ORIGINAL CONTRIBUTION

Histopathologic Analysis of Surgically Resected Lungs of Patients with Non-tuberculous Mycobacterial Lung Disease: a Retrospective and Hypothesis-generating Study

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Non-tuberculous mycobacterial lung disease (NTM-LD) is most commonly due to species within the *Mycobacterium avium* complex (MAC) and *Mycobacterium abscessus* complex (MAbC). Surgical lung resection, typically a lobectomy or segmentectomy, is occasionally undertaken for individuals with recalcitrant but localized NTM-LD. Since the growth characteristics of MAC (slow growers) and MAbC (rapid growers) as well as their drug susceptibility patterns are significantly different, the objective of this study is to characterize and compare the histopathologic features of the resected lungs due to these two major NTM groups. From 1996 to 2017, 356 patients with NTM-LD due to MAC (n=270), MAbC (n=54), or both (n=32) underwent a total of 404 lobar resections (with the lingula counted as a separate lobe) at the University of Colorado Hospital. We analyzed by microscopy the existing surgical lung tissue sections for bronchiolitis, bronchiolectasis, bronchiectasis, non-necrotizing granuloma (airway, parenchymal, and total), necrotizing granuloma (airway, parenchymal, and total), peri-airway fibrosis, fibrous pleuritis, and lymphoid follicles. There were no significant differences in the presence or absence of most of the histopathologic features of surgically removed lungs due to MAC, MAbC, or both MAC + MAbC. However, there were significantly more necrotizing granulomas (airway, parenchymal, and total) and fibrous pleuritis in MAC compared to MAbC lung diseases. Since necrotizing granulomas may be a sign of inadequate control of the infection, we posit that their presence may be an indication of increased chronicity, increased virulence of MAC compared to MAbC, and/or impaired host immunity against the NTM. Futures studies to determine the root cause of such differences in histopathologic findings in MAC versus MAbC lung disease may spawn new leads on differential pathogenic mechanisms with different NTM, with the goal of aiming for more targeted therapy against both the NTM and the lung damage induced by them.

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INTRODUCTION

Non-tuberculous mycobacteria (NTM) are a group of environmental organisms known to cause a panoply of human diseases. While approximately 200 distinct NTM species have been identified, only a minority of them have been documented to cause most human diseases. NTM lung disease (NTM-LD) is the most common manifestation of NTM infections. The majority of infections are caused by species within the *Mycobacterium avium* complex (MAC, comprised of ~10 species) and *Mycobacterium abscessus* complex (MAbC, comprised of three subspecies) [1]. Patients at highest risk for isolated NTM-LD are those with chronic obstructive pulmonary disease (COPD) due to smoking, alpha-1-antitrypsin (AAT) deficiency, or both and/or bronchiectasis due to cystic fibrosis, AAT deficiency, primary ciliary dyskinesia, Sjogren's syndrome, etc. [2-4]. Other less common predisposing conditions include pulmonary alveolar proteinosis and various immune and non-immune gene polymorphisms [5]. A significant number of individuals with NTM-LD who do not have identifiable risk factors often have in common a slender body habitus with thoracic cage abnormalities such as pectus excavatum and scoliosis [6,7].

Since NTM are ubiquitous in the environment, their presence in a respiratory specimen does not necessarily indicate active disease. In a joint consensus paper by the American Thoracic Society (ATS), European Respiratory Society, European Society of Clinical Microbiology and Infectious Disease, and Infectious Disease Society of America (IDSA), establishment of a NTM-LD diagnosis requires three main criteria [8,9]: *(i)* chest imaging study that is compatible with NTM-LD – typically bronchiectasis, inflammatory bronchiolitis ("tree-in-bud" opacities), and nodules ± cavitation ("nodular-bronchiectasis" phenotype) or the upper lobe fibrocavitary disease phenotype; *(ii)* clinical symptoms – typically cough, sputum, anorexia, and fatigue with exclusion of other disease processes; and *(iii)* respiratory cultures confirming the presence of NTM – at least two positive sputum cultures or one bronchoalveolar positive culture – or the presence of lung biopsy findings consistent with NTM-LD [1]. The NTM species identified can also help indicate whether the NTM is the cause of the lung disease; eg, *Mycobacterium kansasii* is highly predictive of true NTM-LD whereas *Mycobacterium gordonae* is almost always a contaminant [10].

The difficulty in treating NTM-LD is rooted in the challenges posed by intrinsic drug resistance, underlying host risk factors, on-going environmental exposure resulting in repeated infections, and occurrence of disease in elderly individuals with their associated co-morbid conditions and decreased tolerance to prolonged, multi-antibiotic cocktails. Despite the best available medical treatment, recurrence of disease is not uncommon and may be due to relapse of the prior infection and/or a new NTM infection acquired from the environment [2]. Hence, in those with severe, localized disease such as cavities and/or areas of advanced atelectasis and bronchiectasis – considered to be protected sites from antibiotics and a source for recrudescence of disease – adjunctive surgical lung resection may be recommended.

It is also unclear whether the lung histopathology differs between NTM species, which may impact response to treatment; eg, well-formed granulomas may provide a haven for the NTM and areas of necrosis may impede penetration by antibiotics. In a biopsy study comparing lymphadenitis (82% from the cervical region) due to *Mycobacterium tuberculosis* or NTM (species not reported), granulomas in the tuberculous lymph nodes were found to have lower number of apoptotic cells and less extensive necrosis than NTM-associated granulomas [11]. The presence of glycopeptidolipids in some strains of *M. abscessus* may also play a role in granuloma formation; conversely, its absence in other strains may prevent granuloma development [12]. A more comprehensive description of the lung histopathology will likely increase our understanding of the pathogenic mechanisms of NTM-LD, which may spawn new targets and approaches to treatment. Thus, the goal of this study is to characterize and compare the lung histopathologic findings of patients with MAC and MAbC lung disease who have undergone lung resections as part of their treatment.

METHODS

Population of NTM Lung Disease Subjects and Their Lung Tissues Analyzed

Following Institutional Review Board approval (HS-3137), a retrospective and cross-sectional analysis was performed to examine the archived lung tissues of 356 patients who underwent lung resection for NTM-LD due to MAC, MAbC, or both between 1996 and 2017 at the University of Colorado Hospital. The vast majority of the patients were referred from National Jewish Health (NJH); in turn, most of the patients were referred to NJH by providers from outside Colorado. Due to the use of a hybrid medical record system during most of the 1996 to 2017 period at NJH – wherein admission and progress notes were handwritten (which were later purged after a certain period of medical chart inactivity) but discharge summaries and letters to referring physicians were dictated and mostly still available electronically – details of their care prior to evaluations at NJH were sparse. One historical information that was lacking in this retrospective review was the NTM treatment regimens

received prior to evaluation at NJH; this was due largely to the fact that prior antibiotic regimens often times involved multiple previous out-of-state providers, other institutions, and various drug cocktails which were often poorly documented, recalled, and/or altered without clear documentation. Hence, dictated summaries, letters, electronically-stored culture results, and brief history from histopathology reports were used to tabulate the demographics and microbiological results. Existing culture data were used to identify patients as having infections from MAC, MAbC, or combined MAC + MAbC. The MAC + MAbC designation was used if the latest sputum cultures were positive for both species before the lung resection. All patients met criteria for NTM-LD based on the 2007 American Thoracic Society and Infectious Diseases Society of America consensus statement [9]. We excluded patients with positive non-NTM bacterial or fungal cultures from sputa or bronchoalveolar lavage within 2 months of surgery as well as excluded patients with a diagnosis of sarcoidosis.

Lung Histopathologic Analysis

All the histopathologic slides were prepared under strict quality control measures in a CAP/CLIAA certified histology laboratory at the University of Colorado Anschutz Medical Campus. All the available H & E lung tissue slides were scanned electronically and then analyzed by two of the investigators (SC and CDC). Up to five histopathologic lung slides for each patient were examined. Each slide was analyzed in its entirety by a pulmonologist and confirmed by a thoracic pathologist. Both analyzed the slides microscopically without knowing the causative NTM. The following histopathologic features were tabulated as yes-no variables: bronchiolitis, bronchiolectasis, bronchiectasis, airway granulomas (necrotizing and/or non-necrotizing), parenchymal granulomas (necrotizing and/or non-necrotizing), peri-airway fibrosis, fibrous pleuritis, and lymphoid follicles. Airway and parenchymal granulomas were distinguished when the boundary of the granuloma was ≤ or > 2 mm from the airway epithelium, respectively.

Statistical Analysis

Bivariate analyses (Chi-square, Fischer's exact test, and Kruskal-Wallis) were used to compare demographic information and histopathology characteristics. Generalized estimating equations (GEE) were used to calculate unadjusted odds ratios for histopathologic characteristics to take into account the lack of independence between slides from the same surgical lung specimen.

RESULTS

The baseline characteristics of the study population are summarized in Table 1. Of the 356 NTM-LD patients who underwent lung resections, 270 were due to MAC, 54 to MAbC, and 32 to both. The MAC and MAC + MAbC groups have more female patients (91.9% and 96.9%, respectively) compared to the MAbC group (79.6%). Regardless of causative NTM, patients were predominantly White – total (81.8%), MAC (82.2%), MAbC (70.4%), and MAC + MAbC (96.9%). The group with MAC lung disease had lower mean body mass index than MAbC or MAC + MAbC. Those with MAC lung disease were significantly more likely to be former smokers; in contrast, those with MAbC lung disease had greater number of current smokers although the number of current smokers were very few. There were no clinically significant differences in the presence of cough, hemoptysis, or relevant past medical history such as COPD, AAT anomalies, gastroesophageal reflux, and prior tuberculosis between the MAC and MAbC groups. In the 23 NTM-LD subjects with heterozygous mutation of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, there was no difference in the frequency of NTM-LD due to MAC vs MAbC but the cystic fibrosis carriers had significantly greater frequency of both MAC + MAbC infections (Table 1).

There were a total of 404 lobectomies (299 from the right lung and 105 from the left lung – with lingula counted as a separate lobe – from 356 patients (Table 1). The right middle lobe, right upper lobe and lingula were the most commonly resected lobes in all three NTM-LD groups. The right lower lobe was resected more frequently in those with MAC lung disease compared to lung disease due to MAbC or MAC + MAbC.

The average number of histology slides examined for each MAC, MAbC, or combined MAC + MAbC patient were 2.8 (757 slides for 270 patients), 2.4 (131 slides for 54 patients), and 3.5 (111 slides for 32 patients), respectively, based on the number of existing H&E slides available (Table 2). Examples of several of the key histopathologic features – namely bronchiolitis, bronchiolectasis, bronchiectasis, necrotizing and non-necrotizing granulomas of the airways, and lymphoid follicle – are shown in Figure 1.

There were no significant differences in prevalence of bronchiolitis, bronchiolectasis, bronchiectasis, non-necrotizing granulomas, peri-airway fibrosis, or lymphoid follicles between MAC, MAbC, and MAC + MAbC groups (Table 2). In contrast, necrotizing granulomas in the airways, parenchyma, and both were significantly more common in the MAC group compared to the MAbC group – 27.3% vs 14.5% (p=0.0018, OR 2.26), 54.5% vs 29% (p= <0.0001, OR 3.15), and 54.6% vs 29%

Table 1. Population Demographics and Clinical Characteristics

	Total population (N=356)	MAC (N=270)	MAbC (N=54)	Both (N=32)	p-value (comparing each category)	p-value (MAC vs MAbC)
Female, N (%)*	321 (90.5)	248 (91.9)	43 (79.6)	31 (96.9)	**0.0165**	**0.0067**
Age, median (IQR)	61 (55-67)	61 (55-67)	59 (53-66)	61 (56-67)	0.4327	0.2216
Ethnicity*					0.5478	0.1751
White	291 (81.8)	222 (82.2)	38 (70.4)	31 (96.9)		
Hispanic	9 (2.5)	8 (3.0)	1 (1.8)	0 (0.0)		
Asian	19 (5.3)	15 (5.6)	4 (7.4)	0 (0.0)		
Indian	1 (0.3)	1 (0.4)	0 (0.0)	0 (0.0)		
African American/Black	1 (0.3)	1 (0.4)	0 (0.0)	0 (0.0)		
Unknown	35 (9.8)	23 (8.4)	11 (20.4)	1 (3.1)		
BMI, median (IQR)	21.3 (19.8-23.3)	21.1 (19.7-22.9)	22.2 (20.0-24.5)	22.0 (20.5-24.8)	**0.0259**	**0.0334**
Nonsmoker	178 (50.0)	134 (49.6)	30 (55.6)	14 (43.8)	0.1710	0.1867
Current smoker	4 (1.1)	1 (0.4)	3 (5.6)	0 (0.0)	**0.0143**	**0.0133**
Former smoker	129 (36.2)	101 (37.4)	12 (22.2)	16 (50)	0.0637	**0.0453**
Weight loss*	76 (21.3)	62 (24.1)	6 (11.1)	8 (25)	0.1912	0.0864
Cough	307 (86.2)	233 (86.2)	43 (79.6)	31 (96.9)	0.5795	1.0000
Hemoptysis	100 (28.1)	71 (26.3)	19 (35.2)	10 (31.3)	0.2093	0.0776
COPD*	22 (6.2)	19 (7.0)	1 (1.9)	2 (5.9)	0.5004	0.3324
Cystic fibrosis* carriers	23 (6.5)	13 (4.8)	4 (7.4)	6 (18.8)	**0.0195**	0.3145
Alpha-1 antitrypsin anomalies*	25 (7.0)	16 (5.9)	6 (11.1)	3 (9.4)	0.2562	0.1270
Asthma*	25 (7.0)	24 (8.9)	1 (1.9)	0 (0.0)	0.0557	0.1450
Prior tuberculosis*	3 (0.8)	1 (0.4)	2 (3.7)	0 (0.0)	0.0786	0.0642
Lung transplant*	1 (0.3)	0 (0.0)	1 (1.9)	0 (0.0)	0.2375	0.1557
Coronary artery disease*	13 (3.7)	7 (2.6)	4 (7.4)	2 (6.3)	0.0824	0.0740
Malignancy*	21 (5.9)	20 (7.4)	0 (0.0)	1 (3.1)	0.0868	0.0518
GERD*	257 (72.2)	195 (72.2)	35 (64.8)	27 (84.4)	0.6953	0.8320
Lobes resected#						
RUL*	75 (21.1)	56 (20.7)	10 (18.5)	9 (28.1)	0.5838	0.7152
RML	178 (50.0)	137 (50.7)	22 (40.7)	19 (59.4)	0.1691	0.0985
RLL*	46 (12.9)	41 (15.2)	2 (3.7)	3 (9.4)	**0.0342**	**0.0161**
LUL*	11 (3.1)	7 (2.6)	4 (7.4)	0 (0.0)	0.1365	0.0946
Lingula*	79 (22.2)	63 (23.3)	9 (16.7)	7 (21.9)	0.5761	0.3706
LLL*	15 (4.2)	9 (3.3)	5 (9.3)	1 (3.1)	0.1334	0.0669

*Analysis with Fischer's Exact Test. #Based on operative report. COPD: chronic obstructive pulmonary disease; LLL: left lower lobe; LUL: left upper lobe; MAbC: *Mycobacterium abscessus* complex; MAC: *Mycobacterium avium* complex; RLL: right lower lobe; RML: right middle lobe; RUL: right upper lobe

Table 2. Lung Histopathologic Characteristics

	Total No. Slides (N=999)	MAC lung slides (N=757)	MAbC lung slides (N=131)	Both MAC+MAbC slides (N=111)	p-value comparing each category	p-value (MAC vs MAbC)
Bronchiolitis	565 (56.6)	431 (56.9)	65 (49.6)	69 (62.2)	0.1333	0.1194
Bronchiolectasis	395 (39.5)	305 (40.3)	44 (33.6)	46 (41.4)	0.3186	0.1470
Bronchiectasis	335 (33.5)	252 (33.3)	49 (37.4)	34 (30.6)	0.589	0.674
Non-necrotizing airway (A) granulomas	218 (21.8)	167 (22.1)	22 (16.8)	29 (26.1)	0.2047	0.1739
Non-necrotizing parenchymal (P) granulomas	287 (28.7)	223 (29.5)	29 (22.1)	35 (31.5)	0.1825	0.0862
Non-necrotizing granulomas (both A & P)	287 (28.7)	223 (29.5)	29 (21.4)	35 (31.5)	0.1825	0.0862
Necrotizing airway (A) granulomas	251 (25.1)	207 (27.3)	19 (14.5)	25 (22.5)	**0.0060**	**0.0018**
Necrotizing parenchymal (P) granulomas	497 (49.8)	412 (54.5)	38 (29.0)	47 (42.3)	**<0.0001**	**<0.0001**
Necrotizing granulomas (both A & P)	498 (49.9)	413 (54.6)	38 (29.0)	47 (42.3)	**<0.0001**	**<0.0001**
Airway granuloma (necrotizing & non-necrotizing)	412 (41.2)	320 (42.3)	41 (31.3)	51 (46.0)	**0.0352**	**0.0182**
Parenchymal granuloma (necrotizing & non-necrotizing)	636 (63.7)	504 (66.6)	61 (46.6)	71 (64.0)	**<0.0001**	**<0.0001**
Peri-airway fibrosis*	49 (4.9)	35 (4.6)	8 (6.1)	6 (5.4)	0.6577	0.5067
Fibrous pleuritis*	99 (11.1)	85 (12.7)	5 (4.4)	9 (8.5)	**0.0179**	**0.0100**
Lymphoid follicles	308 (30.9)	240 (31.8)	37 (28.2)	31 (27.9)	0.5568	0.4193

(p= <0.0001, OR 3.15), respectively (Tables 2 and 3). The total number of airway or parenchymal granulomas – encompassing both necrotizing and non-necrotizing designations – were also more common in the resected lung tissues due to MAC compared to MAbC – 42.3% vs 31.3% (p=0.0182, OR 1.62) and 66.6% vs 46.6% (p<0.0001, OR 2.35), respectively (Tables 2 and 3).

DISCUSSION

In this histopathologic analysis of a relatively large number of surgically resected lungs from patients with NTM-LD, the right middle lobe, lingula, and the right upper lobe were the most commonly resected, reflecting the most severely involved areas and consistent with those of prior surgical case series of NTM-LD patients [13]. Choi and colleagues [14] offered some hypotheses of why these lobes may be more severely affected than other lobes.

While we found that most of the histopathologic features of NTM-LD due to MAC and MAbC are similar (Tables 2 and 3), necrotizing granulomas (airway, parenchymal, and both) were more frequently present among patients with MAC (or MAC + MAbC) lung disease compared to those due to MAbC. There was also a trend toward increased prevalence of non-necrotizing granulomas (airway, parenchymal, and both) in the lung tissues of MAC compared to MAbC lung disease. What could account for the increased necrotizing (and total) granulomas in MAC lung disease over MAbC lung disease? Based on existing literature, we posit the following possibilities in three separate categories:

(1) Longer chronicity of MAC lung disease due to recalcitrance to treatment. Since MAC lung disease is more prevalent and more familiar to physicians in the community as well as having greater treatment options
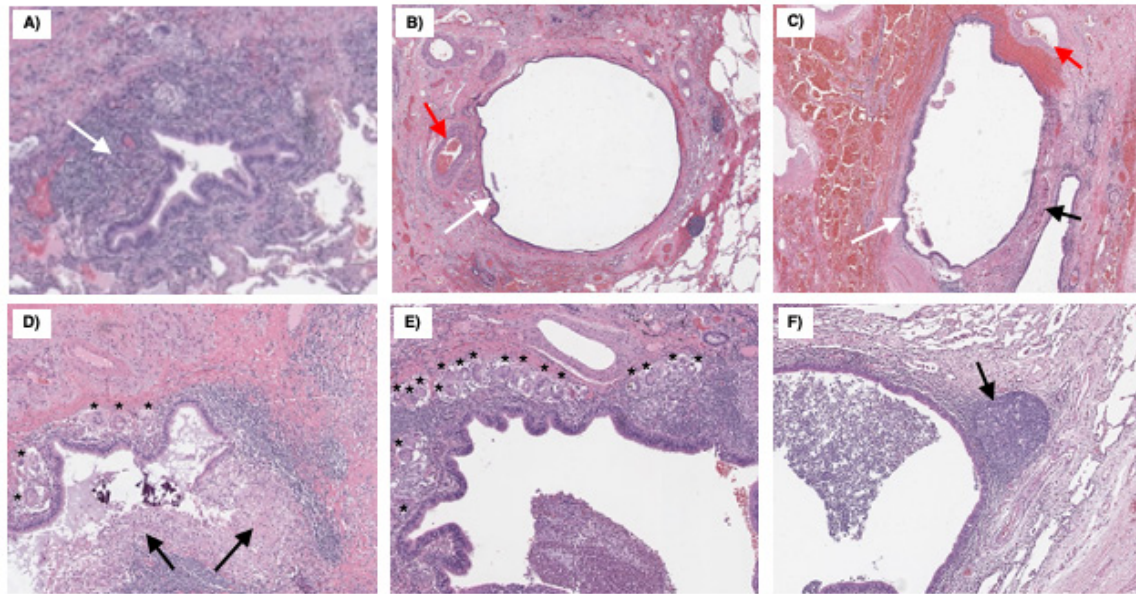
Figure 1. **Representative examples of specific lung histopathologic findings**. **(A)** Bronchiolitis is represented by the infiltration of the walls of small airways by chronic inflammatory cells (white arrow). **(B)** Bronchiolectasis and **(C)** bronchiectasis are diagnosed when the diameter of the bronchus and bronchiole (white arrows), respectively, are greater than that of the lumen of the accompanying pulmonary artery branch (red arrows). Bronchi have cartilage (black arrow) and submucosal glands. **(D)** Necrotizing granulomas of the airways with areas of central necrosis (black arrows) surrounded by a palisading layer composed of epithelioid histiocytes admixed with several giant cells (asterisks) adjacent to and involving the airway walls. **(E)** Non-necrotizing granulomas of the airways with many giant cells (asterisks) but without necrosis. **(F)** Lymphoid follicle (arrow) in the wall of a dilated airway.

than MAbC lung disease, we speculate that those with MAC lung disease were more likely to be recalcitrant to treatment and referred to NJH; ie, have longer chronicity of disease than those with MAbC lung disease. Thus, a plausible reason for the increased frequency of necrotizing granulomas in MAC lung disease may be related to suboptimal control of the infection resulting in a chronic, exuberant pro-inflammatory response, perhaps in an attempt by the host to gain control of the infection but that also resulted in increased necrotizing granulomas. On the other hand, because *M. abscessus* possesses greater intrinsic drug resistance with fewer antibiotic options available and is less familiar to physicians than MAC, perhaps patients with MAbC lung disease were referred earlier in their disease process, accounting for fewer necrotizing granulomas in their lung tissues.

(2) NTM-specific elements. It is becoming increasingly clear that different NTM species, subspecies, and even strains within the same species may induce differential host immune responses [12,15-18]. With the caveat that murine granulomas are generally distinct from those found in humans, a murine model of systemic MAC infection showed that a less virulent *M. avium* strain induced more well-developed liver granulomas than a more virulent strain of *M. avium* [18]. Combined with the finding

that the well-formed granulomas induced by the less virulent *M. avium* were dependent on the presence of CD4+ T cells, tumor necrosis factor (TNF), and interferon-gamma (IFNg), components of the immune system considered to be host-protective against mycobacterial infections, it supports the concept that non-necrotizing granulomas are host-protective [18]. Another mouse study also supports the paradigm that necrotizing granulomas are a sign of inadequate control of the infection. In this study, the I/St mouse strain was shown to be susceptible to *M. tuberculosis* but resistant to *M. avium*; conversely, the B6 mouse strain was susceptible to *M. avium* and resistant to *M. tuberculosis* [19]. Interestingly, necrotizing granuloma only developed in the lungs of the *M. tuberculosis*-susceptible I/St mice and the *M. avium*-susceptible B6 mice, but not in their genetically-resistant mouse counterparts [19]. In a zebrafish model of infection, the presence of glycopeptidolipids in the smooth (S) strain of *M. abscessus* allowed greater phagocytosis and greater propensity for granuloma formation whereas the extracellular cording formed by the glycopeptidolipids-lacking rough (R) strain prevented phagocytosis and induced abscess formation [12,15,17]. Since the S strain of *M. abscessus* often transitions to the more virulent R strain peri-establishment of disease, perhaps the fewer granulomas found

Table 3. Lung Histopathologic Findings of MAC Versus MAbC Lung Disease

Variable	Odds ratio (95% confidence interval)
Bronchiolitis	1.28 (0.78, 2.12)
Bronchiolectasis	1.23 (0.79, 2.07)
Bronchiectasis	0.89 (0.49, 1.61)
Non-necrotizing airway (A) granulomas	1.40 (0.71, 2.74)
Non-necrotizing parenchymal (P) granulomas	1.43 (0.77, 2.63)
Non-necrotizing granulomas (both A & P)	1.43 (0.77, 2.64)
Necrotizing airway (A) granulomas	**2.26 (1.15, 4.42)**
Necrotizing parenchymal (P) granulomas	**3.15 (1.74, 5.69)**
Necrotizing granulomas (both A & P)	**3.15 (1.74, 5.69)**
Airway granulomas (necrotizing & non-necrotizing)	1.62 (0.98, 2.69)
Parenchymal granulomas (necrotizing and non-necrotizing)	**2.35 (1.41, 3.90)**
Peri-airway fibrosis	0.41 (0.23, 2.20)
Fibrous pleuritis	3.14 (0.93, 10.67)
Lymphoid follicles	1.16 (0.65, 2.06)

in the lungs of patients with MAbC organisms is due, in part, to the presence of this immune evasive strategy of the R strain of *M. abscessus*. Thus, differences in cell wall components in the different NTM groups as well as the rate of multiplication between slow-growing MAC versus rapid-growing MAbC may account for differences in necrotizing granuloma formation. Human THP-1 monocytic cells infected with *M. avium* induced higher levels of interleukin-6 (IL-6), IL-12p40, IL-12p70, and TNF than cells infected with *M. intracellulare* or *M. abscessus* [16]. IL-6 and TNF are prototypical pro-inflammatory cytokines and IL-12 is important in the activation and amplification of the T_H1 response, cells, and cytokines known to be important in granuloma formation.

(3) Host-specific elements. In contrast to the aforementioned study of THP-1 cells, *M. abscessus* was found to induce greater levels of TNF, IFNg, and IL-6 than *M. avium* or *M. intracellulare* in primary human peripheral blood mononuclear cells (comprised of both monocytes and lymphocytes) [20]. The difference displayed by the THP-1 cells and the primary cells supports the well-accepted concept that the type and degree of immune responses vary depending on differences in the intrinsic immunity among different individuals. Cigarette smoke and nicotine are also known to impair granuloma formation [21]. While the increased rate of current smoking in those with MAbC lung disease may account for fewer necrotizing (and non-necrotizing) granulomas compared to those with MAC lung disease, the number of current smokers in the MAC group (n=1) and the MAbC group (n=3) are so small that current smoking is unlikely to account for the differences in granuloma formation. While not host factors *per se*, prior infections with other (non-NTM) organisms and prior intake of antibiotics that may alter

the immune response (eg, macrolides, fluoroquinolones) could also influence the lung histopathologic findings that are attributed to the NTM.

Certain aspects of the demographics and past medical history warrant further elaboration in the context of known host risk factors for NTM-LD. The high prevalence of White women (~80-97%) is partly related to referral bias although others have noted in the community that the nodular-bronchiectasis form of NTM-LD, which is the most common radiographic phenotype, is more common in women [22]. The relatively low prevalence of COPD in our population may also be due to referral bias, as most patients who underwent surgery for NTM-LD were middle-age to elderly women with the nodular-bronchiectasis phenotype who less commonly possess the upper lobe fibrocavitary disease typically seen in patients with COPD-associated NTM-LD. While there was no significant difference in the prevalence of COPD among the three NTM groups, there was a trend of more COPD cases in the MAC and MAC + MAbC groups. This latter finding correlate with the finding that patients with MAC and MAC + MAbC lung disease were more likely to be former smokers.

Prior studies correlating radiographic features with histopathology have shed light on the cellular pathogenesis of NTM-LD [23,24]. Computed tomographic findings of bronchiectasis and bronchiolitis correlated with peribronchial and peribronchiolar granulomatous inflammation and airway wall necrosis in resected lung tissues [23]. Therefore, NTM-associated bronchiectasis may develop as a result of either weakened airway walls due to chronic granulomatous inflammation, mucosal ulceration and atrophy, airway obstruction by mucous plugs that leads to bronchial and bronchiolar dilatation

distal to the obstruction, or both processes [25,26]. Lung nodules and cavity walls are also comprised of caseating granulomas; the outer rim of cavity walls may be surrounded by myofibroblasts, the differentiation of which requires transforming growth factor-beta [23].

Despite the airway-centric pathology associated with NTM-LD, the airway epithelium displays an array of host-protective functions, encompassing the mucociliary escalator to help sweep inhaled or aspirated bacteria away from the distal lung and host immunity. These lesser known immune functions of airway epithelial cells include phagocytosing and killing ingested bacteria, producing various antimicrobial peptides, and orchestrating the early host immune response through recruitment and activation of macrophages, dendritic cells, and lymphocytes, through the production and release of cytokines and chemokines. However, progressive NTM lung infection may become established when there is a breach in the integrity and function of the respiratory epithelium and/or a compromise in immune cell function in the airways, particularly in the presence of immune evasive mechanisms by the NTM [27]. Indeed, since exposure to environmental NTM is ubiquitous, those with isolated NTM-LD most likely have some form of airway mucosal defect, whether due to heritable causes (eg, cystic fibrosis, alpha-1-antitrypsin deficiency, primary ciliary dyskinesia, etc.) or acquired (eg, smoking-related emphysema, bronchiectasis from prior infection, etc.). In the context of this current study, a granulomatous response is considered to be a second-line of defense to contain the infection when the initial host immune response fails to eradicate the mycobacteria.

Several limitations of this study were present, most of which hinged on the fact that retrieval of remote data (up to 20+ years) was incomplete due to the retrospective nature of the study and that the majority of the patients were referred from out-of-state with limited follow-up in Colorado. One limitation is that although entire lobes have been resected, only a relatively small portion(s) of the resected lung tissues were made into histopathologic slides following standard protocol by the clinical pathology laboratory; ie, we were limited by the relatively few number of archived lung histologic sections available. In addition, certain lung pathologic features such as bronchiectasis and cavities are better analyzed globally by CT scan rather than the limited representation with histopathologic analyses. It was also difficult to pinpoint the chronicity of the diseased lung that underwent surgical resection since NTM-LD may have been present for some time before the actual diagnoses were made. Because the study was retrospective and most patients were referred from out-of-state, it is quite plausible that other infections or treatments (such as use of glucocorticoids and antibiotics with immunomodulatory effects) prior to the lung surgeries could have altered the lung histopathology. The analyses could also have been more accurate and quantitative if we had employed *two* thoracic pathologists to independently review and numerically score the severity of the histopathologic findings, respectively. However, one caveat to semi-quantifying the number of granulomas is that a recent study showed that granulomas in pulmonary tuberculosis are branching, interconnected structures [28]. This new discovery indicates that discrete appearing granulomas in a two-dimensional analysis are in fact cross-sectional branches of much larger, but fewer granulomas. Since NTM-LD is largely airway-centric, it is likely that this branching morphology of tuberculous granulomas is also seen with NTM-LD. However, because our analyses were qualitative rather than quantitative, our findings are unlikely to be affected by a branching granuloma morphology.

In conclusion, despite significant microbiologic differences between MAC and MAbC, surgically resected lung histopathology is remarkably similar with the exception of a significantly greater prevalence of airway and parenchymal necrotizing granulomas as well as of fibrous pleuritis in lung disease due to MAC compared to that due to MAbC. Further basic and translational research in this area should explore the inflammatory pathways induced in the lungs by different groups of NTM and correlate bronchoalveolar lavage findings with the histopathologic features. Such investigations may shed new light on the determinants of NTM-induced granuloma formation, leading to the identification of better targets for antibiotic or host-directed therapies.

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