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Comparative analysis of non-tuberculous mycobacterial lung disease and lung colonization: a case-control study

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Abstract

Background Non-tuberculous mycobacteria (NTM) are common opportunistic pathogens, and the most common infection site is lung. NTM are found commonly in the environment. Many patients have NTM lung colonization (NTM-Col). NTM lung disease (NTM-LD) have no specific sympotms, though it is hard to differentiate NTM-LD and NTM-Col under this circumstance. The aim of this study is to explore the differences between NTM-LD and NTM-Col for future clinical diagnosis and treatment.

Methods We retrospectively enrolled patients who had a history of NTM isolated from respiratory specimens in Peking Union Medical College Hospital (PUMCH) from January 1st, 2013 to December 31st, 2022. Patients were classified into NTM-LD group and NTM-Col group. Demographic characteristics, clinical manifestations, laboratory tests and imaging findings of the two groups were compared. Comparative analysis was also performed in peripheral blood lymphocyte subsets among three groups.

Results A total of 127 NTM-LD patients and 37 NTM-Col patients were enrolled. Proportion of patients with bronchiectasis was higher in NTM-LD group than in NTM-Col group ($P=0.026$). Predominant NTM isolates were Mycobacterium avium complex (MAC). NTM-LD group had a higher proportion of Mycobacterium intracellulare ($P=0.004$). $CD4^+$T cells counts was lower in NTM-LD group ($P=0.041$) than in NTM-Col group. Imaging finding of bronchiectasis ($P=0.006$) was higher in NTM-LD group than in NTM-Col group. Imaging findings of bronchiectasis (OR = 6.282, $P=0.016$), and $CD4^+$T cell count (OR = 0.997, $P=0.012$) were independent associated factors for differential diagnosis between NTM-LD and NTM-Col.

Conclusion NTM isolates from both NTM-LD and NTM-Col patients were predominantly MAC, with a higher Mycobacterium intracellulare isolation rate in NTM-LD group. Imaging findings of bronchiectasis and lower peripheral blood $CD4^+$T cell count may be helpful to separate the diagnosis of NTM-LD from NTM-Col.

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Keywords Non-tuberculous mycobacteria, NTM Lung disease, NTM lung colonization, Associated factors

Introduction

Non-tuberculous mycobacteria (NTM) includes mycobacterial species other than Mycobacterium tuberculosis complex (MTBC) and Mycobacterium leprae [1]. NTM strains have certain heterogeneity [2]. Mycobacterium abscessus have a higher pathogenic potential, while Mycobacterium gordonae generally lack pathogenicity [3, 4]. NTM is widely present in soil, water, air, and also a common opportunistic pathogen isolated from the human respiratory tract [5]. Some people have colonized NTM flora within respiratory environment [6]. NTM lung disease (NTM-LD) is mainly found in hosts with pulmonary anatomical abnormalities or with immune or genetic disorders [7, 8]. Immunosuppressants, glucocorticoids, azithromycin, and proton pump inhibitors can increase the susceptibility to NTM-LD. Epidemiological survey studies in mainland China have shown that NTM-LD accounted for 6.8% of sputum anti-acid smear-positive patients, while NTM colonization (NTM-Col) or contamination accounted for 1.0% [9].

NTM isolated from airway secretions, along with symptoms such as fever and cough, and pulmonary imaging showing corresponding imaging findings such as bronchiectasis and cavitation, are commonly associated with NTM-LD. Not all NTM-LD cases present with typical clinical features, and infections with other pathogens can also present similar symptoms. The presence of NTM-Col can further complicate the diagnostic process [10–12]. On the other hand, although a portion of NTM-Col patients may develop NTM-LD, many will have spontaneous clearance of NTM from the sputum [13]. For these reasons, differential diagnosis between NTM-LD and NTM-Col would be helpful to doctors in clinical decision-making [14, 15]. However, this is still a challenge.

To explore associated factors for differential diagnosis between NTM-LD and NTM-Col, we conducted a single-center, cross-sectional study to analyze clinical characteristics, laboratory tests, lymphocyte subsets and chest CT imaging manifestations of these two groups.

Methods

Study population

This study was approved by the institutional ethics committee of Peking Union Medical College Hospital (PUMCH). We retrospectively enrolled patients with positive NTM isolates from respiratory specimens in PUMCH from January 1st, 2013 to December 31st, 2022. Eligible respiratory specimens included sputum, bronchial lavage fluid, and bronchoalveolar lavage fluid. The real-time quantitative PCR was applied to detect the DNA of NTM, and then the gene chip method was further used for the identification of NTM species. Based on clinical diagnoses (see diagnostic criteria section), patients were classified into two groups: those with NTM-LD and those with NTM-Col. Exclusion criteria: (a) Disseminated NTM infection; (b) Clinical data is insufficient to confirm whether NTM is pathogenic, or there is a possibility of NTM specimen contamination.

In accordance with a 1:1 ratio with the NTM-LD group, random sampling of the healthy population is conducted, matching for age and gender. The healthy control group samples need to meet the following criteria: (a) No symptoms such as fever, cough, expectoration, and hemoptysis; (b) No active lesions in chest imaging; (c) Lymphocyte subsets results can be queried. Exclusion criteria: (a) Malignancies or severe organ injury; (b) any acute illness within the past three months; (c) Long-term use of steroids, immunosuppressive drugs or biological agents.

Diagnostic criteria

The diagnosis of NTM-LD must meet the following criteria: (a) respiratory or systemic symptoms; (b) radiologic nodular or cavitary opacities on chest radiograph, or a CT scan that shows bronchiectasis with multiple small nodules, with appropriate exclusion of other diagnoses; (c) microbiologic criteria, including (i) Positive culture results from at least two separate expectorated sputum samples. If the results are nondiagnostic, consider repeating sputum acid-fast bacillus (AFB) smears and cultures or (ii) Positive culture results from at least one bronchial wash or lavage or (iii) Transbronchial or other lung biopsy with mycobacterial histologic features (granulomatous inflammation or AFB) and positive culture for NTM or biopsy showing mycobacterial histologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washings that are culture positive for NTM [10, 14, 16].

NTM-Col is defined as follows: There are no respiratory or systemic symptoms caused by NTM-LD, nor any radiographic manifestations associated with NTM-LD, or symptoms and radiographic manifestations can be explained by underlying disease and there is no NTM-LD upon long-term follow-up. Meanwhile, individuals with NTM-Col must meet one of the following conditions: (a) A single sputum specimen with positive NTM culture or molecular biology test, and the identified species is not a commonly contaminated NTM species (Mycobacterium gordonii, Mycobacterium haemophilus and Mycobacterium mucogenicum); (b) At least two separate sputum samples are positive for NTM culture or molecular biology test and are identified as the same organism; (c) At

least one bronchial la vage or bronchoalveolar lavage sample is positive for NTM culture or molecular biology test.

For patients with concurrent pneumonia, or those with difficulty to determine the status of NTM-LD or NTM-Col, two infectious disease specialists should make independent judgments. If their opinions were inconsistent, a third infectious disease specialist should be consulted for assessment.

Clinical information collection

Clinical information such as demographic data, clinical presentation and laboratory tests were extracted from medical records. The subject identification code list and case report form were filled out after extracting the information. The most recent lung CT image within three months before the positive culture was assessed using the modified Reiff scoring system [17, 18]. The system independently scores each lung lobe (the lingual segment of the left lung is regarded as a separate part) with a maximum total score of 18 points and a minimum score of 0 points. The scoring criteria for each lung lobe were as follows: no bronchiectasis 0 points, tubular bronchiectasis 1 point, variceal bronchiectasis 2 points, and cystic bronchiectasis 3 points. For patients who had previously undergone lobectomy, the score of the remaining n lung lobes (parts) was calculated and multiplied by 6/n. Cavities, nodules, consolidations, patchy cords, and ground-glass opacities were also evaluated separately by two infectious disease specialists and one radiologist.

Statistical anaylsis

Statistical analysis were performed with SPSS 27.0 software. The Kolmogorov-Smirnov test was used to identify the normality of continuous data. Numerical variables with normal distributions were presented as mean ± standard deviation, and between-group comparisons were conducted using the t-test. Non-normally distributed continuous data were presented as median and interquartile range [IQR], and between-group comparisons were performed using the Mann-Whitney U test or Kruskal-Wallis H test. Categorical data were presented as frequency (percentage), and between-group comparisons were conducted using the chi-square test or Fisher's exact test. Variables with $P<0.05$ in the univariate analysis were included in the binary logistic regression analysis (backward elimination method, stepwise removal of variables with $P>0.1$). $P<0.05$ was considered statistically significant.

Results

Characteristics of the study population

Retrieving the medical database of PUMCH, a total of 1277 cases were identified from inpatients and outpatients from January 1st, 2013 to December 31st, 2022, which tested positive for mycobacterium culture or had a discharge diagnosis related to mycobacterium. After checking the clinical data, 164 eligible patients were enrolled. They were divided into two groups according to the diagnostic criteria: 127 patients with NTM-LD and 37 patients with NTM-Col. A total of 127 individuals were included in the healthy control group (Fig. 1).

General characteristics of enrolled patients are shown in Table 1. The proportion of patients with NTM-LD combined with bronchiectasis was higher than that in the NTM-Col group (35.4% vs. 16.2%, $P=0.026$). There were no statistically significant difference in other demographic characteristics or underlying diseases between the two groups. Both the NTM-LD group and the NTM-Col group showed a relatively higher prevalence in females and older patients (≥65 years old).

In the healthy control group, there were 53 males (41.7%), with an average age of 55 ± 15 years. There was no significant difference in age and gender distribution between the NTM-Col group and the healthy control group, which allowed for further pairwise analysis between the groups.

Clinical manifestations

NTM-LD was mainly characterized by a chronic course of illness, with a median duration of 20 months from onset to diagnosis. Symptoms included cough (113, 89%), sputum production (103, 81.1%), fever (75, 59.1%), shortness of breath (40, 31.5%), hemoptysis (34, 26.8%), fatigue (31, 24.4%), weight loss which was defined as ≥10% decrease of original weight (29, 22.8%), poor appetite (20, 15.7%), chest pain (16, 12.6%) and night sweats (7, 5.5%). As for physical signs, 15 patients (11.8%) exhibited signs of pleural effusion. There are 18 patients (14.2%) had wet rales, and 2 patients (1.6%) had dry rales.

Microbiological examination

Among the NTM-LD group, 37 cases (37/124, 29.8%) were acid-fast staining positive, with a total of 66 positive acid-fast staining instances (66/439, 15.0%); 118 cases (118/124, 95.2%) were positive in mycobacterial culture, with a total of 255 positive culture instances (255/434, 58.8%); molecular biology testing was positive in 110 cases (110/124, 88.7%), with a total of 179 positive instances (179/413, 43.3%). In the NTM-Col group, 1 case (1/34, 2.9%) was acid-fast staining positive, with a total of 1 positive acid-fast staining instance (1/137, 0.8%); mycobacterial culture showed positive results in 36 cases (36/37, 97.3%), with a total of 39 positive culture instances (39/110, 32.8%); molecular biology testing yielded positive results in 24 cases (24/35, 68.6%), with a total of 28 positive instances (28/95, 43.3%).

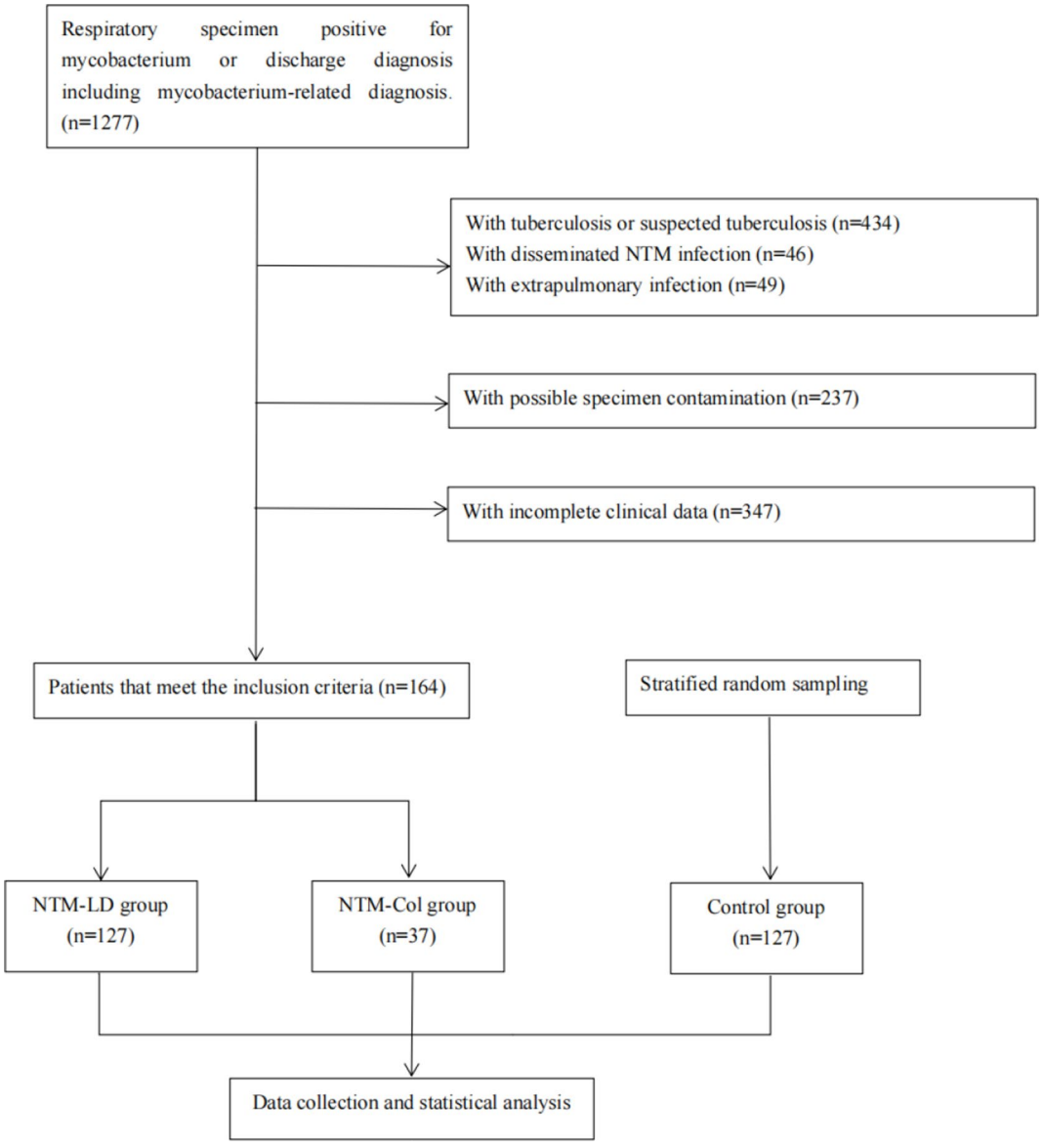

Fig. 1 Flowchart of the study. NTM-LD: NTM lung disease; NTM-Col: fi NTM colonization

The composition of NTM species in the NTM-LD group and NTM-Col group is shown in Table 2. The proportion of MAC (Mycobacterium avium complex) isolates in the NTM-LD group was significantly higher than in the NTM-Col group (55.1% vs. 29.7%, $P=0.006$). After further identification of MAC, there was a higher proportion of Mycobacterium intracellulare isolates in the NTM-LD group (38.6% vs. 13.5%, $P=0.004$), while there was no significant difference in the proportion of Mycobacterium avium isolates between two groups (12.6% vs. 16.2%, $P=0.573$).

Laboratory examination

Routine laboratory tests

The laboratory test results for the NTM-LD group and NTM-Col group are shown in Table 3. The levels of hemoglobin and albumin were higher in the NTM-LD group. There were no significant differences in other routine laboratory tests between the two groups. Patients in both the NTM-LD group and the NTM-Col group did not exhibit a definitive trend of decreased blood cell count or impairment of liver and kidney function. Immunoglobulin levels were within the normal range for both groups, but elevated levels of high-sensitivity C-reactive protein (hsCRP), erythrocyte sedimentation rate (ESR), and ferritin (Fer) were observed in both groups, indicating a high inflammatory state.

Table 1 General characteristics of NTM-LD group and NTM-Col group

	NTM-LD (*n* = 127)	NTM-Col (*n* = 37)	*P* value
Male, n/%	53(41.7)	12(32.4)	0.312
Age, year, mean ± sd	55 ± 16	60 ± 19	0.080
Advanced age (≥ 65), n/%	39(30.7)	16(40.2)	0.157
Postmenopausal women, n/%	42(33.1)	11(29.7)	0.654
Underlying disease			
Rheumatic diseases, n/%	27(21.3)	12(32.4)	0.222
Solid organ tumors, n/%	8(6.3)	3(8.1)	0.785
Hematological malignancies, n/%	3(2.4)	2(5.4)	0.372
HIV infection, n/%	1	0	-
Underlying lung disease, n/%	81(63.8)	20(54.1)	0.175
Bronchiectasis, n/%	45(35.4)	6(16.2)	**0.026**
COPD, n/%	15(11.8)	6(16.2)	0.483
Asthma, n/%	8(6.3)	2(5.4)	0.835
Interstitial lung disease, n/%	19(15.0)	6(16.2)	0.867
Congenital disease, n/%	4[a] (3.1)	1[b] (2.7)	0.885
Others, n/%	6[c] (0.8)	3[d] (8.1)	-
Medication history			
GCs, n/%	21(16.5)	8(21.6)	0.479
GCs dose, mg/d, prednisone equivalent, median(IQR)	20.0(0,40.0)	19.5(15.6,20.0)	0.905
Immunosuppressants, n/%	17(13.4)	6(16.2)	0.665
Biological agents, n/%	3(2.4)	0	0.347
GSs inhalation, n/%	2(1.6)	1(2.7)	0.655
PPI, n/%	7(5.5)	2(5.4)	0.980
Chemotherapy history, n/%	5(3.9)	2(5.4)	0.889
Chest radiotherapy history, n/%	2(1.6)	1(2.7)	0.664
Smoking, n/%	31(24.4)	8(21.6)	0.728

Abbreviations NTM-LD: NTM lung disease; NTM-Col: NTM colonization; COPD: chronic obstructive pulmonary disease; PPI = Proton Pump Inhibitor

Immunosuppressants included Cyclophosphamide (CTX), Cyclosporine A (CsA), Tacrolimus (fk506), Methotrexate (MTX), Leflunomide(LEF), Mycophenolate mofetil (MMF), Azathioprine (AZA),

Notes a. The NTM-LD group includes 2 cases of primary ciliary dyskinesia, 1 case of pulmonary sequestration, and 1 case of congenital multiple pulmonary cysts

b. The NTM-Col group includes 1 case of pulmonary developmental abnormalities

c. Other underlying lung conditions in the NTM-LD group include 3 cases of lung cancer, 1 case of Behçet's disease complicated by bilateral multiple pulmonary artery occlusion, 1 case of ANCA-associated vasculitis with diffuse lung parenchymal lesions, and 1 case of renal anti-glomerular basement membrane disease involving the lungs

d. Other underlying lung conditions in the NTM-Col group include 2 cases of vasculitis-associated diffuse lung parenchymal lesions and 1 case of chronic pulmonary embolism

f. The use of biological agents in the NTM-LD group includes 1 case of Pembrolizumab, 1 case of Vedolizumab, and 1 case of Cabozantinib

T-SPOT.TB

The T-SPOT.TB results for 76 NTM-LD patients and 22 NTM-Col patients are shown in Fig. 2. A total of 21 cases (27.6%) in the NTM-LD group tested positive for T-SPOT.TB. The median number of spot-forming cells (SFCs) for ESAT-6 was 80/106 peripheral blood mononuclear cell (PBMC) and was 64/106 PBMC for CFP-10. A total of 4 cases (18.2%)in the NTM- Col group tested positive for T-SPOT.TB. The median number of SFCs for ESAT-6 was 260/106 PBMC and was 116/106 PBMC for CFP-10. There were no significant differences in positivity rate or the number of SFCs between two groups. Within the MAC subgroup, there was also no statistically significant difference in the T-SPOT.TB positivity rate between NTM-LD and NTM-Col patients.

Peripheral blood lymphocyte subsets

The lymphocyte subsets of NTM-LD group and NTM-Col group are shown in Table 4. The $CD4^+$ T cell count of the NTM-LD group was lower than that of the NTM-Col group (441/μL vs. 626/μL, $P=0.041$). There were no significant difference in the values or proportions of other lymphocyte subsets between these two groups. The NTM-LD group had widespread reduced counts of various lymphocyte subsets. The NTM-LD group had lower B cell ($P\leq0.001$), NK cell ($P=0.001$), T cell ($P=0.01$), $CD4^+$ T cell ($P\leq0.001$), $45RA^+CD4^+$ T cell ($P=0.016$), naïve $CD4^+$ T cell ($P=0.023$), $CD8^+CD28^+$ T cell proportion ($P=0.013$), and CD4/CD8 ratio ($P\leq0.001$) compared to healthy controls, but the $CD8^+CD38^+$ T cell proportion of the NTM-LD group was higher than that of the healthy controls ($P\leq0.001$).

Table 2 The composition of NTM species in the NTM-LD group and the NTM-Col group

NTM species	NTM-LD (*n* = 127)	NTM-Col (*n* = 37)	*P* value
MAC, n/%	70 (55.1)[a]	11 (29.7)	**0.006**
Mycobacterium avium, n/%	16 (12.6)	6 (16.2)	0.573
Mycobacterium intracellulare, n/%	49 (38.6)	5 (13.5)	**0.004**
Mycobacterium chelonae/Mycobacterium abscessus complex, n/%	23 (18.1)[b]	3 (8.1)	0.144
Mycobacterium chelonae, n/%	1 (0.8)	-	-
Mycobacterium abscess, n/%	8 (6.3)	-	-
Mycobacterium kansasii, n/%	7 (5.5)	3 (8.1)	0.564
Other rapidly growing NTM, n/%	6 (4.7)[c]	5 (13.5)[d]	-
Other slow growing NTM, n/%	4 (3.1)[e]	7 (18.9)[f]	-
Unknown NTM, n/%	17 (13.4)	8 (21.6)	-
Culture for less than 7 days, n/%	5 (3.9)	1 (2.7)	-
Culture for more than 7 days, n/%	5 (3.9)	6 (16.2)	-

Abbreviations NTM: Non-tuberculous mycobacteria; NTM-LD: NTM lung disease; NTM-Col: NTM colonization; MAC: *Mycobacterium avium complex*

Notes a. A total of 70 patients in the NTM-LD group were infected with MAC. Among them, 16 cases were definitively identified as *Mycobacterium avium*, 49 cases were definitively identified as *Mycobacterium intracellulare*

b. A total of 21 patients in the NTM-LD group were infected with *Mycobacterium chelonae/Mycobacterium abscessus* complex, with 1 case was definitively identified as *Mycobacterium chelonae*, 8 cases definitively identified as *Mycobacterium abscessus*

c. Other rapidly growing NTM in the NTM-LD group included 4 cases of *Mycobacterium fortuitum*, 1 case of *Mycobacterium wolinskyi*, and 1 case of *Mycobacterium madridense*

d. Other rapidly growing NTM in the NTM-Col group included 2 cases of *Mycobacterium fortuitum*, 2 cases of *Mycobacterium porcinum*, and 1 case of *Mycobacterium flavescens*

e. Other slow-growing NTM in the NTM-LD group included 2 cases of *Mycobacterium kansasii*, 1 case of *Mycobacterium xenopi*, and 1 case of *Mycobacterium intermedium*

f. Other slow-growing NTM in the NTM-Col group included 2 cases of *Mycobacterium scrofulaceum*, 1 case of *Mycobacterium xenopi*, 1 case of *Mycobacterium gordoniae*, 2 cases of *Mycobacterium paracondontium*, and 1 case of *Mycobacterium simiae*

Chest imaging characteristics and comparisons

The prevalence of bronchiectasis (74.8% vs. 51.4%, $P=0.006$) and the severity which measured by modified Reiff score (2 vs. 1, $P=0.011$) in the NTM-LD group is significantly higher than that in the NTM-Col group. We also evaluated other chest imaging performance relevant to NTM infection, including cavity, consolidation, patchy opacity, ground-glass opacity and nodule. There is no significant statistical difference in the occurrence rates of cavity (15/127, 11.8% vs. 3/37, 8.1%, $P=0.529$), consolidation (39/127, 30.7% vs. 12/37, 32.4%, $P=0.843$), patchy opacity (90/127, 70.9% vs. 28/37, 75.7%, $P=0.569$), ground-glass opacity (44/127, 34.6% vs. 15/37, 40.5%, $P=0.514$) and nodule (83/127, 64.5% vs. 27/37, 73%, $P=0.389$) between the two groups.

Multivariate analysis of factors associated with differential diagnosis between NTM-LD and NTM-Col

Based on the univariate analysis, which showed statistical significance for variables such as hemoglobin (HGB) concentration, albumin (ALB) concentration, bronchiectasis signs on chest imaging, and $CD4^+$ T cell count, a multivariable logistic regression analysis was conducted. No clear collinearity was found among the variables. The multivariable analysis revealed that imaging findings of bronchiectasis (OR=6.282, 95%CI: 1.417–27.845, $P=0.016$) and $CD4^+$ T cell count (OR=0.997, 95%CI: 0.995–0.999, $P=0.012$) were independent factors associated with differential diagnosis between NTM-LD and NTM-Col (Table 5).

Discussion

In our study, we found that bronchiectasis was more common in the NTM-LD group than that in the NTM-Col group. Previous studies have found that bronchiectasis is a risk factor for NTM-LD [9, 19, 20]. Furuuchi et al. found that the severity of bronchiectasis [HR=1.32(1.14–1.53), $P=0.001$] and decreased lymphocyte count (<1000/μL) [HR=2.33(1.29–4.22), $P=0.005$] were independent risk factors for recurrence after NTM-LD treatment [21]. Additionally, NTM infection itself may also cause bronchiectasis [22, 23]. Garcia et al. found that NTM-LD group had a higher proportion and more severe bronchiectasis lesions compared to NTM-Col group (17.5% vs. 0%, $P=0.016$). Low body mass index, fever at admission, pulmonary infiltrates, and cavitary lesions were also identified as independent factors associated with NTM-LD [24]. Our study further supports the association between bronchiectasis and NTM-LD. Screening and following up of bronchiectasis will contribute to prevention of NTM-LD [25].

Immunodeficiency is also a risk factor for NTM-LD. The NTM-LD patients in our study had clear evidence of immune dysfunction, showing an overall reduction in lymphocyte subsets. The numbers of B cells, NK cells, T cells, and $CD4^+$ T cells of NTM-LD group in our study were lower than those in healthy controls group, indicating potential compromised innate and acquired immune responses in NTM-LD patients. Previous studies have supported the reduction and dysfunction of $CD4^+$ T cells in patients with NTM-LD. Wobma et al. found that a reduced number of $CD4^+$ T cells is a risk factor for NTM disease in children after stem cell transplantation [26]. Han et al. found increased expression of the co-inhibitory molecules programmeddeath-1 (PD-1), cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and T cell immunoglobulin and mucin domain-3 (TIM-3) in peripheral blood circulating $CD4^+$ T cells of MAC lung disease patients [27].

Table 3 Laboratory examination of patients in the NTM-LD group and NTM-Col group

Laboratory examination	NTM-LD(*n* = 127)	NTM-Col(*n* = 37)	*P* vaule	Normal range
WBC, 10^9/L	6.76(5.19,8.71)	6.87(4.89,10.07)	0.769	3.50–9.50
LYM, 10^9/L	1.47(1.09,1.92)	1.20(0.77,1.72)	0.051	0.8-4.00
NEUT, 10^9/L	5.70 ± 4.19	6.25 ± 4.40	0.499	2.0-7.50
MONO, 10^9/L	0.39(0.28,0.49)	0.44(0.27,0.62)	0.360	0.12–0.80
HGB, g/L	126.9 ± 24.7	111.8 ± 29.0	0.006	110–150
PLT, 10^9/L	274 ± 217	221 ± 118	0.166	100–350
ALT, U/L	15.0(11.0,22.5)	13.5(9.0,23.3)	0.533	7–40
AST, U/L	21.0(17.0,26.0)	21.0(16.5,30.5)	0.906	15–35
GGT, U/L	65.1 ± 107.3	44.5 ± 39.7	0.109	7–45
ALB, g/L	38.5 ± 5.9	35.8 ± 6.0	0.024	35–52
TBIL, umol/l	11.2 ± 6.6	11.7 ± 5.1	0.703	5.1–22.2
DBIL, umol/l	4.1 ± 3.6	4.6 ± 3.3	0.505	0-6.8
LDH, U/L	241.9 ± 137.9	253.8 ± 136.1	0.676	0-250
Cr, umol/l	75.6 ± 65.0	100.5 ± 105.4	0.199	45–84
ESR, mm/h	37.8 ± 34.2	39.8 ± 34.7	0.782	0–20
hsCRP, mg/l	5.9(1.8,37.3)	7.3(1.9,65.7)	0.537	<3.00
Fer, ug/L	306.0(144.0,658.0)	317.5(152.7,583.7)	0.980	14–307
IgA, g/L	2.48(1.67,3.33)	2.28(1.36,2.82)	0.246	0.70-4.00
IgG, g/L	13.79 ± 5.45	12.66 ± 6.87	0.387	7.00–17.00
IgM, g/L	1.00(0.70,1.53)	0.85(0.52,1.41)	0.166	0.40–2.30

Abbreviations NTM: Non-tuberculous mycobacteria; NTM-LD: NTM lung disease; NTM-Col: NTM colonization; WBC: White blood cell; LYM: Lymphocyte; NEUT: Neutrophil; MONO: Monocyte; HGB: Hemoglobin; PLT: Platelet; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; ALB: Albumin; TBIL: Total bilirubin; DBIL: Direct bilirubin; LDH: Lactate dehydrogenase; Cr: Creatinine; ESR: Erythrocyte sedimentation rate; hsCRP: High-sensitivity C-reactive protein; Fer: Ferritin; IgA: Immunoglobulin A; IgG: Immunoglobulin G; IgM: Immunoglobulin M

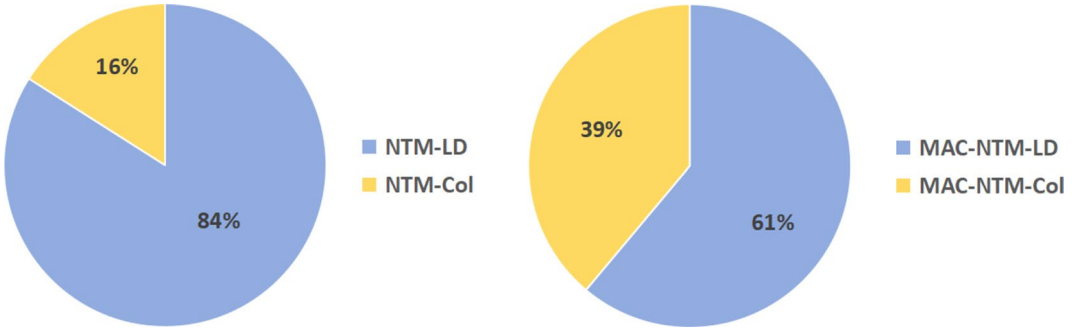

Fig. 2 T-SPOT.TB results in the NTM pulmonary group and NTM colonization group. *Abbreviations* NTM: Non-tuberculous mycobacteria; NTM-LD: NTM lung disease; NTM-Col: NTM colonization; MAC: *Mycobacterium avium complex*

Our study found that a decreased count of $CD4^+$ T cells is an independent risk factor for separating the diagnosis of NTM-LD from NTM-Col. There is few research comparing lymphocyte subsets between patients with NTM-LD and NTM-Col. Pan et al. found that peripheral blood $CD4^+$ T cells and $CD8^+$ T cells in NTM-LD patients had higher expression of TIM-3 molecule on their surface than in individuals with NTM-Col. Surface TIM-3 on these cells has been shown to be associated with cell apoptosis and attenuation of specific cytokines. The number of TIM-3-expressing T cells was found to be higher in patients with lower BMI, advanced disease, and higher bacterial load, and decreased after treatment [28, 29]. The reduction of $CD4^+$ T cells may increase susceptibility to NTM-LD, but it cannot be ruled out the possibility that disease itself leads to of $CD4^+$ T cells depletion. In addition, the number of $CD4^+$ T cells in peripheral blood does not solely reflect pulmonary lymphocyte infiltration. Further researches are needed. Additionally, the proportion of $CD8^+CD38^+$ T cells was higher in the NTM-LD group compared to healthy controls, indicating the presence of activated $CD8^+$ T cell subset, although their specific roles require further study.

There are some limitations of this study. Firstly, it is a single-center retrospective observational study with a relatively small sample size, especially in the NTM-Col group, which may lack statistical power to detect existing differences. Secondly, the NTM-Col group in our study only enrolled individuals with multiple positive microbiological results or those with identified NTM species

Table 4 Distribution of peripheral blood lymphocyte subsets in the NTM-LD group, NTM-Col group and control group

Peripheral blood lymphocyte subsets	NTM-LD(*n* = 127)	NTM-Col(*n* = 37)	Control(*n* = 127)	Pa value*	Pb value*	Pc value*
LYM, /μL	1512 ± 661	1431 ± 933	1642 ± 413	0.561	0.071	0.202
B cell, /μL	87(49,182)	119(76,229)	180(128,247)	0.111	**≤ 0.001**	0.101
NK cell, /μL	159(65,331)	125(63,214)	252(191,342)	0.342	**0.00**1	**≤ 0.001**
T cell, /μL	972 ± 505	1274 ± 652	1154 ± 343	0.057	**0.010**	0.511
$CD4^+$T cell, /μL	441 (262,689)	626(344,1148)	662(486,812)	**0.041**	**≤ 0.001**	0.952
$CD8^+$T cell, /μL	346 (233,552)	505 (281,644)	378(288,481)	0.250	0.72	0.106
Memory $CD4^+$T cell, /μL	262 (148,343)	465 (170,745)	407 (319,515)	0.112	**≤ 0.001**	0.871
$45RA^+CD4^+$T cell, /μL	114 (38,216)	178 (98,340)	219 (119,343)	0.116	**≤ 0.001**	0.807
naïve $CD4^+$T cell, /μL	101 (33,199)	174 (96,331)	201 (101,316)	0.085	**≤ 0.001**	0.935
$CD4^+CD28^+$T cell/$CD4^+$T cell, %	96.6(85.5,98.2)	97.6(86.2,98.5)	97(90.7,99.0)	0.634	0.103	0.605
$CD8^+CD28^+$T cell/$CD8^+$T cell, %	48.1(26.1,67.3)	60.8(38.8,77.0)	57.9(45.1,72.3)	0.149	**0.013**	0.782
$CD8^+DR^+$ cell/$CD8^+$T cell, %	46.3 ± 21.7	40.9 ± 24.7	40.9 ± 17.5	0.431	0.093	0.995
$CD8^+CD38^+$T cell/$CD8^+$T cell, %	54.1 ± 22.2	57.1 ± 16.7	31.2 ± 12.3	0.647	**≤ 0.001**	**≤ 0.001**
$CD4^+$ T cell/$CD8^+$ T cell	1.31(0.78,1.88)	1.47(1.12,2.31)	1.71(1.27,2.25)	0.166	**≤ 0.001**	0.650

Abbreviations NTM: Non-tuberculous mycobacteria; NTM-LD: NTM lung disease; NTM-Col: NTM colonization

Notes * *Pa* represents the *P* value comparing the NTM-LD group to the NTM-Col group, *Pb* represents the *P* value comparing the NTM-LD group to the control group, and *Pc* represents the *P* value comparing the NTM-Col group to the control group

Table 5 Logistic analysis of factors associated with differential diagnosis between NTM-LD and NTM-Col

	Univariate analysis		Multivariable analysis	
	OR (95%CI)	***P* value**	**OR (95%CI)**	***P* value**
HGB concentration	1.024 (1.008–1.039)	0.003	-	-
ALB concentration	1.074 (1.008–1.145)	0.027	-	-
History of bronchiectasis	2.835 (1.100-7.308)	0.031	-	-
Imaging findings of bronchiectasis	2.784 (1.274–6.084)	0.010	6.282 (1.417–27.845)	0.016
NTM identified as MAC	2.903 (1.321–6.376)	0.008	3.700 (0.784–17.460)	0.098
The count of $CD4^+$T cell	0.998 (0.996-1.000)	0.020	0.997 (0.995–0.999)	0.012

Abbreviations NTM: Non-tuberculous mycobacteria; NTM-LD: NTM lung disease; NTM-Col: NTM colonization; HGB: hemoglobin; ALB: albumin; MAC: *Mycobacterium avium* complex

excluding commonly contaminated NTM species; some individuals with NTM-Col who had only one positive microbiological test may not have been enrolled in the study.

Conclusion

In conclusion, NTM isolates from both NTM-LD and NTM-Col patients were predominantly MAC, Mycobacterium chelonae/Mycobacterium abscessus complex, and Mycobacterium kansasii, with a higher Mycobacterium intracellulare isolation rate in the NTM-LD group. Imaging findings of bronchiectasis and lower peripheral blood $CD4^+$ T cell count may be helpful to separate the diagnosis of NTM-LD from NTM-Col.

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Author contributions
XCS and XQL conceived, designed, and supervised this research. QWY, XNS, GRR and BTZ assisted with revisions of protocol. SC and JJZ collected data. SC and LFZ analyzed and performed interpretation of data. SC and JJZ wrote the first draft of the manuscript. XCS and XQL provided comments and modifications to the final version. All authors approved the final manuscript to be published.

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Data availability
Data is provided within the manuscript.

Declarations

Ethics approval and consent to participate
This study was approved by the ethics review board of the Peking Union Medical College Hospital (I-23PJ103) and performed with the appropriate participants' informed consent in compliance with the Helsinki Declaration, according to Ethics in Good Clinical Practice as addressed by Declaration of Helsinki and Greek Regulations.

Consent for publication
Not applicable.

Clinical trial number
Not applicable.

Competing interests
The authors declare no competing interests.

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