Non-tuberculous mycobacterial pulmonary disease

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Despite significant advances the management of nontuberculous mycobacterial pulmonary disease remains challenging; new approaches are needed http://bit.ly/2WPDU8V

Cite this article as: Cowman S, van Ingen J, Griffith DE, *et al*. Non-tuberculous mycobacterial pulmonary disease. *Eur Respir J* 2019; 54: 1900250 [https://doi.org/10.1183/13993003.00250-2019].

ABSTRACT Nontuberculous mycobacterial pulmonary disease (NTM-PD) is a challenging infection which is becoming increasingly prevalent, particularly in the elderly, for reasons which are unknown. While underlying lung disease is a well-established risk factor for NTM-PD, it may also occur in apparently healthy individuals. No single common genetic or immunological defect has been identified in this group, and it is likely that multiple pathways contribute towards host susceptibility to NTM-PD which further interact with environmental and microbiological factors leading to the development of disease.

The diagnosis of NTM-PD relies on the integration of clinical, radiological and microbiological results. The clinical course of NTM-PD is heterogeneous, with some patients remaining stable without the need for treatment and others developing refractory disease associated with considerable mortality and morbidity. Treatment regimens are based on the identity of the isolated species, drug sensitivity testing (for some agents) and the severity of disease. Multiple antibiotics are typically required for prolonged periods of time and treatment is frequently poorly tolerated. Surgery may be beneficial in selected cases. In some circumstances cure may not be attainable and there is a pressing need for better regimens to treat refractory and drug-resistant NTM-PD.

This review summarises current knowledge on the epidemiology, aetiology and diagnosis of NTM-PD and discusses the treatment of two of the most clinically significant species, the *M. avium* and *M. abscessus* complexes, with a focus on refractory disease and novel therapies.

Received: Feb 04 2019 | Accepted after revision: May 31 2019

Introduction

The term "nontuberculous mycobacteria" (NTM) refers to members of the genus *Mycobacterium* which are not part of the *M. tuberculosis* complex, and conventionally also excludes *M. leprae* [1]. They are ubiquitous organisms found in the environment worldwide and owing to their thick lipid-rich cell wall show resistance to extremes of heat and pH, and to many disinfectants and antibiotics [2]. They have been known to cause disease in humans for over 70 years [3] and, while they may cause infection in any bodily site, pulmonary disease (NTM-PD) is the most common manifestation in the immunocompetent [4] and will form the basis for this review.

While over 180 species have been described to date, only a small number have been reported to cause disease in humans [5]. NTM can be divided into rapidly growing mycobacteria, which may form colonies within 7 days, of which the *M. abscessus* complex, *M. chelonae* and *M. fortuitum* are the most clinically relevant; and slow growing mycobacteria, which may take up to 12 weeks to grow, of which the *M. avium* complex (MAC), *M. xenopi, M. kansasii, M. simiae, M. malmoense* and *M. szulgai* are the most important [6–8]. This phenotypic division remains clinically relevant, as important differences in management exists between the two groups [8].

Epidemiology

Estimating the prevalence of NTM-PD poses several challenges; unlike tuberculosis (TB), in most regions it is not a notifiable disease, and as the diagnosis is criteria-based, reports of isolation do not equate with clinical disease. Even allowing for these factors, however, the prevalence appears to vary widely across the globe, with particularly high annual prevalence reported in the USA and Japan of 23–37 and 33–65 cases per 100 000 persons, respectively [9–11]. The prevalence of NTM-PD may also vary markedly within the same country; in a nationwide study of the USA between 1997 and 2007 the estimated period prevalence ranged from 78 cases per 100 000 persons in the Midwest to 396 in Hawaii [9].

The most common species isolated globally are those in the MAC, comprising between 34% and 61% of isolates depending on continent in one systematic review, with the highest proportions found in North America and Oceania (table 1) [12]. A study of 30 predominantly European countries [13] found that *M. kansasii* was most common in Slovakia and Poland, *M. xenopi* was most common in Hungary and *M. abscessus* complex was more common in Taiwan and South Korea. While uncommon worldwide, *M. malmoense* was seen more frequently in Scandinavia with a prevalence of up to 5% in Norway. The proportions of different species may vary even within the same country: one study reported that the proportion of MAC varied between 61% and 91% and *M. abscessus*/*chelonae* between 2% and 18% across different regions of the USA [14], and in Japan a nationwide survey found marked regional differences in the ratio of *M. avium* to *M. intracellulare* and the prevalence of *M. abscessus* and *M. kansasii* [15].

Multiple studies in diverse countries have found an increase in NTM-PD prevalence over time [9–11, 16–24]. Exceptions have been reported: a study in Scotland found no increase in reports of NTM isolation between 2000 and 2010 but suggested that an increase in NTM isolation may have occurred prior to the study period [25], and while a study in Canada found a marked increase in NTM isolation between 1990 and 2006, there was no increase in subjects treated for NTM and a change in laboratory techniques was felt to be responsible [26]. However, while increased case ascertainment through greater disease awareness (and hence greater sampling), improved imaging and culture techniques may play a role, the consistency of this finding across different healthcare systems and time periods suggests a genuine increase. This is supported by reports of an increase in mortality related to NTM-PD over time [11, 27]. The reason for this increase is unclear. The ageing population does not account for the increase, as it remains even when

TABLE 1 Prevalence of different nontuberculous mycobacteria species by continent

Species	Europe	Asia	Africa	North America	Oceania	South America
MAC	34%	34%	49%	51%	61%	34%
M. abscessus		16%			12%	
M. fortuitum	7%	14%		7%	12%	9%
M. gordonae	18%	9%	6%	13%		15%
M. scrofulaceum			6%			
M. kansasii			3%		3%	17%
M. xenopi	15%			11%		
Others	26%	27%	36%	18%	12%	25%

Data from Zweijpfenning *et al.* [12]. MAC: *Mycobacterium avium* complex.

adjusted for age [10, 18, 22]. One possible factor is increasing environmental exposure, which was suggested by data from the USA demonstrating an increase in sensitisation against *M. intracellulare* (assessed *via* skin testing) between 1971–72 and 1999–2000 [28], although the reason for an increase in exposure is not known. Interestingly the increase has not been associated with an increase in TB isolation, which in several centres was reported to have fallen over the same period [16, 18, 22, 24]. One suggested explanation for this is that TB exposure might confer cross-protection against NTM [9, 18], although changing demographic patterns may also contribute to this observation.

Person-to-person transmission

Until recently, it was thought that in contrast to TB, person-to-person spread of NTM did not occur. This was first challenged by a report of *M. abscessus* subsp. *massiliense* developing in four subjects at a cystic fibrosis (CF) centre in the USA following the arrival of a new patient with smear-positive disease. Pulsed-field gel electrophoresis analyses were indistinguishable between isolates, which also displayed amikacin and macrolide resistance [29]. A large retrospective study employing whole genome sequencing of 168 isolates of *M. abscessus* complex in 31 subjects with CF at a UK centre identified two clusters of subsp. *massiliense* showing near-identical sequences, suggesting direct spread between patients on multiple occasions. Isolates from both clusters showed resistance to clarithromycin (and amikacin in one cluster), even in subjects never exposed to these agents. Multiple opportunities for in-hospital transmission were identified although the specific mechanism could not be identified [30]. Subsequent comparison with isolates from the US outbreak revealed high-level relatedness between strains [31]. In a further multicentre global study of whole genome sequencing of 1080 isolates from 517 subjects, dominant circulating clones of subsp. *massiliense* and *abscessus* were identified that exhibited greater virulence than unclustered isolates [32]. Multiple instances of between-person transmission were again identified and opportunities were also identified for potential transmission *via* fomites or aerosols. A separate study has demonstrated that *M. abscessus* displays characteristics favourable to fomite spread [33]. These findings have led to the recommendation for enhanced infection control measures in CF [34]. Outside of CF, however, person-to-person transmission has not been reported.

Aetiology

Environmental factors

NTM are widely distributed throughout the environment, which is thought to represent the reservoir for human infection [2]. Thus, environmental factors may also mediate disease susceptibility. Measures of atmospheric moisture have been linked to increased NTM-PD prevalence [35, 36], presumably through increasing NTM exposure. Further evidence implicating water as a potential route of infection comes from two reports, which found isolates in the domestic water supply of subjects with NTM-PD closely resembling their respiratory isolates [37, 38], and an epidemiological study which found an increased risk of NTM-PD associated with specific sources supplying domestic water [39]. Soil may be another source of infection; there have been reports of the isolation of NTM from soil samples resembling those found in respiratory isolates from NTM-PD cases [40, 41] and exposure to soil has been identified as a risk factor for NTM-PD [42, 43]. Beyond soil and water, NTM have also been recovered from a wide range of other environmental and domestic sources but their role in human disease is uncertain [44].

Host risk factors

A number of risk factors for NTM-PD have been identified, predominantly structural lung disease, such as bronchiectasis, chronic obstructive lung disease (COPD) and interstitial lung disease [45–49]. CF appears to be a particularly strong risk factor for NTM-PD, with a reported prevalence of between 6% and 13%, and a particularly high proportion of *M. abscessus* (ranging from 16% to 68%) [50], which appears particularly well adapted to the CF lung [51, 52]. A high prevalence has also been reported in primary ciliary dyskinesia [53, 54] and a recent novel study found reduced nasal nitric oxide and ciliary beat frequency in subjects with NTM-PD [55].

It has long been recognised that NTM-PD, usually of the nodular bronchiectatic pattern, may also develop in subjects with no previously diagnosed underlying risk factors [56–59]. This was termed the "Lady Windermere" syndrome, after the fastidious character in the eponymous play by Oscar Wilde, as the authors of the original report hypothesised that voluntary cough suppression may be the cause [56]. The majority of subjects are female, postmenopausal, taller and thinner than average, with a higher than average prevalence of thoracic skeletal abnormalities. This phenotype has led to more plausible speculation that hormonal factors, connective tissue abnormalities or low adiposity may play a role in disease susceptibility [60–62].

Increasing age has also been shown to be a strong risk factor for NTM-PD with a dramatic increase in prevalence seen in older age groups [9–11, 17, 18, 20–22, 25]. Many studies have also reported a higher

prevalence observed in females [9–11, 22], although this finding is not reported in studies outside Japan and North America [17, 20, 21, 23–25].

An increased risk of NTM disease has also been demonstrated with the use of immunosuppressant medications, including biological therapy against tumour necrosis factor (TNF)-α and oral corticosteroids [63, 64]. Of potentially greater importance, given the number of affected individuals, are reports suggesting a link between inhaled corticosteroids (ICS) and NTM-PD [49, 65]. Two recent, large case–control studies addressed this question. Both matched controls on key demographics and controlled comparisons for a large number of potential confounders. The first found that ICS use within the past year was associated with an increased risk of NTM-PD (adjusted odds ratio 2.8) in a cohort of subjects with asthma, COPD and bronchiectasis [66]. The second larger study found that current (but not past) ICS use was significantly associated with NTM-PD (adjusted odds ratio of 1.86), but not TB, in a cohort with obstructive lung disease. A significantly higher risk was seen with fluticasone, even when adjusted to beclomethasone dose equivalents [67]. Both studies showed a significant relationship between increasing doses of ICS and the risk of NTM-PD. While there may still be unmeasured differences between groups confounding these analyses, such as the severity of airflow obstruction, together they represent the strongest evidence to date that ICS use may contribute towards the risk of NTM-PD.

Immunological factors

The role of immunodeficiency in predisposing towards NTM disease was first brought to light by the AIDS pandemic, where reduced CD4+ counts were associated with disseminated MAC infection [68]. Disseminated mycobacterial infection is also seen in individuals harbouring mutations affecting the interferon (IFN)-γ–interleukin (IL)-12 pathway, collectively referred to as Mendelian susceptibility to mycobacterial disease. To date, mutations have been identified affecting 11 genes [69]. Outside of this pathway, mutations in *GATA2* have been described, leading to defective haematopoesis and susceptibility to multiple infections, including NTM [70]. More recently, reports emerged of disseminated NTM disease affecting previous healthy, apparently immunocompetent individuals in Thailand and Taiwan traced to an inhibitory anti-IFN-γ autoantibody [71]. These systemic immune defects generally present with disseminated rather than pulmonary disease (although elevated levels of autoantibodies against IFN-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been reported in one series of subjects with NTM-PD [72]). Several studies of individuals with pulmonary disease have examined their immune responses, although the methodologies employed have varied widely and the findings have been inconsistent (table 2).

No evidence of deficiency in CD4+ T-cells has been demonstrated in subjects with NTM-PD [58, 73, 74]. However, several studies have found diminished Th1 responses, showing reduced IL-12 [74, 76, 77, 82] and IFN-γ [58, 62, 73–75, 77, 79, 81] release, although intact or increased responses have also been reported [58, 78, 80, 83]. One study found increased antibodies against IFN-γ in subjects with NTM-PD [72]. TNF-α also plays an essential role in the defence against TB by activating macrophages and maintaining granuloma integrity [84]; several studies have also reported impaired TNF-α release in subjects with NTM-PD [58, 73, 74, 76, 77, 81–83], although intact or elevated responses have also been found [62, 83].

IL-10 is an anti-inflammatory cytokine which plays an important role in regulating the immune responses to prevent immunopathology, although this may be detrimental to the defence against some pathogens, including *M. tuberculosis* [85]. Studies of IL-10 responses in patients with NTM-PD have been inconsistent, with reports of increased [74, 78], unchanged [77] or decreased [62] activity. The release of another anti-inflammatory cytokine, transforming growth factor-β [86], and the expression of the immunoregulatory protein PD-1 [81–83] have also been shown to be increased in subjects with NTM-PD compared with controls.

The role of the pro-inflammatory cytokine IL-17 in the host response to TB is complex; deficiency impairs control of *M. tuberculosis* infection in murine models; however, it has also been implicated in neutrophil-mediated immunopathology [87]. Two studies in subjects with MAC have found impaired IL-17 responses [78, 82] and in subjects with CF and *M. abscessus*, impaired IL-17 release has also been reported [80], although in contrast another report found higher circulating levels [88].

Toll-like receptors (TLRs) play an important early role in the innate host response to mycobacteria [89]. One study demonstrated a reduced TLR2 transcriptional response in patients with NTM-PD [76], and abnormalities in the ciliary beat frequency response to TLR stimulation in epithelial cells from subjects with NTM-PD have also been reported [55].

One study examined the global transcriptional response in NTM-PD through analysis of whole blood gene expression [90]. Genes involved in T-cell signalling were underexpressed in NTM-PD, including *IFNG*,

TABLE 2 Summary of functional immunological studies in subjects with nontuberculous mycobacterial pulmonary disease (NTM-PD)

Study	Population	Methodology			Principal findings in NTM-PD
		Cell type (s)	Mitogen(s)	Readout	
Greinert *et al.* [73] (2000)	32 subjects with NTM-PD#, 30 subjects with MTB, 20 healthy controls	PBMC, whole blood	PHA, anti-CD3 Ab, PPD, NDV, live *M. avium, M. kansasii,* and *M. szulgai*	IFN-γ and TNF-α (ELISA); CD3, CD2, CD4, CD8, CD38, CD20, CD16, CD57, CD45RA, CD45R0, HLA-DR, CD14 (FC)	Compared to MTB controls: Lower anti-CD3 induced TNF-α and IFN-γ production in PBMC; lower PPD induced IFN-γ and TNF-α release in PBMC and whole blood; lower PHA induced TNF-α response in whole blood Compared to healthy controls: Lower anti-CD3 induced IFN-γ production in PBMC
Vankayalapati *et al.* [74] (2001)	26 subjects with NTM-PD# (all MAC), 19 *M. avium*-sensitised healthy controls	PBMC	Heat-killed MAC or MTB	TNF-α, IL-10, IFN-γ, IL-4, IL-18 and IL-12p70 (ELISA); intracellular TNF-α, IL-10 and IFN-γ (FC)	Reduced IFN-γ, TNF-α, IL-12 and IL-18, and increased IL-10 response to MAC
Safdar *et al.* [75] (2003)	2 subjects with nodular NTM-PD¶, 39 healthy controls	PBMC	PMA, anti-CD3 Ab	IFN-γ (ELISA, FC)	Reduced IFN-γ response to PMA and anti-CD3 Ab
Ryu *et al.* [76] (2007)	17 subjects with nodular NTM-PD# and 10 healthy controls	PBM	MAC, LTA	TLR2, IL-12p40, TNF-α (RT-PCR)	Reduced TLR2 expression in response to MAC; reduced resting expression of IL-12p40 and TNF-α and in response to MAC and LTA
Kwon *et al.* [77] (2007)	29 subjects with nodular NTM-PD#, 15 healthy controls	PBMC	PHA, PHA+IL-12, LPS, LPS+IFN-γ	IFN-γ, TNF-α, IL-12p40, IL-10 (ELISA)	Reduced IFN-γ response to PHA+IL-12, reduced TNF-α and IL-12p40 response to LPS+IFN-γ
Kim *et al.* [58] (2008)	55 subjects with NTM-PD, healthy blood bank controls+	PBMC	PHA, PHA+IL-12 heterodimer, LPS, LPS+IFN-γ	IFN-γ, TNF-α, IL-6, IL-10, IL-1b and IL-12 (bioluminescent bead assay); CD2, CD3, CD4, CD8, CD28, CD57, HLA-DR, CD25, CD20, CD16, CD56 (FC); PCR of immunoglobulin heavy chain and T-cell receptor γ-chain	Decreased IFN-γ response to PHA+IL-12 and IL-1B response to LPS; abnormal T-cell receptor γ-chain rearrangement patterns seen in 36%
Lim *et al.* [78] (2010)	17 subjects with NTM-PD, 15 healthy offspring, 13 healthy controls	PBMC	SEB, tuberculin PPD, individualised NTM sensitin PPD, anti-CD28 Ab, anti-CD49d Ab	CD3, CD4, IFN-γ, IL-10, IL-17A (FC); IFN-γ, IL-5, IL-17A, IL-10 (ELISA)	Compared to healthy controls: Increased CD4+ IFN-γ T-cells in response to sensitin; preserved or increased IFN-γ secretion in response to SEB and sensitin; increased IL-10 response to tuberculin and sensitin
Kartalija *et al.* [62] (2013)	47 subjects with NTM-PD, 53 healthy controls	Whole blood	LPS, heat-killed *S. epidermis*, live *M. intracellulare*	IL-1B, IL-6, IL-8, IL-10, IL-12, IFN-γ, TNF-α, RANTES (ELISA)	Decreased IFN-γ response to MAC and LPS; lower unstimulated IL-10 levels and decreased IL-10 response to LPS, *S. epidermis* and MAC
Rae *et al.* [79] (2016)	2 subjects with NTM-PD¶, controls not stated	Whole blood	IL-12+BCG, IL-12+LPS, IL-12+PHA, IL-12 +IL-18	IFN-γ§	Reduced IFN-γ response to IL-12+BCG and IL-12+LPS in both subjects; IFN-γ response to IL-12+PHA and IL-12+IL-18 reduced in one subject but preserved in the other
Becker *et al.* [80] (2016)	3 patients with CF and NTM-PD¶ (all *M. abscessus*), 7 non-CF subjects with NTM-PD¶, 11 healthy controls	PBMC	Heat-killed *M. abscessus*, heat-killed *C. albicans*, LPS, *A. fumigatus* conidia	TNF-α, IL-1B, IFN-γ, IL-17, IL-22 (ELISA)	Increased IL-1B and TNF-α response to *A. fumigatus* in CF NTM-PD group; reduced IL-17 response to *M. abscessus* in both NTM-PD groups; preserved or elevated IFN-γ and IL-22 response to *M. abscessus* in CF NTM-PD group

Continued

TABLE 2 Continued

Study	Population	Methodology			Principal findings in NTM-PD
		Cell type (s)	Mitogen(s)	Readout	
Shu *et al.* [81] (2017)	50 subjects with NTM-PD (all MAC) and 30 healthy controls	PBMC	Heat-killed MAC, MAC sensitin, PHA	CD3, CD4, CD25, CD14, CD19, CD56, PD-1, PD-L1, PD-L2, IFN-γ (FC); IFN-γ, TNF-α and IL-1β (ELISA), apoptosis measured by Annexin V and SYTOX staining	Impaired TNF-α response to all stimuli; impaired IFN-γ response to MAC and MAC sensitin; increased PD-1 and PD-L1 expression in unstimulated CD3, CD4, CD8, CD19, CD56 and CD4+CD25+ T-cells; higher apoptosis status in unstimulated CD4 lymphocytes; higher PD-1 expression and apoptosis in response to MAC stimulation
Shu *et al.* [82] (2018)	50 subjects with NTM-PD (all MAC), 25 MTB controls and 25 healthy controls	Plasma, MDM	Heat-killed MAC, LPS	CD4, IL17, PD-1 (FC); IL-12p40, IL-12p70, IL-23, IFN-γ, IL-17A (ELISA); TNF-α, IL-12p40, IL-12p35, IL-13p19 (RT-PCR)	Compared to MTB controls: Higher resting plasma levels of IL-17 and IL-12p70; lower resting levels of TNF-α and IFN-γ; reduced TNF-α and IL12p40 response to MAC Compared to healthy controls: Higher resting plasma levels of TNF-α, IFN-γ, IL-23 and IL-17; lower resting levels of IL-12p70; reduced TNF-α and IL12p40 response to MAC; reduced IL-17 secretion and increased PD-1 expression on CD4+ IL-17+ T-cells in co-culture assay
Lutzky *et al.* [83] (2018)	6 subjects with CF and active NTM-PD[¶], 8 subjects with CF and successfully treated NTM-PD[¶], 9 CF controls, 10 non-CF subjects with NTM-PD[¶] and 10 healthy controls; all NTM was *M. abscessus*	PBMC	PMA	CD4, CD8, CD16, CD19, CD14, Tim-3, CD25, PD-1, CTLA-4, FOXP3, CD107, CD3, IFN-γ, TNF-α, IL-2 (FC)	CF group: Increased number of resting T_{reg} cells in active NTM-PD compared with both control groups; higher numbers of resting IFN-γ+ CD4 cells and increased CD25 and CTLA-4 expression in resting CD4 cells in active NTM-PD compared to uninfected controls; higher number of CTLA-4+CD4 cells, fewer TNF-α+ CD4 T-cells and more IFN-γ+ CD8 T-cells following stimulation in active or previous NTM-PD compared with uninfected controls Non-CF NTM-PD: Increased number of resting T_{reg} and CD25+PD-1+ cells; increased resting CD25, CTLA-4 and PD-1 expression; increased TNF-α+CD8+ T-cells in response to PMA

Selected methodological details and findings are given; for full methods and findings please refer to the original papers. Subjects with NTM-PD were diagnosed by the American Thoracic Society (ATS) 2007 criteria unless otherwise stated. MTB: *M. tuberculosis*; PBMC: peripheral blood mononuclear cells; PBM: peripheral blood monocytes; PHA: phytohemagglutinin; Ab: antibody; PPD: purified protein derivative; NDV: Newcastle disease virus; IFN: interferon; TNF: tumour necrosis factor; HLA-DR: human leukocyte antigen DR isotype; MAC: *M. avium* complex; IL: interleukin; LPS: lipopolysaccharide; FC: flow cytometry; RANTES: regulated on activation, normal T-cell expressed and secreted; LTA: lipoteichoic acid; MDM: monocyte-derived macrophages; PMA: phorbal 12-myristate 13-acetate; BCG: bacillus Calmette–Guérin; PD-L1: programmed death ligand 1; SEB: staphylococcal enterotoxin B; CF: cystic fibrosis; CTLA-4: cytotoxic T-lymphocyte antigen 4; T_{reg}: regulatory T-cells. [#]: ATS 1997 criteria; [¶]: other diagnostic criteria, or not stated; [+]: number of subjects not stated; [§]: method not stated.

which encodes IFN-γ. In subjects with NTM-PD, increased expression of genes involved in innate immunity and inflammation was associated with increased mortality, whereas expression of genes relating to T- and B-cell function were associated with survival.

The interpretation of these data is clouded by the potential immunomodulatory effect of mycobacterial infection on the immune response [91], as well as differences in study populations and the methodologies used. The weight of the evidence thus far suggests downregulation of the immune response in NTM-PD, although whether this is truly a predisposing factor or a result of disease remains unresolved. Furthermore, it is likely that immune responses vary according to the pattern or severity of disease and the NTM species.

Genetics

As with *M. tuberculosis* [92], it has been suggested that there may be a genetic contribution to disease susceptibility, supported by reports of familial clustering of NTM-PD [93]. Several studies have examined candidate genes related to the host response, with mixed results (table 3). Studies of human leukocyte antigens have variously associated the DR6 and A26 alleles and the A2-B21 haplotype with NTM disease [106, 114–116]. Mutations in the murine *Nramp1* gene confer susceptibility to mycobacterial disease; heterozygosity for three polymorphisms in the human homologue *NRAMP1* were associated with NTM-PD in one cohort [97], but conflicting findings were seen in a second study which reported a higher prevalence of homozygotes for two of these polymorphism in NTM-PD [102]. Two earlier studies found no relationship [94, 96]. While an earlier small study found no link between two polymorphisms in *IFNGR1* and NTM-PD [94], a recent study found a higher prevalence of the T-56C polymorphism [109]. Mutations linked with NTM-PD have also been reported in *IL10, IL28, TNFA* and an intron II of *TLR2* [105, 107, 111], but studies of *IL12RB1*, *MBL* and two polymorphisms in *TLR2* previously linked to mycobacterial disease found no association [99, 102, 103].

The high incidence of NTM-PD seen in CF has led to *CFTR* being proposed as a candidate gene for disease susceptibility, and the incidence of *CFTR* mutations in patients with NTM-PD disease is significantly higher than the general population. The reported proportion of NTM-PD patients with *CFTR* mutations ranges from 21% to 44% and the proportion with a sweat chloride >60 mmol·L^{-1} ranges from 3% to 22% [46, 58, 98, 100]. Studies in Asian populations with a low prevalence of CF have also demonstrated an association between *CFTR* polymorphisms and NTM-PD [101, 108]. One retrospective study addressed the issue of whether *CFTR* mutation merely predisposes towards bronchiectasis, which is in itself a risk factor for NTM-PD, or is independently associated [46]. The authors found a significantly higher incidence of *CFTR* mutation in patients with NTM-PD and bronchiectasis *versus* bronchiectasis alone (22% *versus* 5%), despite a similar proportion of each group receiving screening.

Other genes which have been linked to NTM-PD include alpha-1 antitrypsin (*AAT*) in two studies [98, 117], the vitamin D receptor (*VDR*) in one study [95], although this has not been replicated in Korean and Japanese populations [102, 104], and the macrophage stimulating 1 receptor (*MSTR1*) in a cohort of subjects with NTM-PD and thoracic skeletal abnormalities, where it was associated with reduced IFN-γ responses [112].

Two recent studies have utilised whole exome sequencing to search for genetic factors conferring susceptibility to NTM-PD. The first study performed whole exome sequencing on 69 subjects with NTM-PD and 18 unaffected relatives [110], focusing on genes in four candidate categories: immune, cilia, connective tissue and *CFTR*. NTM-PD cases were more likely to have variants in all categories compared with population controls. Interestingly, a higher number of variants were also seen in unaffected family members of NTM-PD cases compared with controls, except for immune-related genes where variants were observed almost exclusively in NTM-PD cases, perhaps indicating a particularly important role in mediating susceptibility to disease. *CFTR* variants were seen in 26% of subjects with NTM-PD (and 44% of unaffected relatives) compared with a population prevalence of 6%. An unbiased exome-wide search was also conducted to find any novel genes associated with NTM-PD which identified 89 genes, 53% of which belonged to one of the candidate categories.

The second study was conducted on the same cohort and used a genome-wide linkage analysis of nine families with NTM-PD, identifying a 20cM region of chromosome 6 with evidence of linkage [113]. At the gene level this was most significant for *TTK* which encodes a protein involved in cell division and replication, but linkage was also seen with *TPBG*, *ORC3* and *ANKRD6*. In a further genome-based association analysis, five genes reached genome-wide significance, including *MAP2K4*, which plays a role in TLR signalling, and *IFNLR1*, which encodes part of the receptor for IFN-λ.

As with the literature on immune defects in NTM-PD, interpretation of the result of these studies is complicated by the differing methodology used between studies performed in different populations of

TABLE 3 Summary of genetic studies in nontuberculous mycobacterial pulmonary disease (NTM-PD)

Study	Gene(s) examined	Population	Country	Methodology	Principal findings in NTM-PD
Huang *et al.* [94] (1998)	*NRAMP1, IFNGR1*	8 subjects with NTM-PD#, 22 healthy controls	USA	*NRAMP1*: PCR, HPLC and partial sequencing to detect G105D polymorphism; PCR and RFLP of D543N and 3′UTR polymorphisms; PCR and microsatellite sizing of promoter region polymorphism. *IFNGR1*: PCR and RFLP for nucleotide 395 polymorphism; PCR and HPLC for nucleotide 131 polymorphism	No polymorphisms were associated with NTM-PD
Gelder *et al.* [95] (2000)	*VDR*	56 subjects with NTM-PD¶ (all *M. malmoense*), 101 healthy controls	UK	PCR and RFLP for *FokI*, *ApaI* and *TaqI* polymorphisms	Higher prevalence of *TaqI* t and *ApaI* A alleles and lower prevalence of *FokI* f alleles in NTM-PD
Tanaka *et al.* [96] (2000)	*NRAMP1*	4 pairs of related subjects with NTM-PD¶	Japan	Sequencing of *NRAMP1*	Heterozygous missense mutation at codon 419 in one subject; no other relevant polymorphisms identified
Koh *et al.* [97] (2005)	*NRAMP1*	41 subjects with NTM-PD#, 50 healthy controls	South Korea	PCR and RFLP of INT4, D543N and 3′UTR polymorphisms	Higher rate of heterozygotes at INT4 (G/C), D543N (G/A) and 3′UTR (TGTG ins/del) in NTM-PD
Kim *et al.* [98] (2005)	*CFTR*	85 subjects with NTM-PD#	USA	Oligonucleotide probe panel (86 mutations)	Variants seen in 15 subjects (ΔF508, w1282x, R117H and D11152H)
Fowler *et al.* [46] (2006)	*CFTR*	98 subjects with bronchiectasis (10 with positive sputum NTM culture)	UK	Screening panel for ΔF508, G85E, R117H, 621 +1G>T, G551D, R553X, G542X, N1303X, and ΔI507 variants	Variants found in 2/9 subjects with NTM isolated compared with 4/77 of non-NTM subjects
Ryu *et al.* [99] (2006)	*TLR2*	80 subjects with nodular NTM-PD# and 84 healthy volunteers	South Korea	PCR and RFLP of Arg677Trp and Arg753Gln polymorphisms	No polymorphisms were associated with NTM-PD
Ziedalski *et al.* [100] (2006)	*CFTR*	50 subjects with bronchiectasis, of whom 30 had NTM-PD#	USA	PCR-based commercial screening assay (>1300 mutations)	Variants seen in 14/30 NTM-PD subjects and 10/20 non-NTM subjects
Mai *et al.* [101] (2007)	*CFTR*	300 subjects with NTM-PD# (all MAC), 100 healthy controls	Japan	PCR and RFLP to detect M470V polymorphism in exon 10, PCR and sequencing of poly-T and TG repeats in intron 8 (IVS8)	Higher rate of IVS8T5 allele in NTM-PD
Tanaka *et al.* [102] (2007)	*NRAMP1, VDR, MBL*	111 subjects with NTM-PD# (all MAC), two groups of 177 and 247 healthy controls	Japan	*NRAMP1*: PCR and sequencing of INT4, 3′UTR and D543N polymorphisms *VDR*: PCR and RFLP of *FokI* and *TaqI* polymorphisms *MBL*: PCR and RFLP of rs7096206 and rs1800450 SNPs	Higher rates of homozygotes for major alleles of D543N (G/G) and 3′UTR (TGTG ins/ins) of *NRAMP1* in NTM-PD; no polymorphisms in *VDR* or *MBL* were associated with NTM-PD
Kim *et al.* [58] (2008)	*CFTR*	63 subjects with NTM-PD, 32 healthy controls	USA	Commercial screening panel and sequencing of exons 1–27, 5′ and 3′ untranslated regions, 15 intronic bases flanking each exon and 1000 bp 5′ of exon 1	Variants found in 36.5% of subjects with NTM-PD compared with 15.6% of controls, including delF508, R117H, V754M, D1152H, R75Q, S1235R, G576A, R668C, R31C and R1162L
Park *et al.* [103] (2008)	*IL12RB1*	128 subjects with nodular NTM-PD, 240 healthy controls	South Korea	PCR and sequencing of +705A/G, +1158T/C, and +1196G/C SNPs	No polymorphisms were associated with NTM-PD
Park *et al.* [104] (2008)	*VDR*	124 subjects with nodule NTM-PD#, 127 healthy controls	South Korea	PCR and RFLP for *FokI* and *TaqI* polymorphisms	No difference between groups

Continued

TABLE 3 Continued

Study	Gene(s) examined	Population	Country	Methodology	Principal findings in NTM-PD
Yim *et al.* [105] (2008)	*TLR2*	193 subjects with nodular NTM-PD[#] 191 controls	South Korea	PCR of 150 bp region surrounding GT repeat	Short GT repeats more common in NTM-PD; S-alleles more common in NTM-PD compared with controls in MAC, but not *M. abscessus*
Matsuyama *et al.* [106] (2010)	*CFTR, NRAMP1*[+]	Five members (3 affected) of family with outbreak of MAC NTN-PD[¶]	Japan	CFTR: Commercial screening panel and PCR and sequencing of poly-T and TG repeats in intron 8 (IVS8) NRAMP1: PCR and sequencing of INT4, 3′UTR and D543N polymorphisms	No polymorphisms were associated with NTM-PD
Affandi *et al.* [107] (2013)	*TNFA, BAT1, IL1A, IL2, IL4, IL18, CCL2, VDR, CD14, IL10, IL12B, SLC11A1, IL28B,*	79 subjects with NTM-PD (all MAC), 188 healthy controls	Australia	Taqman probes for 16 known SNPs (rs1800629, rs1799964, rs9281523, rs17561, rs2069762, rs2243250, rs187238, rs1946518, rs1024611, rs10735810, rs2569190, rs1800896, rs3212227, rs17235409, rs8099917, rs12979860)	SNPs in *IL12B* (rs8099917), *TNFA* (rs1799964) and *IL10* (rs1800896) associated with NTM-LD
Jang *et al.* [108] (2013)	*CFTR*	300 subjects with NTM-PD, 446 healthy controls	South Korea	Whole exon sequencing (60 NTM-PD subjects); MALDI-TOF MS of identified variants	Higher frequency of Q1352H variant in NTM-PD
Farnia *et al.* [109] (2017)	*IFNGR1*	80 subjects with NTM-PD and 80 healthy volunteers	Iran	PCR and RFLP of T-56C SNP	Significantly higher frequency of C-allele in NTM-PD
Szymanski *et al.* [110] (2015)	Exome-wide	69 subjects with NTM-PD, 18 unaffected family members	USA	Whole exome sequencing	Significantly higher number of variants in immune (including *STAT1, IRF8, MPEG1, CARD9*), cilia (including *RSPH1* and *MST1R)* and connective tissue (including *FBN2* and *COL5A1*) related genes; *CFTR* variants seen in 23% of NTM-PD subjects and 44% of family controls
Halstrom *et al.* [111] (2017)	*IL10*	124 subjects with NTM-PD, 229 healthy controls	Australia	Custom TaqMan Genotyping plates for 7 known SNPs (rs3024498, rs1518111, rs3021094, rs3024491, rs1800872, rs1800871, rs3024497)	One SNP (rs1518111) associated with NTM-PD
Becker *et al.* [112] (2017)	*MST1R*	11 subjects (2 related) with nodular NTM-PD and pectus excavatum and scoliosis	USA	Whole exome sequencing	Rare missense variant (p.V900M) in *MST1R* seen in both related subjects and 2 subjects with sporadic disease; further analysis revealed the same variant in 6 relatives of the familial cases.
Chen *et al.* [113] (2017)	Exome-wide	9 families with NTM-PD (16 affected, 20 unaffected), 57 subjects with sporadic NTM-PD	USA	Whole exome sequencing, variant-level and gene-level linkage analysis	Variant-level analysis identified a region of chromosome 6 with evidence of linkage; gene-level analysis of chromosome 6 found suggestive linkage of the *TTK* gene as well as *TPBG, ORC3* and *ANKRD6*; further genome-wide analysis identified variants in *MAP2K4, RCOR3, KRT83, IFNLR1* and *SLC29A1* associated with NTM-PD

Selected methodological details and findings are given; for full methods and findings please refer to the original papers. The nomenclature for genes and variants is that used in the original studies. Subjects with NTM-PD were diagnosed by the American Thoracic Society (ATS) 2007 criteria unless otherwise stated. RFLP: restriction fragment length polymorphism; SNP: single nucleotide polymorphism; HPLC: high performance liquid chromatography; bp: base pair; MAC: *M. avium* complex; MALDI-TOF MS: matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. [#]: ATS 1997 criteria; [¶]: other diagnostic criteria, or not stated; [+]: now termed *SLC11A1*.

varying ethnicities. It may also be difficult to untangle whether such mutations merely predispose to bronchiectasis, which is in itself a risk factor for NTM-PD, or make an independent contribution to disease susceptibility. The weight of the evidence thus far implicates *CFTR* mutations as a risk factor, but the data for other genes is inconsistent. It seems increasingly likely that the genetic contribution to NTM-PD is complex and involves multiple variants in multiple pathways, making the identification of a target for intervention challenging.

Diagnosis

The diagnosis of NTM-PD depends upon the integration of clinical, radiological and microbiological findings, summarised in the American Thoracic Society and Infectious Diseases Society of America (ATS/IDSA) 2007 criteria which have become the accepted disease definition [8]. The presenting symptoms of NTM-PD are nonspecific [118] but the radiological presentation is more suggestive, typically falling into two patterns: bronchiectasis, with nodules ("nodular bronchiectatic" disease) or cavitation with fibrosis ("fibrocavitary" disease) (figure 1) [119, 120].

Detection of NTM

To be able to apply current diagnostic criteria, a minimum of three respiratory samples should be obtained. Storage of sputum samples at either room temperature or in a refrigerator does not affect the yield of microscopy and culture of MAC bacteria, which enables home sampling and sample submission to centralised laboratories by mail [121]; this has not been investigated for other NTM. In nodular bronchiectatic disease, with its generally lower bacterial load, small cohort studies have suggested that the sensitivity of bronchoalveolar lavage sample cultures is higher than that of sputum culture [122, 123]. In a small study of 26 patients with suspected MAC nodular bronchiectatic lung disease, bronchoalveolar lavage yielded positive cultures in 13, *versus* only six by sputum cultures [122]. Automated liquid culture systems have become the reference method for mycobacterial culture. Still, incubation of samples on both liquid and solid media at 37°C increases the sensitivity of culture (figure 2) [124].

Identification of NTM isolates

Molecular tests, being either line probe assays or gene sequencing-based approaches, are the favoured method for NTM identification. Several commercial tests detecting the most frequent species exist, although all detect a limited number of species or species complexes and have suboptimal specificity; for example, most tests fail to discriminate the species of MAC [124, 125]. Matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) mass spectrometry has revolutionised clinical bacteriology and is also useful for NTM identification, although it is a labour-intensive method and its sensitivity and specificity rely on the quality of protein extraction and the mass spectra databases of the system used [126]. Next generation sequencing of whole genomes is likely to supersede current molecular identification algorithms in the centres that have access to this technology.

Correct identification of NTM species is of paramount importance, as the different species differ in their ability to cause pulmonary disease. Identification of species well known to cause pulmonary disease (MAC,

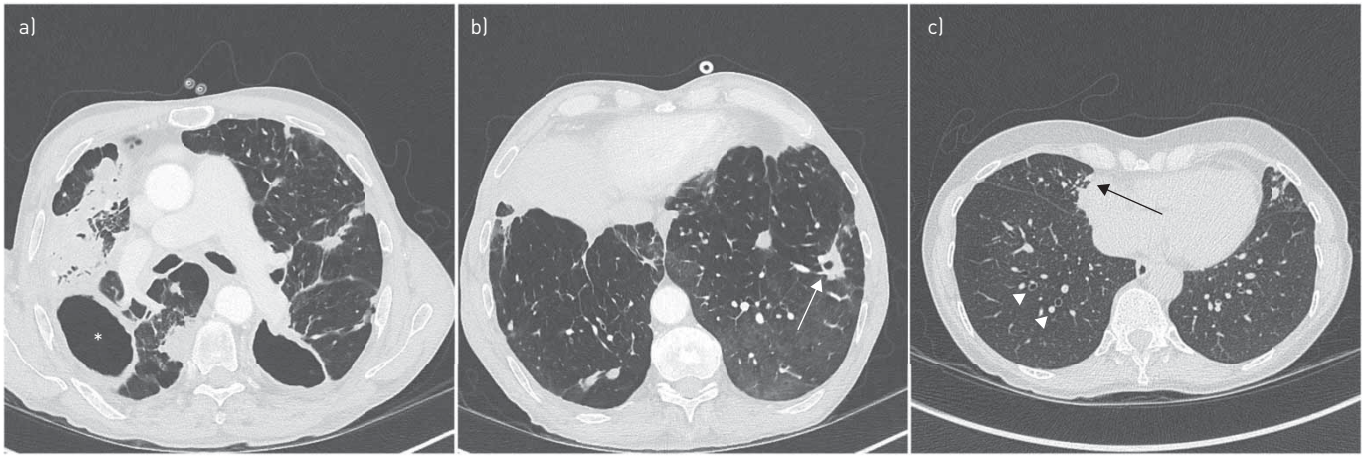

FIGURE 1 The appearance of pulmonary nontuberculous mycobacterial disease on high-resolution computed tomography: a) fibrocavitary disease, b) cavitating nodules and c) nodular bronchiectatic disease in the middle lobe and lingua in a patient with "Lady Windermere" syndrome. Asterisk: cavity; white arrow: cavitating nodule; arrowheads: bronchiectasis; black arrow: nodules.

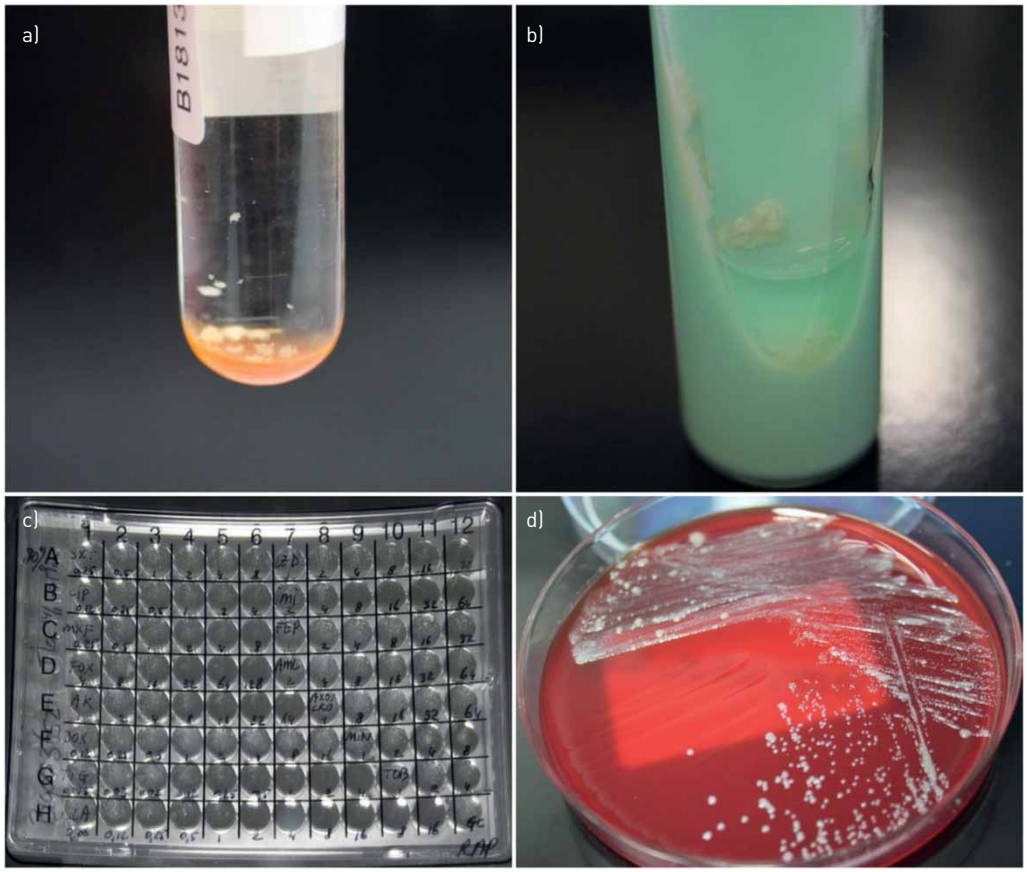

FIGURE 2 The microbiological diagnosis of pulmonary nontuberculous mycobacterial disease. a) Growth of *Mycobacterium abscessus* in broth medium. Mycobacteria mostly grow in clumps or flakes, at the bottom of the tube. b) *Mycobacterium abscessus* growing on Lowenstein–Jensen medium. The cream coloured and waxy colonies set *M. abscessus* and other nonchromogenic nontuberculous mycobacteria clearly apart from *M. tuberculosis*, which grows as white and drier, cauliflower-shaped colonies. c) *Mycobacterium abscessus* susceptibility testing by broth microdilution. d) Smooth *Mycobacterium abscessus* colonies on a sheep blood agar plate.

M. abscessus, M. malmoense, M. xenopi) should prompt intensive investigation, whereas samples yielding typically saprophytic species such as *M. gordonae* and *M. chelonae* might not require that, depending on the clinical background [127].

Culture-free NTM detection

While the culture of respiratory samples remains the cornerstone of diagnosis, culture-free methods offer the potential to provide a more rapid diagnosis and avoid the challenges of NTM culture.

Similar molecular methods to those used in species identification may be applied to DNA extracted from respiratory samples to directly detect and identify NTM without the need for culture [128–133]. However, the reported sensitivity is low, detecting NTM in only 29–76% of culture positive samples [128–130, 132], although better detection rates are reported in more heavily smear-positive samples. Most reported methods also target a limited range of species [128, 129, 132] and so will also miss rare or novel species. Nevertheless, as sequencing technology steadily advances, optimisation of these techniques may overcome these challenges and they could potentially form a part of future clinical practice.

Several studies have looked at the use of serological testing to detect NTM-PD. In a large meta-analysis of a predominantly Japanese cohort, an assay measuring IgA against glycopeptidolipid (GPL) antigens present in the cell wall of MAC reported an overall sensitivity of 69.6% and specificity of 90.6% [134]. However, significant cross-reactivity was seen with rapidly growing mycobacteria and up to 7% of healthy controls also reported a positive result. Within the CF population, measurement of IgG against the mycobacterial A60 antigen was reported to show a sensitivity of 86.7% and specificity of 95.1% for the diagnosis of *M. abscessus* disease [135], and a multi-antigen IgG assay against *M. abscessus* reported a

sensitivity of 95% and specificity of 73% [136]. Both studies suggested a correlation with disease activity although the numbers were small.

While serology will not replace microbiological methods, it may have a useful role in certain circumstances, for example when obtaining repeated respiratory samples is not possible. It may be useful in disease surveillance as a means of identifying those at high risk of disease development [136], or even potentially in the monitoring of disease activity and response to treatment, although further work needs to be done in this area.

Drug susceptibility testing

Broth microdilution (figure 2c) is the recommended platform for drug susceptibility testing of NTM; technical guidance documents have been published by the Clinical Laboratory Standards Institute [137] and endorsed by the ATS/IDSA guideline on diagnosis and treatment of NTM-PD [8]. Testing susceptibility of NTM to macrolides and amikacin (and rifampicin for *M. kansasii*) is of clear clinical importance, as resistance to these drugs is associated with poor outcomes of treatment with these agents. For MAC, there is a clear correlation between baseline macrolide susceptibility of the causative strain and the outcome of treatment with rifampicin–ethambutol–macrolide regimens [138, 139]. In a case series from Japan, culture conversion rates were 72% overall, but only 25% in patients whose primary MAC isolates were already macrolide resistant [139]. For amikacin, it was recently established that isolates with minimal inhibitory concentrations (MICs) >64 $mg{\cdot}L^{-1}$ typically show resistance-conferring mutations in the 16S rDNA (rrs) gene and are isolated from patients with extensive exposure to aminoglycosides [140]. Similarly, the recent clinical trial of amikacin liposome inhaled suspension (ALIS) (previously known as liposomal amikacin for inhalation) reported failure of treatment associated with baseline or acquired amikacin resistance, evidenced by MICs >64 $mg{\cdot}L^{-1}$ [141]. Based hereupon, the tentative breakpoint for resistance is an MIC >64 $mg{\cdot}L^{-1}$; such MICs should lead to cessation of amikacin therapy, as it is unlikely to improve outcomes [140, 141]. Tentative breakpoints for linezolid and moxifloxacin are also provided by the Clinical Laboratory Standards Institute; for these, *in vitro–in vivo* correlations have not been established [137, 142].

For *M. abscessus*, too, *in vitro–in vivo* correlations are most evident for macrolides and amikacin. Resistance to macrolides may be evident on initial testing (constitutive resistance, mediated by mutation in the 23S ribosomal RNA gene) or develop after incubation in macrolide-containing media after at least 14 days (inducible resistance, mediated by the *erm(41)* gene) [142]. Inducible macrolide resistance is present in most strains of *M. abscessus* subsp. *abscessus* and subsp. *bolletii* but absent in *M. abscessus* subsp. *massiliense*, owing to a large deletion in the *erm(41)* gene. In case series, it has become evident that the outcomes of macrolide-based treatment regimens are poorest in patients infected by *M. abscessus* strains with inducible macrolide resistance (25%–30% culture conversion) and best in those with *M. abscessus* subsp. *massiliense* (70–80% culture conversion) [143, 144]. In a trial of ALIS, outcomes in *M. abscessus* disease were poor and treatment failure occurred with the emergence of mutational resistance [145]. For other important drugs, including tigecycline and the β-lactams cefoxitin and imipenem, such studies are lacking, although pharmacodynamic models show that their efficacy is eventually annulled by emergence of resistance [146, 147].

For *M. kansasii*, susceptibility testing to rifampicin and macrolides is recommended, but only for rifampicin, there is evidence of treatment failure, with the emergence of rifampicin resistance [148]. For other NTM species, *in vitro–in vivo* correlations have not been studied, but drug susceptibility testing to macrolides, fluoroquinolones, linezolid, tetracyclines and aminoglycosides is used to guide treatment regimens.

Treatment

A challenging aspect of NTM disease is that, unlike TB, the diagnosis of disease does not necessitate treatment. A large study of patients with MAC found that nearly one quarter remained stable after 3 years without treatment [149], and in a series restricted to nodular bronchiectatic MAC half remained stable without treatment after a mean of 32 months follow-up [150]. A retrospective study of prognostic factors in MAC disease reported that 80% of subjects were initially treated with observation, of whom only 14% went on to receive any treatment [151]. The choice of initial observation was not associated with adverse outcomes. A series of subjects with *M. abscessus* also found more than half did not require treatment over a median follow-up of 1265 days [152]. In addition, treatment is associated with a high rate of adverse events with no guarantee of cure. The decision on who to treat and when to start treatment is therefore of great importance.

One important factor is the identity of the isolated species, as the clinical relevance varies between species [12]. The radiological pattern of disease is also highly important as cavitation has been repeatedly associated with disease progression in MAC [149–151]. Further reported multivariate predictors of disease

progression requiring treatment in one study of MAC were increased age, lower body mass index (BMI), presence of systemic symptoms, positive sputum smear and the number of involved lobes [149].

Predictors of mortality may also help guide treatment decisions, although it must be borne in mind that these will not identify subjects in whom treatment is more likely to be successful, but rather those with a poor prognosis even with treatment. Such factors include: cavitary, consolidative or infiltrative radiological patterns, male sex, increasing age, low income, higher healthcare utilisation, low BMI, low forced vital capacity, haemoptysis, low albumin, lymphopaenia, raised erythrocyte sedimentation rate, anaemia, previous TB, the presence of comorbidities, immunosuppression, chronic pulmonary aspergillosis and pulmonary hypertension [151, 153–160]. A summary of the factors to be considered in the decision to treat is shown in figure 3.

Before starting treatment, the management of the underlying lung disease and associated comorbidities should be optimised, including treatment of other pathogens, such as *Pseudomonas*. Consideration should also be given to the ultimate goal of treatment. While in most cases treatment may be given with curative intent, in some circumstances a cure may not be achievable and treatment aimed at stabilising disease progression and controlling symptoms. In this scenario a less efficacious but better-tolerated regimen may be selected, particularly in the elderly, who are at increased risk of drug intolerance. In contrast, in the context of CF, infection with *M. abscessus* may preclude transplantation and an aggressive approach aimed at maximising the chance of cure at the expense of an increased risk of adverse events may be chosen. In each case the management plan should be formulated in partnership with the patient, with a shared understanding of the rationale for treatment, its limitations and the risk of adverse events.

There is a relative lack of randomised controlled trials in the treatment of NTM-PD. The majority of evidence is provided by cases series, and for less common species there is no evidence to guide treatment. The aforementioned ATS/IDSA statement on NTM-PD [8] gave recommendations on treatment of major NTM species that have been widely adopted, and an updated guideline in association with the European Respiratory Society and European Society of Clinical Microbiology and Infectious Diseases is in preparation. Recommendations have also recently been published by the British Thoracic Society and several other specialist societies have also issued guidelines [161–163].

M. avium complex

The standard of care for the treatment of MAC is a macrolide-containing regimen, typically in combination with rifampicin and ethambutol, although reported outcomes vary. The efficacy of this combination was demonstrated by a large retrospective series which found it achieved maintained culture conversion in 82% of 180 subjects with macrolide susceptible nodular bronchiectatic disease, although subsequent relapse was seen in 48% [164]. Outcomes in a more heterogeneous population were reported in a meta-analysis encompassing 1462 subjects across 16 studies (not including the above), finding a more modest overall culture conversion rate of 60% [165]. The use of a range of companion drugs and dosing regimens, the varying inclusion of fibrocavitary and macrolide resistant disease and exclusion of patients not completing treatment in the former study may go some way to explaining this discrepancy [166].

The treatment of refractory and macrolide resistant disease remains challenging. For refractory MAC lung disease, two recent studies of inhaled liposome encapsulated amikacin have shown improved

Host factors	Disease severity	Disease progression	Clinical relevance
Age Increasing risk of intolerance and adverse events **Comorbidities** **Drug intolerances** Consider dose reduction or thrice-weekly regimens Consider interactions with other drugs, *e.g.* azoles **Patient wishes** **Aim of treatment** Aiming for cure or disease control?	**Radiological** Fibrocavitary disease **Clinical** Weight loss, fever, haemoptysis, respiratory failure Biochemical markers **Microbiological** Smear positivity	**Radiological** Development of cavitation or fibrosis, increasing nodules or tree-in-bud changes **Clinical** Worsening symptoms, development of new symptoms, weight loss **Microbiological** Development of new or increasing smear positivity	**NTM species** Some species more pathogenic than others **Immunosuppression** Primary immunodeficiency HIV infection Immunosuppresive therapy Anti-TNF-α therapy Corticosteroids **Lung transplantation** Need for *M. abscessus* eradication

FIGURE 3 Factors to consider when deciding on the initiation of treatment for pulmonary nontuberculous mycobacterial (NTM) disease. TNF: tumour necrosis factor.

microbiological outcomes compared with continuation of standard multidrug therapy alone [141, 145]. For macrolide resistant MAC disease, two studies have shown that a combination of parenteral antibiotic administration and surgical resection of involved lung is associated with favourable microbiological outcome [167, 168], while antibiotic therapy was associated with treatment failure and high mortality. A study using combinations of rifabutin, clofazimine, linezolid and moxifloxacin reported similarly poor sustained culture conversion in only 18% of patients [169].

M. abscessus *complex*

The M. *abscessus* complex is comprised of three subspecies: subsp. *abscessus*, subsp. *massiliense* and subsp. *bolletii*. Subspecies determination of *M. abscessus* complex isolates is necessary to guide therapy, as two subspecies (subsp. *abscessus* and subsp. *bolletii*) have an active inducible macrolide resistance gene (*erm*41) and are macrolide resistant, while subsp. *massiliense* has an inactive *erm*41 gene and is macrolide susceptible. It is imperative that *M. abscessus* complex isolates should be identified to subspecies and that macrolide susceptibility is also determined phenotypically in all *M. abscessus* complex isolates.

Due to the intrinsic drug resistance of the *M. abscessus* complex, treatment is difficult and regimens typically require large numbers of agents with an induction phase of parenteral therapy lasting several weeks to possibly months. A series of 41 subjects treated with a macrolide plus prolonged parenteral therapy with amikacin, with or without imipenem or cefoxitin (median 230 days), achieved sputum conversion in 81% with a 12% relapse rate during follow-up [170]. Another retrospective study found a conversion rate of only 48% using combinations of amikacin, imipenem and a macrolide plus surgery where indicated [171].

As mentioned previously, a strong link has been observed between macrolide resistance in *M. abscessus* complex and treatment failure. A meta-analysis of treatment outcomes revealed that in contrast to the disappointing sustained sputum conversion rates of undifferentiated *M. abscessus* complex (59%), studies reporting the specific treatment response in subsp. *massiliense* reported sputum conversion rates of up to 79%. In contrast the conversion rate seen in subsp. *abscessus* was very poor (35%) [172]. While this offers the hope that less intensive treatment regimens may be used in subsp. *massiliense* infection [173], since the majority of isolates belong to subsp. *abscessus* there is a pressing need for better treatment regimens in this group.

The treatment of refractory or macrolide resistant disease also remains challenging. Tigecycline has *in vitro* bactericidal activity [146] and treatment was associated with a clinical response in 44% of patients in a small series but there are no data on microbiological outcomes [174]. Clofazimine-containing regimens have been reported to reduce semiquantative culture scores and achieved culture conversion in 24–50% [175, 176].

New approaches to treatment

Oxazolidinones

The oxazolidinone linezolid has *in vitro* activity against many NTM [177, 178]. There are several reports of its successful use in NTM disease [179–186], including a series of 16 patients with disseminated disease due to anti-IFN-γ antibodies where a 50% response rate was seen with linezolid-containing regimens [187]. Side-effects were reported in 45% of subjects in the largest case series to date [188]. The newer agent tedizolid shows greater *in vitro* activity [189–191], and one report suggests it is better tolerated [192], although there is only one case report to date of successful clinical use [193].

Inhaled amikacin

Delivering amikacin through inhalation offers the advantage of limiting systemic toxicity and achieving high drug levels at the site of disease. Data from a retrospective study of 20 subjects with refractory MAC and *M. abscessus* disease found the addition of inhaled amikacin achieved culture conversion in 25% and an improvement in symptoms in 45% [194]. More recently, a randomised controlled trial of the addition of ALIS to an existing regimen for refractory MAC and *M. abscessus* disease found that more patients receiving ALIS achieved >1 negative culture by day 84 (32% *versus* 9%), which was maintained until day 168 in 79% in those who converted to negative [145]. A greater response was seen in MAC, and a subsequent phase 3 trial found a significantly higher 6-month culture conversion rate with the addition of ALIS (29%) *versus* placebo (8.9%) to guideline based therapy [141]. ALIS has now been approved for use for this indication in the USA. A trial in *M. abscessus* subsp. *abscessus* is ongoing (NCT03038178).

Antituberculous drugs

The new agent bedaquiline was developed for multidrug-resistant TB (MDR-TB) and is bacteriostatic against a range of NTM, including *M. abscessus* [195] and MAC [196–198]. However, several species,

notably *M. xenopi*, show resistance conferred by a mutation in the *atpE* gene [198, 199], and emergence of resistance conferred by mutation in the *mmpT5* gene has been reported in *M. intracellulare* [200]. There has been only one report of its use in NTM-PD, where in 10 subjects with refractory disease receiving 6 months of treatment a radiological improvement was seen in 40% and an improvement in semiquantiative culture scores was seen in 50%, but none achieved sustained culture conversion [201]. Another new agent for MDR-TB, delamanid, shows *in vitro* activity against MAC [202]; however, there are no reports regarding its clinical efficacy.

Mefloquine is an antimalarial agent which has antimycobacterial activity [203] and shows *in vitro* and *in vivo* activity against *M. avium* [204]. One case report describes its successful use as part of a regimen in refractory MAC disease [183] but its clinical efficacy was unclear in another report [205]. The neuroleptic thioridazine also has antimycobacterial activity, has shown *in vitro* activity against *M. avium* and accumulates in high concentrations within macrophages, but is limited by neurological and cardiac toxicity [206] and there are no reports of its clinical use in NTM-PD.

Drugs in development

The ketolide antibiotic solithromycin shows activity against *M. avium* and has the advantage of being resistant to erythromycin resistance methylases (such as that encoded by the *erm(41)* gene) [207], although it displays significant hepatotoxicity [208].

The search for drugs to treat MDR-TB may also identify further agents with useful activity against NTM. A study which screened 129 compounds previously identified as active against *M. tuberculosis* identified 10 compounds showing activity against NTM. These included three oxazolidinones and one macrolide, but further diverse possible targets were identified including a cytoskeletal protein (FtsZ) DNA gyrase (GyrB), dihydropholate reductase (DHFR), RNA polymerase, the ABC transporter and a mycolic acid transporter (MmpL3) [209]. The latter has been repeatedly identified as a potential therapeutic target [210] and several agents targeting MmpL3 have been shown to display *in vivo* activity against NTM [211, 212].

Beta-lactamase inhibition

Overcoming antimicrobial resistance mechanisms may be another route to expanding the available armamentarium. Deficiency of the beta-lactamase Bla_{Mab} renders *M. abscessus* susceptible to amoxicillin and ceftaroline, and allows synergy between imipenem and amikacin, which in combination displayed bactericidal activity [213]. Addition of the beta-lactamase inhibitor avibactam increased the intracellular activity of ceftaroline in this study, and it has also been shown to increase the activity of imipenem [214] and carbapenems including ertapenem [215]. The commercially available combination ceftazidime/avibactam also shows *in vitro* activity against MAC [216].

Immunomodulation

Another treatment approach is to utilise the immune response to fight infection. The largest trial of such treatment looked at immunotherapy with *M. vaccae* in a randomised controlled trial [217] and found no benefit. Case reports have suggested benefit from adjuvant IFN-γ (usually in the context of immunodeficiency) [218–222], but data from clinical trials in NTM-PD are limited and inconsistent. The addition of inhaled IFN-γ showed no benefit in one trial [223], but in another parenteral therapy was associated with a significantly higher response to treatment (83% *versus* 36%) and higher rate of sputum conversion (92% *versus* 50%), although the baseline drug regimen was unconventional [224]. A recent case report found no benefit in the supplementation of IFN-γ to two subjects with impaired baseline IFN-γ responses [79]. Two reports have also found benefit from the addition of subcutaneous or inhaled GM-CSF in CF and *M. abscessus* infection [225, 226], potentially through activation of macrophages and improved cellular immunity. Nitric oxide plays a key bactericidal and bacteriostatic role in the host defence against mycobacteria [227], and one case report of two subjects with CF demonstrated a marked fall in *M. abscessus* sputum load during treatment with inhaled nitric oxide [228]. One interesting report showed a clinical and microbiological response to the addition of thalidomide in a subject with disseminated MAC [229]. The authors suggest that selective inhibition of TNF-α and amelioration of immune-mediated pathology was responsible.

Summary

Despite an increasing body of research and international collaboration, fundamental questions remain unanswered in NTM-PD. The prevalence is increasing but the reason for this is unclear, and the lack of notification in most areas complicates even estimating the exact disease prevalence. A concerning finding of recent studies has been the evidence of between-person transmission of NTM, although as the route by which infection is acquired (or spread) is uncertain it is not possible to formulate evidence-based

recommendations for infection control. There is an urgent need for better understanding of these factors, particularly in the context of CF.

No single unifying host factor has been identified as conferring susceptibility and it seems likely that a variety of different pathways increase an individual's risk of infection, which combined with environmental factors culminates in the development of NTM-PD. Mutations in CFTR have been consistently linked with disease, and the results provided by exome sequencing regarding variants in cilia, immune and connective tissue associated genes also provide a useful avenue for further research.

Once NTM-PD is diagnosed the decision to treat is challenging and work in this area should focus on the development of markers of disease activity and predictors of progression, in order to better target who to treat and who to observe. Treatment outcomes remain suboptimal and it is disappointing that more than 10 years have elapsed since the ATS guidelines were published in 2007, but treatment for MAC has not progressed, with failure rates of 18–40%. The management of *M. abscessus* has been informed by our increasing understanding of its taxonomy, including the discovery of inducible macrolide resistance, and reports of multidrug regimens with prolonged parental therapy have demonstrated the ability to achieve maintained sputum conversion, although outcomes in macrolide resistant disease remain dismal.

In refractory disease the development of ALIS represents the first agent specifically licensed for use in NTM-PD, the result of the collaborative effort of more than 100 centres across the world. Other agents such as tigecycline, oxazolidinones and bedaquiline show promising *in vitro* efficacy and have already entered clinical use, but the evidence base is lacking at present. However, while recent studies have rightly focused on the treatment of refractory disease, there is also the need for better first-line treatment options.

Conflict of interest: S. Cowman has nothing to disclose. J. van Ingen has nothing to disclose. D.E. Griffith reports grants, personal fees for consultancy and non-financial support from Insmed Inc., outside the submitted work. M.R. Loebinger reports personal fees for advisory board work and lecturing from Insmed, personal fees for advisory board work from Savara, outside the submitted work.

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