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Microbiome Investigation of the Lower Airways of Bronchiectasis Patients with Serum Cytokine and Chemokine Content into the Pathogenesis of Bronchiectasis

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ABSTRACT

Bronchiectasis is a chronic respiratory condition characterized by persistent airway inflammation and recurrent infections, yet its underlying pathogenesis remains incompletely understood. This study aimed to investigate the roles of the lower respiratory tract microbiome and serum cytokine/chemokine profiles in the pathogenesis of bronchiectasis.

In this retrospective study, we enrolled 285 bronchiectasis patients admitted to our hospital between January 2024 and June 2025. Participants were categorized into an acute exacerbation group (n=158) and a clinically stable group (n=127). We compared the two groups in terms of respiratory pathogens, immune function indicators (CD3+, CD4+, CD8+, CD4+/CD8+ ratio, white blood cell count, and neutrophil count), pro-inflammatory cytokines (interleukin-6, tumor necrosis factor-alpha, and interleukin-17A), anti-inflammatory cytokines (interleukin-10 and interleukin-4), acute-phase reactants (C-reactive protein, procalcitonin, and serum amyloid A), and chemokines (monocyte chemoattractant protein-1). The involvement of these factors in disease pathogenesis was analyzed.

Significant differences were observed between the groups in the rates of hypoalbuminemia, the presence of dyspnea and hemoptysis, and the oxygenation index in arterial blood gas analysis. Sputum cultures were positive in 103 (65.19%) patients in the acute exacerbation group, compared to 58 (45.67%) in the stable group. Immune markers CD3+, CD4+, CD8+, and the CD4+/CD8+ ratio were lower during acute exacerbation, while WBC and NEUT levels were elevated. Pro-inflammatory cytokines (interleukin-6, tumor necrosis factor-alpha, and interleukin-17A) and acute-phase reactants (C-reactive protein, procalcitonin, and serum amyloid A) were significantly higher during exacerbation, whereas anti-inflammatory cytokines (interleukin-10 and interleukin-4) were lower. Monocyte chemoattractant protein-1 levels were also elevated during exacerbation.

Dysbiosis of the lower respiratory tract microbiome, immune dysfunction, and exacerbated inflammatory responses are interrelated and collectively contribute to the pathogenesis of bronchiectasis.

Keywords: Bronchiectasis; Inflammation; Immunity, Cellular; Cytokines; Bronchi; Microbiota

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INTRODUCTION

In recent times, the prevalence of bronchiectasis has been on the rise worldwide, which may be attributed to advancements in imaging techniques and improved awareness of the disease.[1] However, this trend has also led to an increased socioeconomic burden and healthcare costs.[2] Studies have indicated a rise in mortality rates among patients with bronchiectasis, with mortality reaching 16% to 24.8% during a 4- to 5-year follow-up period.[3] The onset and progression of bronchiectasis are closely associated with a vicious cycle of infection and inflammation. When the airways are infected by bacteria, an inflammatory response is triggered, resulting in impaired mucociliary clearance and airway damage. This makes the airways more susceptible to further infections, thereby precipitating acute exacerbations of bronchiectasis.[4] In recent years, despite continuous advancements in clinical diagnostic and therapeutic techniques, the application of medications such as antibiotics and bronchodilators has significantly improved short-term symptom control in patients. Nevertheless, the frequency of acute exacerbations and the rate of disease progression in bronchiectasis have not been effectively curbed.[5] The root cause is that the disease's pathogenesis remains incompletely understood, which severely restricts the development of targeted prevention and treatment approaches.

Currently, the underlying pathogenesis of bronchiectasis remains incompletely understood.[6] Among the numerous theories, the most well-known is the "vicious cycle" hypothesis proposed by Cole in 1986.[7] This theory encompasses inflammatory responses, airway infection, impaired mucociliary clearance, and structural damage resulting from various factors. It posits that these factors interact with each other, forming a vicious cycle, and the initiation of any single link can drive disease progression.[8] However, although weakening one link, such as through the use of antibiotics or airway clearance therapies, can improve clinical outcomes in bronchiectasis to some extent, these measures have failed to fundamentally halt disease progression.[9,10] Therefore, the "vicious cycle" hypothesis is still insufficient to fully explain the complex mechanism underlying the recurrent onset of bronchiectasis. In recent years, scholars such as Flume[11] have proposed a new concept called the "vicious vortex." This concept emphasizes that the factors associated with the disease are interconnected and do not follow a fixed cyclic order. When mucociliary clearance is impaired and airway secretions are retained, the normal host defense mechanisms are disrupted, making the airways more susceptible to chronic infections.[12] During this process, bacterial pathogens interact with the host, and their immunomodulatory properties can promote long-term bacterial survival, triggering an inflammatory response. This leads to airway damage and abnormal remodeling, ultimately inducing bronchiectasis.[13] Thus, bronchiectasis is the result of the interaction among pathogens, the host microbiome, and host inflammatory and immune regulation. The "vicious vortex" concept not only clarifies that bronchiectasis is triggered by multiple key factors through complex interactions but also better explains why single-agent therapies have only a minor effect on improving clinical outcomes in bronchiectasis. Therefore, describing it as a "vortex" theory is more reasonable.[14] Among these factors, the imbalance of the lower respiratory tract microbiome, immune dysfunction, and the cytokine/chemokine-mediated inflammatory cascade are considered core elements of this network. However, there are still many controversies regarding the interactions among these three factors and their specific roles in the onset and progression of the disease.

In bronchiectasis, most bacteria that trigger pulmonary inflammation exist in a symbiotic form within the upper respiratory tract microbiome, primarily concentrated in the nasopharynx. When specific bacteria within the microbiome migrate to the lower respiratory tract, they may induce an inflammatory response, and such bacteria are defined as potential pathogenic microorganisms.[15] Researches have confirmed that infection with potential pathogenic microorganisms in the airways is a core driving factor in the progression of bronchiectasis. Notably, there are complex intercellular signaling mechanisms within bacterial populations. These mechanisms can precisely regulate bacterial viability, colonization efficiency, and potential host invasion behaviors, thus contributing significantly to the initiation and progression of bronchiectasis.[16] Abnormal immune function plays a non-negligible role in the course of bronchiectasis. Previous studies have found that imbalances in the proportions of peripheral blood T lymphocyte subsets and neutrophil dysfunction are common in patients with bronchiectasis. These immune deficiencies may lead to a decreased ability of the host

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to clear pathogens, promoting chronic infection.[17] Meanwhile, abnormal activation of immune cells can trigger excessive inflammatory responses, forming a vicious cycle.[18] Pro-inflammatory cytokines boost inflammation by triggering key signaling cascades, thereby increasing airway mucus secretion and fibrosis. Conversely, anti-inflammatory cytokines help maintain immune homeostasis by suppressing the release of pro-inflammatory factors.[19] Chemokines, on the other hand, can selectively attract immune cells such as monocytes and macrophages to the inflamed area, where they contribute to pathogen elimination and tissue repair.[20]

In view of the current research status mentioned above, this study focuses on the associations among the lower respiratory tract microbiome, serum cytokines and chemokines, and immune function in patients with bronchiectasis. We retrospectively included 285 bronchiectasis patients, dividing them into an acute exacerbation group and a clinically stable group. By comparing the clinical characteristics, microbiome composition, immune indicators, and inflammatory cytokine levels, we aim to uncover the synergistic roles of these three factors in the pathogenesis of the disease. The conduct of this research not only helps to elucidate the pathophysiological essence of bronchiectasis but also provides a theoretical basis for the development of novel prevention and treatment strategies centered on regulating microbiota balance, targeting inflammatory cytokines, or improving immune function. It holds promise for opening up new avenues to reduce the frequency of acute exacerbations, slow down disease progression, and improve patient prognosis.

MATERIALS AND METHODS

Subjects

This study is a retrospective clinical controlled study, with data collection and analysis conducted by researchers who were not involved in the patients' treatment. The study enrolled 285 bronchiectasis patients hospitalized between January 2024 and June 2025, categorizing them into an acute exacerbation group (n=158) and a clinically stable group (n=127). We collected patients' symptoms upon admission, imaging findings, and initial laboratory indicators such as complete blood count, biochemical tests, and arterial blood gas analysis. Differences in respiratory pathogens, immune function indicators, serum cytokines, and chemokines between the two groups were compared, and their roles in the pathogenesis of bronchiectasis were analyzed. The flowchart is shown in Figure 1.

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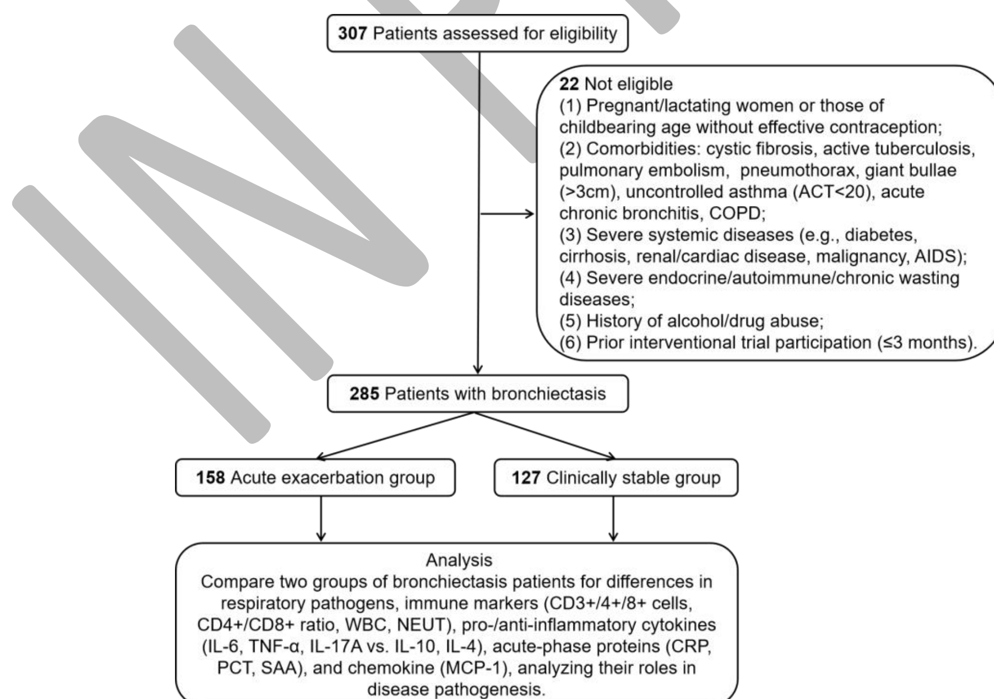

Figure 1. Research flowchart.

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Inclusion Criteria

(1) Age≥18 years old; (2) Diagnosed with bronchiectasis[21]; (3) Patients in the acute exacerbation phase meet the criteria for acute exacerbation,[22] which require a patient to have a deterioration for at least 48 hours in three or more of the following key symptoms: cough, sputum volume and/or consistency, sputum purulence, dyspnea and/or exercise tolerance, fatigue and/or malaise, hemoptysis. Patients assigned to the clinically stable group had no evidence of acute exacerbation for at least 4 weeks prior to enrollment and were in their baseline state of health, with no changes in their chronic respiratory symptoms or requirement for additional antibiotics beyond their maintenance therapy; (4) Specimens are collected prior to anti-infective treatment.

Exclusion Criteria

Exclusion criteria were as follows[23]: (1) Pregnant, lactating, or women of childbearing age who have not adopted effective contraceptive measures; (2) Comorbid with cystic fibrosis, active tuberculosis, pulmonary embolism, pneumothorax, multiple giant bullae (>3 cm), uncontrolled asthma (ACT score<20), acute exacerbation of chronic bronchitis, or chronic obstructive pulmonary disease; (3) Suffering from other severe systemic diseases, such as diabetes, liver cirrhosis, kidney disease, heart disease, malignancy, or AIDS; (4) Comorbid with other severe endocrine diseases, autoimmune diseases, or chronic wasting diseases; (5) History of alcohol or illicit drug abuse; (6) Subjects who have participated in other interventional clinical trials within the 3 months prior to enrollment; (7) Receiving long-term macrolide therapy (defined as regular use for≥3 months prior to enrollment).

Ethical Statement

This clinical trial complies with applicable ethical standards, such as the Declaration of Helsinki,[24] and obtained approval from the hospital's ethics committee before it began. Researchers must explain the study's goals, methods, and possible risks to participants in detail and secure their written informed consent.

Sample Size Calculation

This study is a retrospective clinical controlled trial, and the sample size was calculated using G-Power software.[25] The effect size was set at 0.5 (medium effect), based on previous similar studies investigating immune and inflammatory markers in bronchiectasis patients,[26] which commonly reported moderate to large differences in cytokine levels and immune parameters between stable and exacerbation phases. The significance level (α) was set at 0.05 (two-tailed), and the statistical power (1−β) was set at 0.95. These parameters indicated a requirement of 210 participants. Ultimately, 285 participants were included in this study, exceeding the target sample size and meeting the statistical requirements of the research.

Observation Indicators

(1) Lower respiratory tract microbiota detection: To minimize oropharyngeal contamination and ensure the collection of high-quality lower respiratory tract specimens, a standardized sputum induction and processing protocol was strictly followed. Patients were instructed to brush their teeth thoroughly and rinse their mouths with water upon waking, before breakfast. Immediately prior to sputum expectation, patients performed oral hygiene again by rinsing their mouths three times with sterile physiological saline. They were then instructed to take deep breaths and cough deeply to produce sputum from the lower airways. The first sputum expectoration was discarded to reduce oral cavity contamination. The subsequent deep-cough sputum sample was collected into a sterile, wide-mouthed, leak-proof container.

Immediately, a sputum smear was prepared and stained with Gram staining for microscopic examination at 15× magnification. A total of 30 fields were observed. A sputum sample was considered qualified if there were≥25 polymorphonuclear leukocytes per field and≤10squamous epithelial cells per field. The sample was then inoculated within 1 hour. To improve the detection rate of potential pathogens, sputum collection was performed consecutively for 3 days for each patient. A positive culture result was defined as the presence of a significant bacterial load (≥ 10^7 CFU/mL in a single culture or >10^4 CFU/mL of the same dominant species in two cultures) from any of the three samples. It should be noted that the day-to-day reproducibility of culture results was not formally analyzed in this study. The samples were cultured at 37°C for 24–48 hours, followed by isolation, purification, and microscopic examination. A bacterial colony count of≥10^7 CFU/mL in a single culture or>10^4 CFU/mL of the same dominant

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bacterial species in two cultures was defined as pathogenic bacteria. A colony count of <10^4 CFU/mL was considered indicative of oropharyngeal flora or contaminants. The isolated pathogenic bacteria were suspended in 0.45% saline and identified using the Beckman Coulter AutoSCAN-4 Microbiology Analyzer (USA).

The distinction between colonization and infection for fungal isolates such as *Candida* species was based on a combination of quantitative culture results, microscopic examination of sputum samples, and clinical context. A fungal colony count of ≥ 10^4 CFU/mL in a qualified sputum sample (with ≤ 10 squamous epithelial cells and ≥ 25 polymorphonuclear leukocytes per low-power field), accompanied by signs of acute infection and the absence of other predominant bacterial pathogens, was considered indicative of potential fungal infection rather than mere colonization. Isolates not meeting these criteria were regarded as colonizing flora or contaminants.

(2) Immune function detection: Peripheral venous blood (5 mL) was collected from patients in the fasting state in the morning using EDTA-K2 anticoagulant tubes. The tubes were gently inverted to mix the contents, and $CD3^+$, $CD4^+$, $CD8^+$, white blood cell (WBC) count, and neutrophil (NEUT) count were measured within 2 hours. The $CD4^+/CD8^+$ ratio was calculated. $CD3^+$, $CD4^+$, and $CD8^+$ were analyzed using the BD FACSCanto Flow Cytometer (USA), with data acquisition performed using FACSDiva v8.0.1 software. Gating strategies were based on guidelines from the International Council for Standardization in Haematology (ICSH). WBC and NEUT counts were measured using the Sysmex XN-9000 Automated Hematology Analyzer (Japan), employing a combination of electrical impedance and semiconductor laser flow cytometry. The gating strategy for flow cytometric analysis of T lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$) was performed as follows: Lymphocyte Gate: Forward scatter (FSC) vs. side scatter (SSC) dot plots were used to gate the lymphocyte population based on size and granularity. Singlets Gate: FSC-height vs. FSC-area plots were used to exclude doublets and cell aggregates, ensuring analysis of single cells. $CD3^+$ T Cell Gate: From the singlet-lymphocyte population, $CD3^+$ cells were gated using fluorescence channel for CD3-fluorochrome conjugate. $CD4^+$ and $CD8^+$ Subsets: Within the $CD3^+$ population, $CD4^+$ and $CD8^+$ T cells were further distinguished using their respective fluorochrome-conjugated antibodies. $CD4^+$ T cells: helper T cells-$CD8^+$ T cells: cytotoxic T cells $CD4^+/CD8^+$ Ratio Calculation: The ratio was derived from the absolute counts of $CD4^+$ and $CD8^+$ T cells obtained from the gated populations. All gating procedures adhered to the recommendations of the International Council for Standardization in Haematology (ICSH). Data were acquired using FACSDiva v8.0.1 software and analyzed with FlowJo v10.8.1.

(3) Inflammatory marker detection: Peripheral venous blood (5 mL) was collected from patients in the fasting state in the morning using vacuum tubes without anticoagulant. Pro-inflammatory cytokines [interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interleukin-17A (IL-17A)] and anti-inflammatory cytokines [interleukin-10 (IL-10) and interleukin-4 (IL-4)] were measured using the MAGLUMI 4000 Automated Chemiluminescence Analyzer. Quality control was performed using manufacturer-provided controls (low, medium, and high concentrations) for each batch of assays to ensure accuracy and precision. The intra-assay and inter-assay coefficients of variation (CV) were maintained below 5% and 10%, respectively, for all cytokines measured. C-reactive protein (CRP) and procalcitonin (PCT) levels were measured using the Roche Cobas c702 Automated Biochemistry Analyzer (Switzerland). Serum amyloid A (SAA) levels were determined using the Hitachi 7600 Automated Biochemistry Analyzer (Japan). Monocyte chemoattractant protein-1 (MCP-1) levels were measured using the Abbott Architect Chemiluminescent Immunoassay Analyzer (USA).

Statistical Analysis

Data analysis used SPSS 25.0. Normally distributed data (e.g., IL-6, TNF-α, IL-17A) were reported as mean ± SD and compared via t-tests. Non-normal data were shown as median (IQR) and compared using Mann-Whitney U tests. Categorical data (e.g., gender) were presented as n (%) and compared via chi-square tests. Significance was set at $p<0.05$.

RESULTS

Comparison of Baseline Data of Patients

Table 1 compares the baseline characteristics. The results revealed no significant differences in terms of gender, age, body mass index (BMI), length of hospital

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stay, location of bronchiectasis (unilateral/bilateral), smoking history, alcohol consumption history, or underlying medical conditions (hypertension, hyperlipidemia, diabetes) (p>0.05).

Comparison of Patient Symptoms with Concomitant Diseases

Table 2 illustrates the differences in symptoms and comorbidities. The incidence of dyspnea (28.48% vs. 15.75%, p=0.011) and hemoptysis (23.42% vs. 11.02%, p=0.007) was significantly higher in the acute exacerbation group. Hypoalbuminemia (12.03% vs. 4.72%, p=0.030) was also more prevalent in this group, while no significant differences were observed in electrolyte disturbances (p=0.090) and respiratory failure (p=0.217).

Table 1. Baseline characteristics [mean ± SD, n (%)]

Variables	Acute exacerbation group (n=158)	Clinically stable group (n=127)	Test	χ^2/t	*p*
Gender			Chi-Square test	0.577	0.447
Male	70 (44.30)	62 (48.82)			
Female	88 (55.70)	65 (51.18)			
Age, y	55.62 ± 6.88	56.35 ± 7.24	Independent-samples t test	0.865	0.388
BMI, kg/m²	22.34 ± 1.71	22.46 ± 1.82	Independent-samples t test	0.572	0.568
Length of stay, d	11.56 ± 2.33	11.02 ± 2.69	Independent-samples t test	1.153	0.250
Dilated bronchi			Chi-Square test	0.217	0.642
Unilateral	74 (46.84)	63 (49.61)			
Bilateral	84 (53.16)	64 (50.39)			
History of smoking	38 (24.05)	35 (27.56)	Chi-Square test	0.455	0.500
Drinking history	32 (20.25)	27 (21.26)	Chi-Square test	0.044	0.835
Hypertension	29 (18.35)	22 (17.32)	Chi-Square test	0.051	0.821
Hyperlipidemia	27 (17.09)	25 (19.69)	Chi-Square test	0.318	0.573
Diabetes	23 (14.56)	24 (18.90)	Chi-Square test	0.963	0.326

BMI: body mass index.

Table 2. Comparison of patient symptoms with concomitant diseases [n (%)]

Variables	Acute exacerbation group (n=158)	Clinically stable group (n=127)	Test	χ^2	*p*	OR (95% CI)
Dyspnea	45 (28.48)	20 (15.75)	Chi-Square test	6.484	0.011	2.131 (1.182, 3.841)
Hemoptysis	37 (23.42)	14 (11.02)	Chi-Square test	7.361	0.007	2.468 (1.268, 4.805)
Electrolyte imbalance	21 (13.29)	9 (7.09)	Chi-Square test	2.878	0.090	2.010 (0.886, 4.557)
Hypoalbuminemia	19 (12.03)	6 (4.72)	Chi-Square test	4.690	0.030	2.757 (1.066, 7.125)
Respiratory failure	18 (11.39)	9 (7.09)	Chi-Square test	1.522	0.217	1.686 (0.730, 3.892)

CI: confidence interval; OR: odds ratio.

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Comparison of Arterial Blood Gas Analysis Results of Patients

Table 3 compares the arterial blood gas parameters. The results revealed that the oxygenation index in the acute exacerbation group was significantly lower than that in the stable group (330.44 ± 28.65 vs. 364.18 ± 24.36 mm Hg, $p<0.001$). However, no significant differences were observed in terms of pH, $PaCO_2$, and HCO_3^- levels ($p>0.05$). These findings indicate that during acute exacerbations, patients experience a marked decline in pulmonary gas exchange capacity, with more severe impairment of oxygenation function. Clinically, a lower oxygenation index serves as an objective indicator of hypoxemia severity. In bronchiectasis exacerbations, monitoring the OI can help clinicians assess the need for supplemental oxygen therapy, guide the intensity of respiratory support, and serve as a prognostic marker for longer hospital stays or increased risk of respiratory failure.

Comparison of the Flora in the Lower Respiratory Tract of Patients

Table 4 compares the lower respiratory tract microbiota. Among the 158 patients in the acute exacerbation group, 103 (65.19%) had positive sputum cultures, while among the 127 patients in the clinically stable group, 58 (45.67%) had positive sputum cultures, with a statistically significant difference ($\chi^2=10.916$, $p=0.001$). A total of 130 pathogenic bacteria were co-cultured in the acute plus recombinant group, and 78 pathogenic bacteria were co-cultured in the clinical stable group. The most prevalent pathogenic bacteria detected in both groups were *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Candida albicans*, with no statistically significant differences in their constituent ratios ($p>0.05$). In addition to the distribution of pathogens, the antibiotic susceptibility testing of the predominant Gram-negative bacteria was performed using the Kirby-Bauer disk diffusion method, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Among the 66 *Pseudomonas aeruginosa* isolates from the acute exacerbation group, high resistance rates were observed for several commonly used antibiotics: levofloxacin (42.4%, 28/66), ceftazidime (39.4%, 26/66), and piperacillin-tazobactam (36.4%, 24/66). Notably, 18.2% (12/66) of the isolates were identified as multidrug-resistant (MDR), defined as resistance to at least three different classes of antimicrobial agents. In the clinically stable group, the 30 *P. aeruginosa* isolates exhibited lower but still considerable resistance rates: levofloxacin (30.0%, 9/30), ceftazidime (26.7%, 8/30), and piperacillin-tazobactam (23.3%, 7/30), with 10.0% (3/30) being MDR. The difference in the prevalence of MDR *P. aeruginosa* between the acute exacerbation and stable groups was no statistically significant ($\chi^2=0.519$, $p=0.471$).

Comparison of Immunological Markers in Patients

Table 5 analyzes the immune function indicators. The numbers of $CD3^+$, $CD4^+$, and $CD8^+$ T cells, as well as the $CD4^+/CD8^+$ ratio, were significantly lower in the acute exacerbation group compared to the stable group (all $p<0.001$). This suggests severe suppression of T-cell immune function during the acute exacerbation phase, which may render patients more susceptible to infections or impair their ability to clear pathogens, thereby exacerbating the disease. Additionally, WBC and NEUT were significantly elevated (both $p<0.001$), indicating a robust inflammatory response during the acute exacerbation phase. This heightened inflammation may lead to tissue damage and worsen the pathological changes associated with bronchiectasis.

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Table 3. Comparison of arterial blood gas analysis results of patients (mean ± SD)

Variables	Acute exacerbation group (n=158)	Clinically stable group (n=127)	Test	t	*p*	Mean Difference (95% CI)
pH	7.44 ± 0.04	7.43 ± 0.05	Independent-samples t test	1.125	0.262	0.006 (-0.004,0.016)
$PaCO_2$, mm Hg	42.65 ± 2.88	42.21 ± 2.75	Independent-samples t test	1.306	0.193	0.439 (-0.223,1.101)
HCO_3^-, mmol/L	28.19 ± 2.17	27.78 ± 2.34	Independent-samples t test	1.490	0.137	0.400 (-0.128,0.928)
Oxygenation index, mm Hg	330.44 ± 28.65	364.18 ± 24.36	Independent-samples t test	10.553	<0.001	-33.738 (-40.031,-27.445)

CI: confidence interval; HCO_3^-: bicarbonate; $PaCO_2$: partial pressure of carbon dioxide.

Table 4. Comparison of the flora in the lower respiratory tract of patients [n (%)]

Variables	Acute exacerbation group (n=130)	Clinically stable group (n=78)	Test	χ^2	*p*
Gram negative bacteria			Chi-Square test	1.259	0.750
Pseudomonas aeruginosa	66 (50.77)	30 (38.46)			
Klebsiella pneumoniae	8 (6.15)	4 (5.13)			
Acinetobacter baumannii	15 (11.54)	5 (6.41)			
Other	5 (3.85)	4 (5.13)			
Gram positive bacteria			Chi-Square test	0.545	1.000
Staphylococcus aureus	3 (2.31)	4 (5.13)			
Streptococcus pneumoniae	2 (1.54)	3 (3.85)			
Other	1 (0.77)	3 (3.85)			
Fungi			Chi-Square test	0.551	0.911
Candida albicans	22 (16.92)	17 (21.79)			
Aspergillus flavus	5 (3.85)	4 (5.13)			
Other	3 (2.31)	4 (5.13)			

Table 5. Comparison of immunological markers in patients (mean ± SD)

Variables	Acute exacerbation group (n=158)	Clinically stable group (n=127)	Test	t	*p*	Mean Difference (95% CI)
$CD3^+$ T cells, /µL	635.66 ± 152.49	783.17 ± 234.65	Independent-samples t test	6.399	<0.001	-147.509 (-192.885,-102.132)
$CD4^+$ T cells, /µL	332.49 ± 92.33	532.13 ± 182.58	Independent-samples t test	11.974	<0.001	-199.640 (-232.458,-166.823)
$CD8^+$ T cells, /µL	298.66 ± 54.66	378.61 ± 73.41	Independent-samples t test	10.532	<0.001	-79.948 (-94.890, -65.006)
$CD4^+/CD8^+$ ratio	1.15 ± 0.40	1.48 ± 0.66	Independent-samples t test	5.148	<0.001	-0.325 (-0.450, -0.201)
WBC, $\times 10^9$/L	16.85 ± 4.28	9.46 ± 5.72	Independent-samples t test	12.482	<0.001	7.390 (6.225, 8.555)
NEUT, $\times 10^9$/L	9.88 ± 3.41	5.78 ± 2.91	Independent-samples t test	10.783	<0.001	4.110 (3.360, 4.860)

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Comparison of Pro-inflammatory Cytokines in Patients

Table 6 demonstrates the differences in inflammatory markers. The levels of pro-inflammatory cytokines were significantly elevated in the acute exacerbation group, including IL-6 (42.59 ± 15.78 vs. 17.28 ± 6.61 pg/mL), TNF-α (38.72 ± 11.24 vs. 13.53 ± 7.87 pg/mL), and IL-17A (142.88 ± 25.33 vs. 78.45 ± 13.42 pg/mL), with statistically significant differences between the groups (all p<0.001).

Comparison of Anti-inflammatory Cytokines in Patients

Table 7 compares the levels of anti-inflammatory factors. Notably, IL-4, a key cytokine associated with T-helper 2 (Th2) immune responses, was also significantly elevated during exacerbations. The results revealed that the levels of IL-10 (21.62 ± 6.51 vs. 9.32 ± 3.71 pg/mL) and IL-4 (105.88 ± 19.65 vs. 57.33 ± 14.85 pg/mL) were significantly higher in the acute exacerbation group compared to the stable group (both p<0.001).

Comparison of Reaction Proteins in Patients During Acute Phase

Table 8 compares the levels of inflammatory markers. The acute exacerbation group exhibited significantly higher levels of CRP (96.22 ± 37.81 vs. 34.25 ± 12.07 mg/L), PCT (0.10 ± 0.02 vs. 0.05 ± 0.01 ng/mL), and SAA (44.65 ± 12.13 vs. 25.69 ± 8.99 mg/L) compared to the stable group (all p<0.001). These findings indicate a more pronounced systemic inflammatory response in patients during the acute exacerbation phase.

Comparison of Chemokines in Patients

Table 9 shows that the level of MCP-1 in the acute exacerbation group of bronchiectasis (149.23 ± 26.75 ng/L) was significantly higher than that in the clinically stable group (97.52 ± 18.74 ng/L), with a statistically significant difference (p<0.001). This result suggests that MCP-1 may play an important role in the inflammatory process during acute exacerbations of bronchiectasis. Elevated MCP-1 levels indicate active monocyte/macrophage recruitment to the airways, which could contribute to sustained inflammation and tissue injury. Clinically, MCP-1 may serve as a potential biomarker for monitoring exacerbation severity and inflammatory burden.

Table 6. Comparison of pro-inflammatory cytokines in patients (mean ± SD)

Variables	Acute exacerbation group (n=158)	Clinically stable group (n=127)	Test	t	*p*	Mean Difference (95% CI)
IL-6, pg/mL	42.59 ± 15.78	17.28 ± 6.61	Independent-samples t test	16.908	<0.001	25.300 (22.355, 28.245)
TNF-α, pg/mL	38.72 ± 11.24	13.53 ± 7.87	Independent-samples t test	21.390	<0.001	25.180 (22.863, 27.497)
IL-17A, pg/mL	142.88 ± 25.33	78.45 ± 13.42	Independent-samples t test	25.890	<0.001	64.430 (59.531, 69.329)

CI: confidence interval; IL: interleukin; TNF-α: tumor necrosis factor α.

Table 7. Comparison of anti-inflammatory cytokines in patients (mean ± SD)

Variables	Acute exacerbation group (n=158)	Clinically stable group (n=127)	Test	t	*p*	Mean Difference (95% CI)
IL-10, pg/mL	21.62 ± 6.51	9.32 ± 3.71	Independent-samples t test	18.936	<0.001	12.290 (11.013, 13.568)
IL-4, pg/mL	105.88 ± 19.65	57.33 ± 14.85	Independent-samples t test	23.042	<0.001	48.550 (44.403, 52.697)

CI: confidence interval; IL: interleukin.

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Table 8. Comparison of reaction proteins in patients during acute phase (mean ± SD)

Variables	Acute exacerbation group (n=158)	Clinically stable group (n=127)	Test	t	*p*	Mean Difference (95% CI)
CRP, mg/L	96.22 ± 37.81	34.25 ± 12.07	Independent-samples t test	17.751	<0.001	61.970 (55.098, 68.842)
PCT, ng/mL	0.10 ± 0.02	0.05 ± 0.01	Independent-samples t test	23.977	<0.001	0.046 (0.043, 0.050)
SAA, mg/L	44.65 ± 12.13	25.69 ± 8.99	Independent-samples t test	14.684	<0.001	18.970 (16.427, 21.513)

CI: confidence interval; CRP: C-reactive protein; PCT: procalcitonin; SAA: serum amyloid A.

Table 9. Comparison of chemokines in patients (mean ± SD)

Variables	Acute exacerbation group (n=158)	Clinically stable group (n=127)	Test	t	*p*	Mean Difference (95% CI)
MCP-1, ng/L	149.23 ± 26.75	97.52 ± 18.74	Independent-samples t test	18.442	<0.001	51.710 (46.191, 57.229)

CI: confidence interval; MCP: monocyte chemoattractant protein.

DISCUSSION

Bronchiectasis is a chronic respiratory disease characterized by persistent airway inflammation and recurrent infections, yet its pathogenesis remains unclear.[27] This study analyzed the lower respiratory tract microbiome, serum cytokines, and chemokines in patients. Results show that microbiome dysbiosis, immune dysfunction, and enhanced inflammatory responses are interconnected and collectively drive disease pathogenesis. These findings confirm the chronic inflammatory nature of bronchiectasis and reveal that microbial dysbiosis with activation of the inflammatory cytokine network occurs during acute exacerbations, offering new insights into the transition from stable to exacerbation phase.

Clinical presentations of bronchiectasis exacerbations often overlap with those of chronic obstructive pulmonary disease (COPD) and asthma, complicating diagnosis. Shared features like elevated IL-6, TNF-α, and neutrophil activation blur distinctions, but bronchiectasis exacerbations are more frequently bacterial-driven with pronounced microbial dysbiosis. Comprehensive microbiological and inflammatory profiling is essential for accurate diagnosis and targeted therapy.

Acute exacerbations of bronchiectasis are challenging due to complex pathogens, rising antibiotic resistance, and symptom overlap with COPD and asthma, often necessitating broad-spectrum antimicrobial therapy that complicates treatment and disease control.[28] Terpstra et al[29] found that both Gram-negative bacteria, Gram-positive bacteria, and fungi could be detected during both acute exacerbation and stable phases in bronchiectasis patients, with mixed infections or unidentified pathogens being common phenomena. The findings of this study are consistent with these previous studies. Among the 158 patients in the acute exacerbation group, 103 (65.19%) had positive sputum cultures, while 58 (45.67%) of the 127 patients in the clinically stable group had positive sputum cultures, suggesting that pathogenic bacterial colonization and disruption of microbial homeostasis are prevalent in the respiratory tracts of patients during both acute exacerbation and stable phases. However, the positive sputum culture rate was higher in patients during the acute exacerbation phase compared to those in the stable phase. *Pseudomonas aeruginosa* is the most significant pathogenic threat in respiratory infections. Due to persistent chronic inflammation and impaired immune function, patients with bronchiectasis are highly susceptible to *P. aeruginosa* infection and struggle to resist its rapid proliferation.[30] This bacterium can

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directly damage airway epithelial cells and the basement membrane by secreting virulence factors such as elastase and alginate, leading to airway injury.[31] Additionally, *P. aeruginosa* can evade clearance by the host immune system by forming biofilms, thereby promoting chronic infection.[32] This study further classified the types of pathogens and revealed that *P. aeruginosa*, *Acinetobacter baumannii*, and *Candida albicans* were the most frequently detected pathogens. The overgrowth of these pathogens may disrupt the balance of the airway microbiota, triggering abnormal immune responses in the host. Notably, microbial dysbiosis is not merely a result of pathogenic infection but rather involves an imbalance in the overall microbial community structure.[33] During the stable phase of the disease, although the detection rate of pathogens is relatively low, patients may still harbor latent microbial colonization. At this stage, a relative balance exists between the host's immune defenses and microbial invasion. However, once this balance is disrupted, pathogens proliferate extensively, triggering an inflammatory cascade that ultimately leads to disease progression to the acute exacerbation phase. Therefore, the dynamic changes in the lower respiratory tract microbiome are closely associated with the evolution of bronchiectasis, and these changes may significantly impact disease progression. Cheng et al's study[34] conducted an in-depth longitudinal analysis of the lower respiratory tract microbiome in critically ill patients and found reduced microbial diversity in bronchiectasis patients, with opportunistic pathogens such as *P. aeruginosa* predominating. Hong et al[35] further pointed out that colonization by opportunistic pathogens in the lower respiratory tract can induce inflammatory responses, disrupt the airway mucosal barrier, and exacerbate airway damage. Furthermore, our antibiotic susceptibility analysis revealed a higher prevalence of multidrug-resistant (MDR) *P. aeruginosa* isolates during acute exacerbations compared to the stable phase. This elevated resistance pattern, particularly to fluoroquinolones and third-generation cephalosporins, aligns with previous reports highlighting the challenge of managing chronic *P. aeruginosa* infections in bronchiectasis, which are often associated with biofilm formation and long-term antibiotic exposure. The emergence of MDR strains during exacerbations may limit therapeutic options, potentially leading to prolonged infection, sustained inflammatory stimulation, and worse clinical outcomes. This underscores the necessity for routine antibiotic susceptibility testing in clinical practice to guide appropriate antimicrobial therapy and suggests that strategies aimed at preventing or eradicating *P. aeruginosa* colonization before the development of resistance could be crucial in managing bronchiectasis.

It is important to note that our microbiological analysis relied on conventional sputum culture, which has inherent limitations in detecting fastidious or anaerobic bacteria. Anaerobic bacteria, such as those from the genera *Prevotella*, *Fusobacterium*, and *Veillonella*, are increasingly recognized as potential contributors to airway inflammation and disease progression in bronchiectasis, particularly in cases associated with aspiration or impaired mucosal immunity.[36,37] These organisms are difficult to culture using standard aerobic techniques and may thrive in the hypoxic environment of damaged airways. Their absence in our culture results does not preclude their involvement in the pathophysiology of bronchiectasis. Future studies employing culture-independent methods, such as 16S rRNA gene sequencing or metagenomic sequencing, would provide a more comprehensive profile of the lower airway microbiome, including anaerobic and uncultivable species, and clarify their role in disease exacerbation and chronicity.

This study demonstrates that patients with bronchiectasis exhibit significant immune dysfunction, particularly during acute exacerbations. During these exacerbations, patients have lower $CD3^+$, $CD4^+$, and $CD8^+$ T cell counts and a reduced $CD4^+/CD8^+$ ratio compared to stable phases ($p<0.05$). Meanwhile, their peripheral blood WBC and NEUT levels are significantly elevated ($p<0.05$). These findings suggest that cellular immune function is impaired in patients with bronchiectasis, and that the immune system may be in a hyperactivated state. This imbalance may exacerbate airway inflammation and tissue damage.[38,39] As central cells in adaptive immunity, $CD4^+$ T cells regulate immune responses by secreting cytokines, and their reduced levels lead to weakened immune regulatory function, making it difficult to effectively control pathogen infections. Meanwhile, $CD8^+$ T cells possess cytotoxic effects, and their decreased levels may impair the clearance of infected cells, resulting in persistent pathogen colonization. A reduced $CD4^+/CD8^+$ ratio further reflects disrupted immune homeostasis, consistent with previous reports of immune senescence or exhaustion in patients with bronchiectasis.[40]

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Furthermore, our findings of reduced $CD3^+$, $CD4^+$, and $CD8^+$ T-cell counts along with a decreased $CD4^+/CD8^+$ ratio during acute exacerbations may also reflect underlying immunosenescence, particularly in older subgroups. Immunosenescence-the age-related decline in immune function-is characterized by T-cell exhaustion, reduced T-cell diversity, and chronic low-grade inflammation (inflammaging). Older individuals with bronchiectasis may be especially vulnerable to accelerated immune aging due to recurrent infections and chronic inflammation, which could further impair pathogen clearance and exacerbate disease progression. Although our study did not specifically analyze age-stratified immunosenescence markers, future investigations should consider evaluating senescent T-cell populations, telomere shortening, or senescence-associated cytokines to elucidate the contribution of immunosenescence to bronchiectasis pathophysiology, particularly in the elderly.

Furthermore, beyond the quantitative changes in $CD4^+$ and $CD8^+$ T cells, the functional balance between effector T cells and regulatory T cells (Tregs) is crucial for maintaining immune homeostasis. Tregs, a specialized subset of $CD4^+$ T cells characterized by the expression of the transcription factor FoxP3, play a critical role in suppressing excessive immune responses and preventing immunopathology. In the context of bronchiectasis, an imbalance between pro-inflammatory Th17 cells and Tregs has been implicated in the pathogenesis of chronic inflammatory diseases. A potential defect in Treg number or function could lead to a failure in adequately controlling the inflammatory response triggered by persistent bacterial colonization, thereby contributing to the sustained airway inflammation and tissue damage characteristic of bronchiectasis.[41] Conversely, persistent antigenic stimulation from chronic infection might also lead to an exhausted or dysfunctional Treg phenotype, further compromising immune regulation. Therefore, future investigations specifically assessing Treg frequency and function, alongside the Th17/Treg ratio, are warranted to elucidate their precise role in the immune dysfunction observed in bronchiectasis.

Additionally, the elevation in WBC and NEUT levels suggests a robust immune response during acute exacerbations. Although neutrophil infiltration can help eliminate pathogens, excessive accumulation may also contribute to airway inflammation and tissue damage through the release of neutrophil extracellular traps (NETs).[42] NETs are web-like structures composed of DNA, histones, and granular proteins that trap and kill pathogens. However, excessive NET formation can also contribute to epithelial injury, impair mucociliary clearance, and perpetuate inflammation by exposing autoantigens and activating further immune responses. In bronchiectasis, the high neutrophil burden observed during exacerbations may lead to increased NET release, which could amplify airway damage and facilitate chronic infection—thereby reinforcing the vicious vortex of inflammation and infection. Our findings of significantly elevated neutrophils and pro-inflammatory cytokines are consistent with a state of neutrophilic inflammation where NETosis may be upregulated. Notably, during acute exacerbations, the occurrence of hypoalbuminemia was significantly more frequent in patients than in the stable phase ($p<0.05$). Albumin is not only an important nutritional indicator but also possesses immunomodulatory and anti-inflammatory properties. Mechanistically, hypoalbuminemia may contribute to immune suppression through several pathways. Firstly, albumin binds and inactivates various pro-inflammatory mediators; its deficiency can lead to increased circulating levels of unbound inflammatory molecules, potentially exacerbating inflammation and contributing to immune cell exhaustion. Secondly, albumin is a primary carrier for hormones and essential molecules that support immune cell function; low levels may disrupt this transport, impairing lymphocyte activation and proliferation. Thirdly, severe hypoalbuminemia often reflects a systemic catabolic state and nutritional deficiency, which can directly suppress bone marrow production of immune cells and impair their effector functions. Finally, the resulting osmotic imbalance and potential endothelial dysfunction can further compromise the trafficking and homing of immune cells to sites of infection. Therefore, hypoalbuminemia may not merely be a marker of illness severity but could actively contribute to the observed immune dysfunction, creating a vicious cycle that hampers pathogen clearance and exacerbates disease progression in bronchiectasis. Therefore, correcting immune dysfunction may represent a critical therapeutic target in the management of bronchiectasis.

Moreover, the study revealed that during acute exacerbations, patients had significantly elevated levels of pro-inflammatory cytokines IL-6, TNF-α, and IL-17A compared to the stable phase, while anti-inflammatory cytokines IL-10 and IL-4 were notably reduced.

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Interestingly, IL-4, a canonical Th2 cytokine, was significantly elevated during exacerbations. These results suggest that patients with bronchiectasis may experience an exacerbation of inflammatory responses during acute exacerbations. Pro-inflammatory cytokines play a crucial role in the onset and progression of airway inflammation. IL-6 is a pleiotropic cytokine that promotes the activation and proliferation of inflammatory cells while also inducing the synthesis of acute-phase reactive proteins.[43] TNF-α is an important inflammatory mediator that can directly damage airway epithelial cells and promote the aggregation of other inflammatory cells.[44] IL-17A, primarily secreted by Th17 cells, induces the chemotaxis and activation of neutrophils, thereby exacerbating airway inflammation.[45] The anti-inflammatory cytokines IL-10 and IL-4 are crucial for modulating inflammatory responses. IL-10, primarily produced by regulatory T cells (Tregs), macrophages, and dendritic cells, plays a pivotal role in maintaining immune homeostasis by suppressing the activation of NF-κB and JAK-STAT signaling pathways in various immune cells. This suppression leads to decreased production of key pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β. Furthermore, IL-10 inhibits antigen presentation and co-stimulatory molecule expression on antigen-presenting cells, thereby dampening T-cell activation and Th1/Th17 responses, which are implicated in neutrophilic inflammation in bronchiectasis. A deficiency in IL-10 would disrupt this critical feedback inhibition, leading to uncontrolled activation of the innate and adaptive immune systems. This results in exaggerated and persistent neutrophilic airway inflammation, enhanced tissue damage via matrix metalloproteinases, and impaired bacterial clearance due to collateral damage to the airway epithelium and mucociliary apparatus. IL-4, on the other hand, primarily participates in Th2 immune responses and stimulates B cell proliferation and antibody production.[46] The observed elevation of IL-4 may reflect a compensatory mechanism attempting to counterbalance the excessive Th1/Th17-driven inflammation. Alternatively, it could indicate a distinct endotype of bronchiectasis characterized by a Th2-high inflammatory phenotype, which might be associated with specific clinical features or pathogen profiles. This Th2 skewing, while potentially modulatory in intent, could also contribute to disease pathology by promoting eosinophilic inflammation or altering antibody responses, warranting further investigation. In patients with bronchiectasis, reduced levels of anti-inflammatory cytokines may be insufficient to counterbalance the overproduction of pro-inflammatory cytokines, thereby exacerbating the inflammatory cascade. Additionally, this study observed markedly increased levels of acute-phase proteins (CRP, PCT, SAA) and the chemokine MCP-1 during acute exacerbations compared to stable phases. The rise in these biomarkers further supports intensified inflammatory activity in bronchiectasis exacerbations.[47] CRP, PCT, and SAA are commonly used inflammatory markers, and their elevated levels typically indicate the presence of an inflammatory response in the body. MCP-1 is an important chemokine that attracts monocytes and macrophages to aggregate at the site of inflammation, thereby exacerbating the inflammatory response. In patients with bronchiectasis, the elevation of MCP-1 levels may be associated with the colonization of respiratory pathogens and the activation of immune cells. The significant elevation of MCP-1 during acute exacerbations holds important clinical implications. As a key chemokine responsible for recruiting monocytes and macrophages to sites of inflammation, elevated MCP-1 levels likely reflect an intensified innate immune response aimed at controlling infection but also contributing to collateral tissue damage. Persistent MCP-1 elevation may perpetuate a chronic inflammatory state, facilitating the vicious cycle of infection and inflammation characteristic of bronchiectasis progression. From a clinical perspective, measuring MCP-1 levels could aid in identifying patients with heightened inflammatory activity, potentially predicting those at higher risk for frequent exacerbations or poorer outcomes.

Previous studies have also found that among patients with bronchiectasis, due to differences in pathogen infection characteristics and immune function levels, there is a high degree of heterogeneity in clinical symptoms, inflammatory cytokine levels, and immune cell changes.[48–51] However, most previous research has primarily focused on either the microbiome or inflammatory factors alone. In contrast, this study comprehensively analyzed the relationship between the lower respiratory tract microbiome and serum cytokine and chemokine levels, revealing their interactive roles in the pathogenesis of bronchiectasis. By examining the relationship between the lower respiratory tract microbiome, immune function, and inflammatory responses, we found that the colonization of respiratory

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pathogens may activate immune responses, leading to excessive release of inflammatory cytokines and thereby exacerbating airway inflammation and tissue damage. This perspective offers potential targets for future therapeutic strategies. Additionally, this study identified immune dysfunction abnormalities in patients during the acute exacerbation phase, a topic rarely explored in previous research. By analyzing immune function indicators, we further elucidated the pathophysiological changes in bronchiectasis patients during acute exacerbations, providing a more comprehensive understanding of the disease's pathogenesis.

These findings highlight the potential for personalized treatment in bronchiectasis. Significant patient heterogeneity in pathogen colonization, T-cell suppression, and cytokine elevation suggests that a one-size-fits-all approach is suboptimal. Personalized management could stratify patients by pathobiological endotypes: those with T-cell immunodeficiency and high P. aeruginosa burden may benefit from immunomodulatory therapy plus targeted anti-pseudomonal strategies; those with a hyperinflammatory phenotype may require aggressive anti-inflammatory interventions, including biologics. Monitoring serum chemokines like MCP-1 could guide anti-inflammatory treatment intensity. Future research integrating multi-omics with clinical data is needed to define endotypes and test tailored regimens. This precision medicine approach aims to modify disease course by targeting specific pathogenic drivers.

This study only analyzed a portion of serum cytokines and chemokines. Future research could further expand the scope of detection to comprehensively dissect the inflammatory response characteristics of bronchiectasis. Additionally, this study did not include analysis of viral pathogens, which are known to contribute significantly to acute exacerbations in bronchiectasis. The absence of viral detection (e.g., via PCR or metagenomic sequencing) limits our understanding of the full microbial landscape during exacerbations and its interaction with the host immune response. This study relied on conventional sputum culture for microbial analysis, which may underestimate microbial diversity and fail to detect fastidious or unculturable organisms. Additionally, this study did not conduct in-depth molecular biological analyses of the lower respiratory tract microbiome, nor did it formally assess the day-to-day reproducibility of the conventional sputum culture results obtained over the three-day collection period. Future studies could employ molecular techniques such as 16S rRNA gene sequencing or shotgun metagenomics to provide a more comprehensive profiling of the respiratory microbiome, including taxonomic composition, microbial community dynamics, and functional genetic potential. Such approaches could elucidate the role of commensals, pathogens, and microbial interactions in disease exacerbation and progression. Furthermore, future studies should consider incorporating radiological severity, such as distinguishing between unilateral and bilateral disease, as a key stratification factor. The extent of anatomical involvement may influence microbial load, host immune responses, and inflammatory cascades, potentially revealing distinct endotypes within the bronchiectasis population. Investigating these subgroups could provide deeper insights into personalized management strategies. Future research could incorporate therapeutic interventions to dynamically observe changes in the lower respiratory tract microbiome, immune function, and inflammatory responses, thereby further validating the conclusions of this study and offering more robust support for optimizing clinical treatment strategies.

In conclusion, by analyzing the relationship between the lower respiratory tract microbiome and serum cytokine and chemokine levels in bronchiectasis patients, this study revealed their interactive roles in the pathogenesis of the disease. The findings indicate that disturbances in the lower respiratory tract microbiome, abnormal immune function, and exacerbated inflammatory responses in bronchiectasis patients are interconnected and collectively contribute to the disease's pathogenesis. Future research can further delve into the interaction mechanisms among these factors and integrate therapeutic interventions to provide novel insights and approaches for the treatment of bronchiectasis.

STATEMENT OF ETHICS

This study was approved by the Ethics Committee of The Second Affiliated Hospital of Jiaxing University (Approval No.: 2024-205).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Not applicable.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

AI ASSISTANCE DISCLOSURE

Not applicable.

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