway secretions in this study were quite different from those previously reported. The main pathogens identified were GNB, such as *Klebsiella pneumoniae*. Focusing on the pathogens of POP, GNB was the most common agents in these patients, similar to those of previous reports (6,18). The reason for this difference is also unclear. Considering the overuse of antibiotics is quite a serious problem in China, we prudently presume that this could be an important reason. However, data on the occurrence of airway colonization in Chinese patients are still rare, and whether this small population represents the real situation is uncertain. Therefore, this presumption needs further confirmation, and we hope that additional data on airway colonization in Chinese patients with NSCLC can be obtained in the future.

The pathogens of POP are frequently different from those in preoperative colonization (6,17). We also observed the same phenomenon in this study. Airway colonization altered during the perioperative period, and the proportion of more antibiotic-resistant pathogens, such as *Acinetobacter baumannii*, increased. The occurrence of more resistant pathogens may be caused by the screening effect of prophylactic antibiotics. The reasons for the alteration have rarely been described. We presume that decreased airway

Table 5 Risk factors for POP

Variables	OR	95% CI	P value
Univariate			
Gender (M/F)	0.323	0.067-1.565	0.253
Age (<60, 60-70, ≥70)	1.654	0.817-3.349	0.162
BMI (<25, ≥25)	0.754	0.215-2.643	0.018
Smoking index (0, 0-400, ≥400 cigarette-years)	1.429	0.787-2.598	0.241
FEV1 (≥80%, 70-80%, <70%)	1.364	0.712-2.611	0.349
MVV (≥70%, <70%)	2.547	0.298-21.737	0.644
DLco (≥70%, <70%)	4.580	1.360-15.428	0.025
Duration of the operation (≤3, >3 hrs)	3.889	1.128-13.411	0.050
Blood loss (≤300, >300 mL)	1.333	0.243-7.329	1.000
Surgical approaches (VATS/open)	0.882	0.250-3.116	1.000
Preoperative airway colonization	9.333	2.564-33.973	0.001
Multivariate			
DLco (≥70%, <70%)	5.838	1.318-25.854	0.020
Duration of the operation (≤3, >3 hrs)	6.366	1.349-30.033	0.019
Preoperative airway colonization	9.448	2.206-40.465	0.002

BMI, body mass index; CI, confidence interval; DLco, diffusing capacity of the lung for carbon monoxide; F, female; FEV_1, forced expiratory volume in one second; M, male; MVV, maximum ventilatory volume; OR, odds ratio; VATS, video assisted thoracic surgery.

colonization during the operation was due to the use of prophylactic antibiotics. However, prolonged endotracheal intubation may increase the risk of bacterial colonization, which is in accordance with the increasing trend as observed of the samples collected before removal of the intubation.

Airway bacterial colonization is a frequent feature in patients with stable chronic lung diseases (14). In previous studies on lung cancer patients, the comorbidity of COPD (6), central location of the tumor, and overweightness (BMI >25 $kg \cdot m^{-2}$) (19) have been described as independent risk factors of airway bacterial colonization. Our data showed that advanced age and smoking were independent risk factors of airway bacterial colonization. Only 21.7% of our patients had COPD, and few of them had severe COPD. The proportion of overweight patients was only 29.5% in this group. Both comorbidity of COPD and overweightness were not identified as independent risk factors of airway bacterial colonization. This may be due to their small proportions in the currently observed group. The incidence rate of postoperative pulmonary complications was 23.1% in the present group, similar to those in previous reports (1-4). Multivariate analysis of our data also revealed that preoperative airway bacterial colonization was an independent risk factor of POP, as well as %Pre DL_{CO} <70% and prolonged operative duration. According to these reasons, NSCLC patients with high risk factors should have microbiological study of the airway secretions before surgery, and this may guide the perioperative preventive measures for POP.

First- or second-generation Cephalosporins, mainly targeting Gram-positive cocci, are the most commonly recommended prophylactic antibiotics for lung surgery (21), but GNB contributed the most towards the occurrence of POP in our patients as well as in some other reports (6,18). Evidence on the constitution of the most appropriate antibiotic prophylaxis for lung cancer surgery is still insufficient, and the implications of preoperative airway colonization on the selection of prophylactic antibiotics are unknown. A further investigation into the airway flora in more patients with NSCLC is needed, and this may help us revise the strategy of perioperative prophylactic medication.

There are also some limitations in this study. We were unable to study the sputum flora after surgery in all patients, but only in patients with signs of POP. Confined to the limitations of clinical practice, the methodology of sample collecting varied among the four sequential time point. Moreover, the sample size obtained for the study is relatively small.

Conclusions

The airway colonized pathogens changed and more antibiotic-resistant GNB emerged during the perioperative period. Advanced age and smoking are risk factors of airway bacterial colonization in patients with lung cancer. Decreased DL_{CO}, preoperative airway bacterial colonization, and prolonged operation time are risk factors of POP. According to this pilot study, special attention should be paid to GNB when patients suffered from pneumonia after lung cancer surgery.

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Author's contribution: M.J.D. designed and carried out the study, collected and analyzed data, and wrote the paper; L.L.X. designed and carried out the study, interpreted data; T.M.L. carried out the study, collected and analyzed data; X.N.H. carried out the study and collected data; P.Q. analyzed data and revised the paper; L.C.W. revised the paper; M.L. carried out the study and analyzed data; S.H. carried out the study and collected data; C.G.W. designed the overall study and carried out the study, analyzed and interpreted data, and revised the paper.
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