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Treating nontuberculous mycobacteria in children with cystic fibrosis: a multicentre retrospective study

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Abstract

Background: Respiratory infection with nontuberculous mycobacteria (NTM) in children with cystic fibrosis (CF) has increased in prevalence. The condition is difficult to diagnose and treatments are complex with limited evidence to guide practice. This study describes the approaches to diagnosis, management and consequences of treatment in a multi-centre cohort of children with CF in the UK.

Methods: Retrospective data were collected from 11 CF specialist centres from patients less than 17 years old, treated for NTM infection between 2006-2017. Descriptive statistics were used to describe the clinical characteristics of children treated. Treatment regimens, adverse events and success of treatment, with respect to lung function and culture conversion, were evaluated.

Results: Data from 70 patients treated for NTM pulmonary disease were collated (60 *Mycobacterium abscessus complex* (MABSC); 10 *M. avium complex* (MAC)). Older age and previous diagnosis of allergic bronchopulmonary aspergillosis were all significantly associated with NTM. There was a wide variance in drug choice and side effects were reported with all agents. NTM eradication occurred in 80% of patients with MAC and 48% with MABSC, with variable outcomes on lung function.

Conclusions: Diagnosis and treatment of NTM infection in children with CF is challenging. Treatment success is not guaranteed, particularly for MABSC. Large clinical trials are urgently required to evaluate treatment regimes and their suitability and efficacy in children.

Introduction

Cystic fibrosis (CF) remains the most common inherited life-limiting condition in Europe, North America and Australia with an incidence of around 1 in 2,500 (1). Respiratory infection with nontuberculous mycobacteria (NTM) has become a subject of increasing clinical concern in people with CF over the last decade. During this time the prevalence of NTM infection has risen in children with CF from 1.3% in the United Kingdom (UK) CF Registry in 2010 to over 3% from 2015 onwards (2,3).The two most common types of NTM isolated are *Mycobacterium abscessus* complex (MABSC) and *Mycobacterium avium* complex (MAC) (4).

MABSC infection is of particular concern because it is associated with a more rapid decline in lung function compared to other pathogens (5,6) and is a strong relative contraindication to lung transplantation in many centres (7,8). Furthermore, it has been reported that MABSC may be transmitted between individuals with CF (9,10).

The clinical management of people with CF who isolate NTM represents an area of evolving expertise and practice (11,12). Guidelines have been published by the United States CF Foundation and European CF Society in 2016 and by the British Thoracic Society (non-CF specific) in 2017 (13,14). However, there is a lack of clinical evidence, especially in children. A single centre study recently reported experience of treating 26 children with MABSC with eradication achieved in 65% using a standardised regime(15).

There are no paediatric-specific criteria for NTM pulmonary disease (NTM PD) and most clinicians use the adult-based 2007 American Thoracic Society (ATS)/Infectious Disease Society of America definition, which involves clinical, radiological and microbiological criteria (16). Treatment for NTM is a major burden for patients as it requires prolonged courses of multiple antibiotics, many of which are associated with frequent and multiple adverse effects. Evidence to support the effectiveness of individual regimes is lacking (12,13).

The aim of this study was to collect 'real-world' data from multiple centres on the treatment of NTM in children with CF. Specifically, we report the antibiotic regimes used, their tolerability and effectiveness as well as patient outcomes across 11 large centres. These data will increase knowledge about how to best manage NTM infection in children with CF and guide areas for future research.

Methods

Directors of 11 large centres, caring for approximately 45% of children with CF in the UK, were approached and agreed to participate. Children aged >17 years, who received active therapy for respiratory culture of any species of NTM between 01/04/2006–05/07/2017, were included.

Data were collected by a member of the clinical team at each site and recorded on a RedCAP on-line database using a custom-designed proforma (Supplementary Figure 1). This included anonymised demographics, relevant co-morbidities, lung function, respiratory sample type, microbiological details, NTM treatment and adverse effect data (collected by chart review).

Results were summarised in tables as numbers and proportions for categorical data and median values with interquartile ranges for continuous data. Univariate tests were used to compare characteristics of groups where applicable. All analyses were carried out using the R statistical language, SPSS 24 and Graphpad Prism 6.

The study was performed as a multi-site audit of practice and was registered as a national audit. Ethical approval was not required as data were anonymised with no identifiable information collected.

Results

Demographics and clinical characteristics of children treated for NTM infection

Eleven centres collected data from 70 children. The median age was 11 years (interquartile range [IQR] 8-13 years), 57% were female and median body mass index was 16.6 kg/m^2 (IQR 15-18.4). Patient characteristics are summarised in Table 1.

Characteristics	n	%	Median (IQR)
Total	70	100	-
Age (years)	70	100	11 (8-13)
Sex (Female)	40	57	-
Body mass index, (kg/m^2)	70	100	16.6 (15-18.4)
Microbiology in the year prior to NTM diagnosis			
Pseudomonas aeruginosa	33	47	-
Staphylococcus aureus	33	47	-
Aspergillus sp.	27	39	-
Stenotrophomonas maltophilia	3	4	-
Haemophilus influenzae	3	4	-
Candida sp.	3	4	-
Comorbidities			
Allergic bronchopulmonary aspergillosis*	12	17	-
Pancreatic insufficiency	66	94	-
CF-related diabetes	8	11	-
CF-related liver disease	17	24	-
Medications received in the year prior to NTM diagnosis			
Oral proton pump inhibitor	31	44	-
Oral corticosteroids	16	23	-
Macrolide	30	43	-

Table 1. Characteristics of children treated for NTM infection.

* defined as a clinical episode in the year preceding NTM diagnosis. Abbreviations: NTM nontuberculous mycobacteria; CF cystic fibrosis.

In the year prior to NTM diagnosis, the majority of patients (70%) had grown >2 other recognised CF pathogens with the commonest being *Pseudomonas aeruginosa* (47%), *Staphylococcus aureus* (47%) and *Aspergillus* sp. (39%). Less common

pathogens included *Stenotrophomonas maltophilia, Haemophilus influenzae* and *Candida* sp in 4% of individuals. In the preceding year 44% had received a proton pump inhibitor, 43% had received a macrolide antibiotic and 23% had received oral corticosteroids. The most common comorbidities were pancreatic insufficiency (94%), CF-related liver disease (24%), allergic bronchopulmonary aspergillosis (17%) (defined as a clinical episode in the year preceding NTM diagnosis) and CF-related diabetes (11%).

Symptoms most likely to be reported when NTM was first detected were increased cough (66%), increased sputum (39%), breathlessness (20%) and systemic symptoms (predominantly weight loss, 16%). Twenty percent of patients were reported to be asymptomatic.

FEV_1 at diagnosis of NTM infection was significantly lower than best recorded 'baseline' FEV_1 in the preceding year for both MAC (65[8] vs 76[6]%; p=0.002) and MABSC (76[3] vs 85[3]%; p<0.0001), and remained lower across all but one (3 months for MAC) subsequent time-points (Figure 1). FEV_1 at diagnosis of NTM infection and at 'baseline' was generally lower in those children with MAC compared to MABSC.

NTM species isolated and treated

Sixty patients were treated for MABSC (58 *M. abscessus abscessus*; 2 *M. bolletii*) and 10 for MAC (7 *M. avium*; 3 *M. intracellulare*). A breakdown of MABSC and MAC infections by centre is shown in Supplementary Table 1. Eighteen (30%) patients with MABSC and one (10%) with MAC were smear positive. *ERM* gene status was only reported in 2. Twelve (17%) patients isolated >1 NTM species (data only analysed for first NTM infection). Eight patients treated for MABSC isolated a MAC species before

(n=4) or after (n=4) MABSC was detected. Four patients treated for MAC isolated a MABSC species before (n=2) or after (n=2) MAC was detected.

NTM diagnosis

In 56% of patients NTM was detected as part of screening when asymptomatic, in 27% because of respiratory symptoms, and in 17% as part of routine screening in patients who had respiratory symptoms. Diagnostic samples were obtained by spontaneous expectoration (37%), bronchoalveolar lavage (34%), and induced sputum (24%). The method was not recorded in 5%.

Most patients who were commenced on treatment met ATS diagnostic criteria. Overall, 51 (73%) patients met ATS diagnostic criteria and 19 (27%) patients did not. Of those who met ATS diagnostic criteria, 47 (92%) and four (8%) were treated for MABSC and MAC respectively. Of those who did not meet criteria, 13 were treated for MABSC and 6 were treated for MAC. Of those not meeting criteria, ten (53%) had no radiological changes identified, four (21%) had only one sputum sample in which NTM was detected (rather than two) and five (26%) had both no radiological changes and NTM detected in only one sputum sample. Radiographic changes were identified by radiologist reporting.

Characteristics of those that fulfilled NTMPD diagnostic criteria compared with those that did not are summarised in Supplementary Table 2 for both MABSC and MAC groups.

Treatment

Treatment regimens used for MAC

Nine out of 10 patients who isolated MAC were treated according to published guidelines with a daily oral macrolide, rifampicin and ethambutol (Supplementary Figure 2). Regarding macrolide treatment, five patients (50%) received clarithromycin, three patients (30%) azithromycin, one (10%) erythromycin and in one patient, the macrolide given was unknown. Median (range) total duration of treatment was 275 (180-450) days.

Treatment regimens used for MABSC

All patients who were treated for MABSC had intravenous induction treatment followed by maintenance therapy. MABSC induction regimes are shown in Figure 2. Half of induction regimes fitted within current, as of January 2021, guidelines, with 57% of these involving one intravenous antibiotic in addition to amikacin, and 43% involving two additional intravenous antibiotics. Non-guideline directed, alternate regimes were administered in 45% of patients (unknown induction regimen in 3 children). Regarding macrolide treatment, most received clarithromycin (86%) or azithromycin (14%).

There were 30 different maintenance schedules excluding dose differences (Figure 3). Seventeen (28%) patients received nebulised amikacin, a macrolide plus 2 or more additional antibiotics (in accordance with guidelines), and 39 (65%) patients received an alternate regime differing to ATS/ECFS guidance (maintenance treatment unknown for 4 patients).

Adverse effects associated with NTM treatment

There were 60 adverse events recorded in the clinical records (92% MABSC and 8% MAC) involving 15 separate drugs (Supplementary Table 3), 35 (58%) were caused by induction drugs and 25 (42%) maintenance. Thirty percent resulted in drug

cessation, and 10% in a preparation or dose change. Nausea and vomiting were the most common adverse events (25 events). Intravenous cefoxitin was associated with the highest number of adverse events leading to alteration of therapy.

Treatment outcomes

We took a pragmatic approach to defining culture conversion. Culture conversion was presumed to have occurred if there were no positive samples in the six months after induction treatment. If this continued to be the case in the remainder of the follow-up period, eradication was presumed to have occurred. Recurrence was taken as a positive culture for the same NTM species after conversion (i.e. following the first six months after induction treatment). Disease was classified as refractory if there was a failure to culture convert after 12 months of treatment or a failure to remain clear of NTM. Minimum time of follow-up was 12 months.

Figure 4 shows outcomes for those patients treated for MAC. Eight patients (80%) culture converted with treatment and none reisolated MAC. Two (20%) patients did not convert and subsequently developed refractory disease. Of the eight patients in whom MAC was ultimately eradicated lung function was stable or improved in six, and worse, defined by drop in lung function by >10% during 12 months following diagnosis of NTM disease, in two patients. Two out of four patients who fulfilled NTMPD criteria and all six patients who did not fulfil NTMPD criteria ultimately eradicated MAC.

Figure 5 shows outcomes in those patients treated for MABSC. Thirty-five (58%) patients culture converted with treatment within six months of detection of whom MABSC recurred in 13 patients. In the 29 (48%) patients in whom MABSC was ultimately eradicated, lung function was stable or improved in 15, worse in 10, and unknown in four patients. In the 25 (42%) patients who ultimately had refractory

disease, lung function was stable or improved in seven, worse in 12 and unknown in six patients. Six patients were unclassified due to incomplete follow up data. Amongst patients treated for MABSC, differences between patients in the eradicated versus refractory group were analysed but no association with *Pseudomonas* growth, ABPA, pre-diagnosis lung function, BMI, age or sex were found.

Those who received guideline directed induction regimes had a higher percentage of MABSC eradication compared to those who did not (57% *vs.* 33%). In both groups, the majority of patients fulfilled NTMPD criteria (73% *vs.* 85%). Those who received amikacin-containing induction regimes had a higher percentage of eradication (50%), whereas none of the patients whose induction regimes lacked amikacin eradicated MABSC. Amongst those who received macrolide-containing induction regimes, those containing clarithromycin were associated with a higher proportion of culture clearance than those who received azithromycin (53% *vs.* 40%) (Supplementary Figure 3). A breakdown of children treated for MABSC and MAC respectively according to whether or not NTMPD diagnostic criteria were met is provided in Supplementary Tables 4 and 5. Out of the 29 that eradicated MABSC, 21 (72%) patients had met ATS diagnostic criteria.

Discussion

We present a retrospective analysis of data from 11 centres describing experience of treating NTM infection in children with CF. *M. abscessus abscessus* and *M. avium* accounted for most isolates. Treatment regimes varied in terms of drugs used, doses and length of course. Eradication occurred in 80% of patients with MAC and 48% with MABSC and had variable effects on lung function. Adverse events were common and often resulted in medications being stopped or preparations/doses changed.

Lung function was significantly lower at NTM diagnosis than the best recorded in the previous year. Most patients had respiratory symptoms at diagnosis, although 20% (14 out of 70) were asymptomatic. These findings highlight the potential importance of early detection of NTM using proactive screening. Induced sputum may allow improved surveillance with repeated and easy access to the lower airway in all age groups (17,18).

Treatment aimed at NTM eradication is intensive and lengthy (4). Ninety percent of patients with MAC infection received guideline directed treatment. Around half of patients with MABSC received guideline directed induction and two thirds maintenance treatment. Outcomes varied depending on the NTM being treated. Thus, MAC eradication rates were relatively high (80%) whereas MABSC eradication occurred in only 48%. It is important to note that the children in this audit were treated over a long period of time and for the majority this was prior to the publication of the guidelines by the CF Foundation and European CF Society in 2016 and by the British Thoracic Society in 2017 (13,14). Clinical practice has changed and evolved during

the course of time and a single centre paediatric study has reported a higher rate of MABSC eradication of 65-73% between 2011-2018 (15).

Reaching a diagnosis of NTM-PD in people with CF is complex, and particularly so in children. Just over a quarter of our cohort started NTM treatment without meeting all the radiological and microbiological criteria published by the ATS, CF Foundation and European CF Society (13(16)). NTM treatment was commenced in nine children supported by microbiological but not radiographic findings. Others were treated based on just one positive culture but significant radiographic changes.

The true clinical significance of NTM infection is not entirely clear at present with a range of outcomes reported, from no effect to severe lung function decline (5,19–21). A longitudinal analysis of lung function in Danish CF patients suggested that excessive loss of lung function may be mitigated by MABSC clearance (6). In our data, lung function stability or improvement was seen in 75% of those in whom MAC was eradicated and in 52% for MABSC eradication. In contrast, refractory MABSC infection was associated with lung function deterioration in 72%.

Side effects and adverse events associated with treatment were common. They were most often seen with MABSC treatment, particularly during the induction phase, with cefoxitin being the antibiotic most often associated with treatment discontinuation (~30%). Gastrointestinal disturbances, particularly nausea and vomiting were the most frequently reported. We suspect that the rate of adverse events are likely to be an underestimate as these are recognised to be under-reported (22). Although there are on-going observational studies on children with NTM disease (PREDICT, PATIENCE

and FORMAT) (23,24), large well conducted clinical trials are urgently required both in the adult and paediatric CF population to evaluate optimal treatment regimens, duration and to define treatment success.

A strength of this work is that it provides detailed 'real world' information from multiple centres representing the largest report of the experience of treating children with CF for NTM infection to date. However, this also leads to the major limitations of this analysis, specifically the retrospective observational nature of the data, the protracted time period over which cases were studied and some children who received treatment for NTM infection may not have been identified. Data on children who isolated NTM but did not receive treatment were also not collected. The sampling and treatment strategies were not standardised across centres. Techniques for culturing NTM and practices for routine surveillance in respiratory samples evolved during the study period as did clinical thresholds for commencing NTM treatment (25,26). However, these data reflect real life practice and outcomes as experienced by clinicians for these patients.

Conclusion

This large retrospective multi-centre study of children with CF treated for NTM infection highlights the significant scale and burden in this age-group along with variations in practice and associated challenges in sampling and detection of NTM, management and treatment. These data will help to inform the future clinical management of children with NTM infection. There is a clear requirement for urgent research to facilitate the development of paediatric-specific protocols.

What is already known on this topic

- Infection with NTM is a major concern for people with CF, their families and clinicians
- Rates of infection have increased over the last decade
- Treatment requires prolonged courses of multiple antibiotics that are frequently associated with adverse effects, there is a dearth of evidence to inform practice in children

What this study adds

- We report the largest dataset of the experience of multiple paediatric CF centres in treating children with NTM
- Widely varying treatment regimes were used
- Overall NTM eradication occurred in 80% of children with MAC and 48% of children with MABSC

Competing interests

MB: Not related to this study, has been CI on investigator-led research grants from Pfizer and Roche Diagnostics; speaker fees paid to Newcastle University from Novartis, Roche Diagnostics and TEVA; travel expenses to educational meetings from Boehringer Ingelheim and Vertex.

MFT: Not related to this study, investigator-led research grant from Pfizer

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Author's contribution

Conception and design, PSM, MB, & MFT

Acquisition of data, GLS, PSM MB, MFT, RJL and all members of the NTM collaborators group

Analysis and interpretation of data, GLS, PSM, MB, MFT, RJL and NZA.

Manuscript preparation, GLS, PSM, MFT, MB and NZA

Manuscript review, all authors including NTM collaborators group

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Figure Legends

Figure 1: Median FEV_1 over time, from baseline (best in year preceding NTM isolation) through point of NTM isolation, induction, maintenance treatment and follow up for MAC and MABSC. FEV_1 at baseline was statistically significantly higher than at diagnosis (Wilcoxon matched pairs signed rank test, p = 0.002 MAC; p<0.0001 MABSC) and remained so across all other time-points. The majority of MAC patients did not receive a regime that included induction, therefore lung function recorded within the first 3 months after commencing treatment is used for the end of induction time point. Not all patients had FEV_1 available at all times points. There was a minimum of 3 time points for each patient. Error bars represent interquartile range.

Abbreviations: FEV_1: forced expiratory volume in 1 second; NTM: Nontuberculous mycobacteria; MAC: *Mycobacterium avium* complex; MABSC: *Mycobacterium abscessus* complex.

Figure 2: Different MABSC induction regimes used. Regimes have been categorised as guideline directed if they included amikacin plus one or two additional intravenous antibiotics and a macrolide. Regimes have been categorised as alternative if they were an alternate regime out with American Thoracic Society or European Cystic Fibrosis Society guidance.

Abbreviations: MABSC: *Mycobacterium abscessus* complex.

Figure 3: Different MABSC maintenance regimes used. Regimes have been categorised as guideline directed if they included nebulised amikacin, a macrolide and two or more additional antibiotics. Regimes have been categorised as alternative if they were an alternate regime out with American Thoracic Society or European Cystic Fibrosis Society guidance.

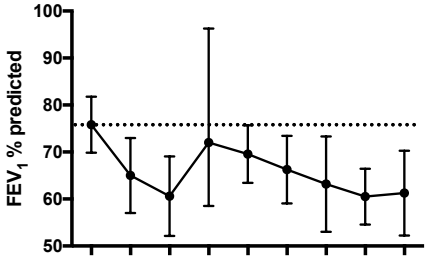
Abbreviation: MABSC: *Mycobacterium abscessus* complex.

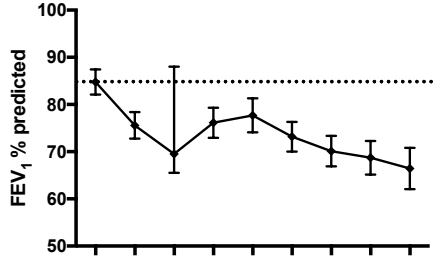
Figure 4: Culture conversion and lung function outcomes for those children treated for MAC.

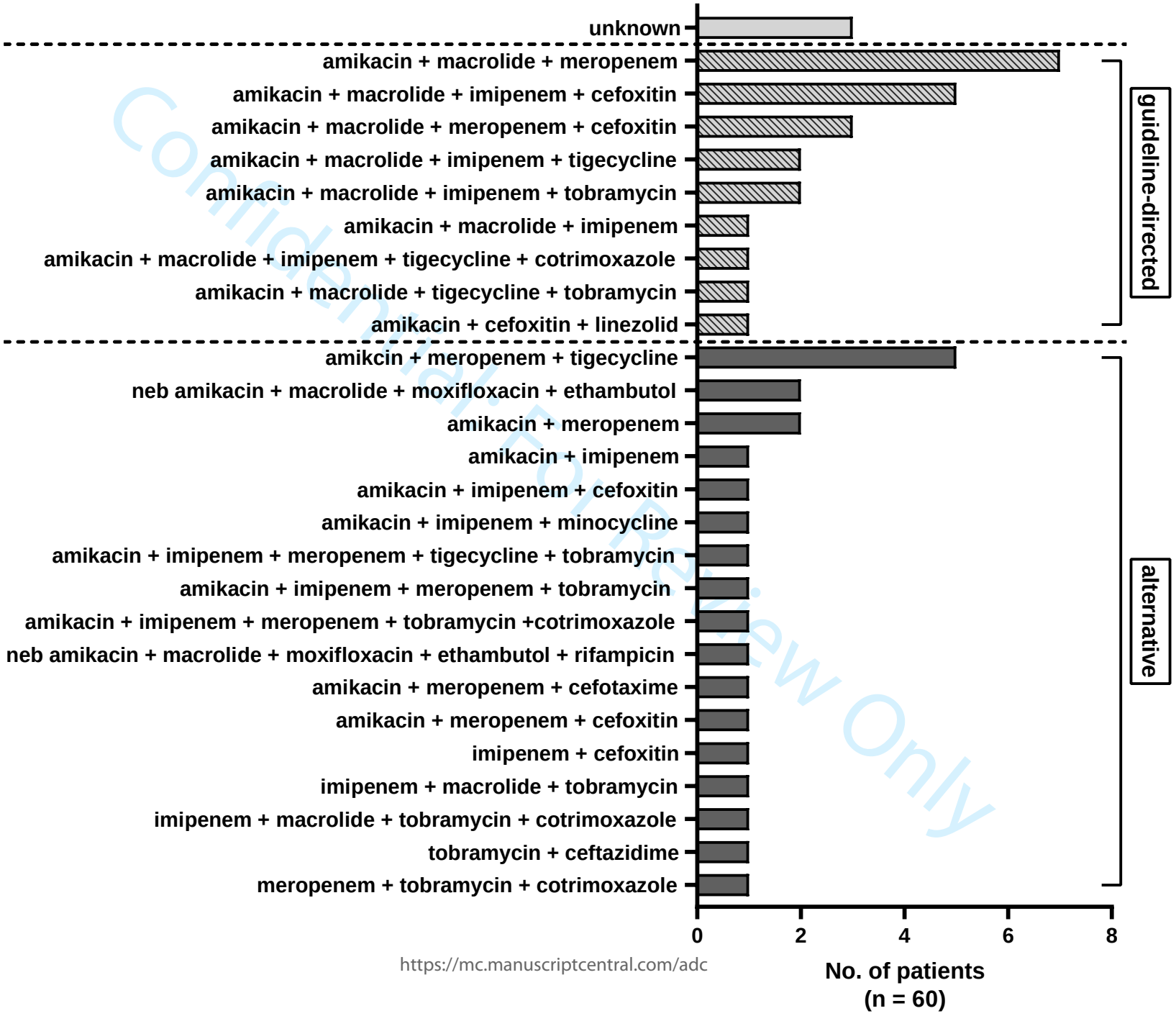
Abbreviations: LF: lung function; MAC: *Mycobacterium avium* complex

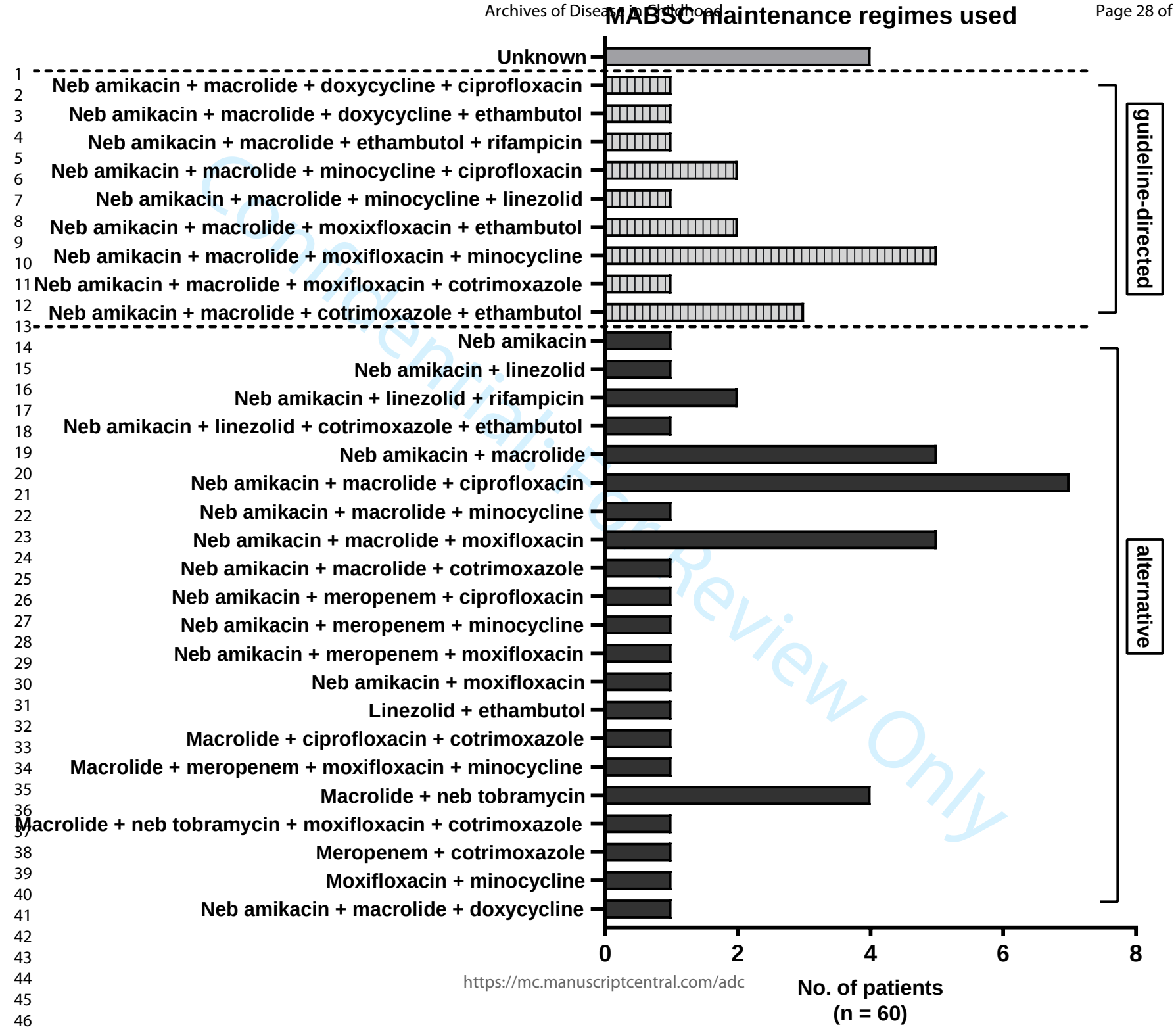
Figure 5: Culture conversion and lung function outcomes for those patients treated for MABSC. Unclassified refers to those patients unable to be classified due to incomplete follow up data.

Abbreviations: LF: lung function; MABSC: *Mycobacterium abscessus* complex.









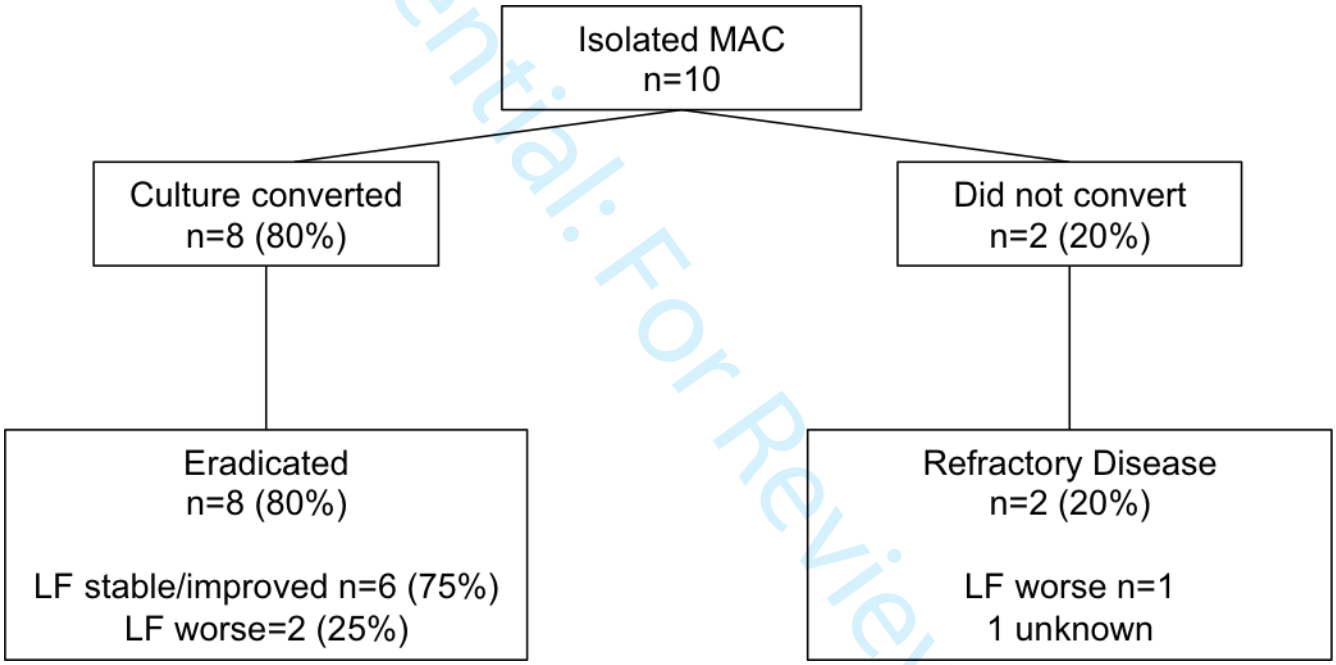
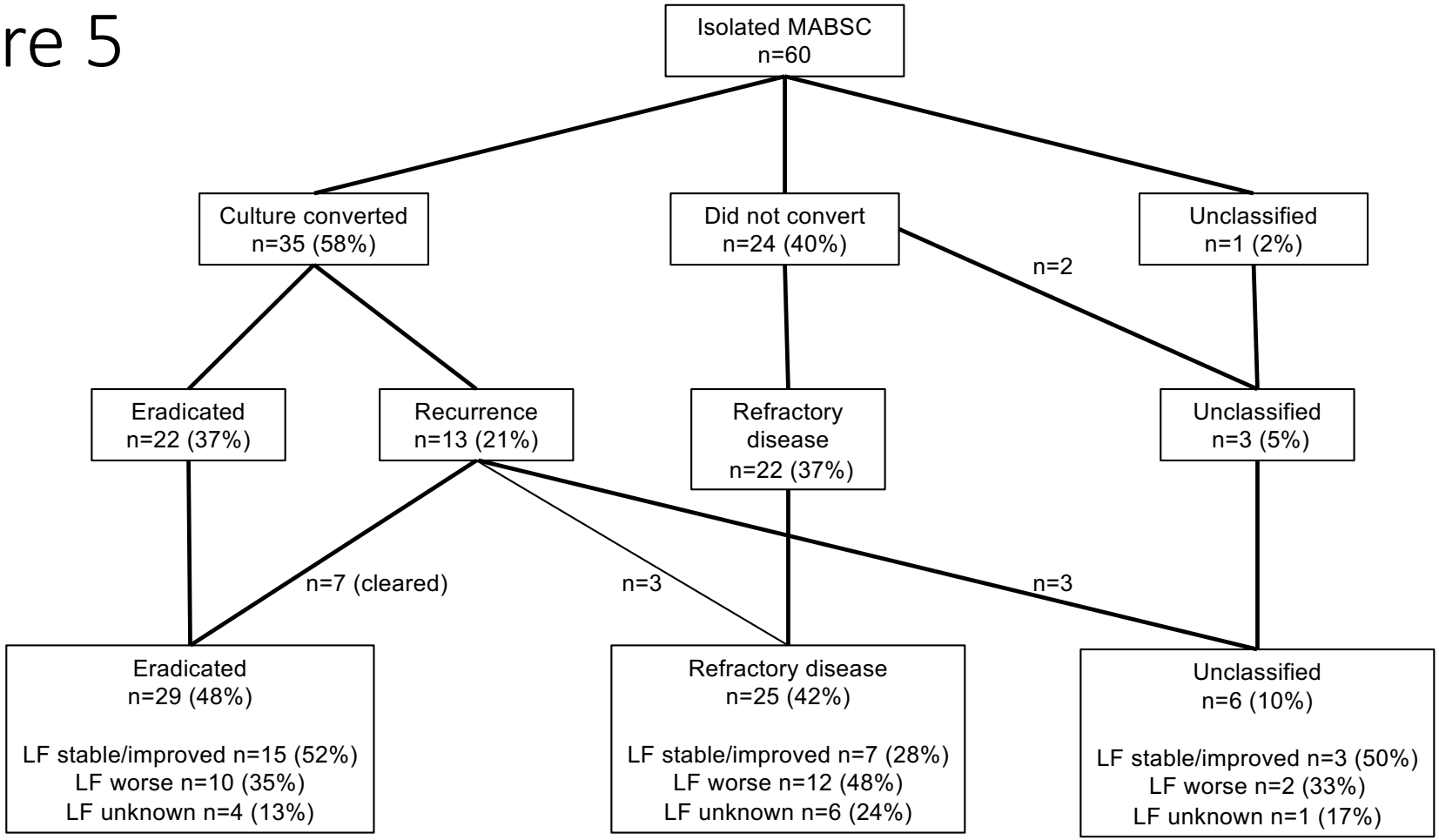


Figure 5


Isolated MABSC
n=60
Culture converted
n=35 (58%)
Did not convert
n=24 (40%)
Unclassified
n=1 (2%)
n=2
Eradicated
n=22 (37%)
Recurrence
n=13 (21%)
Refractory
disease
n=22 (37%)
Unclassified
n=3 (5%)
n=7 (cleared)
n=3
n=3
Eradicated
n=29 (48%)
LF stable/improved n=15 (52%)
LF worse n=10 (35%)
LF unknown n=4 (13%)
Refractory disease
n=25 (42%)
LF stable/improved n=7 (28%)
LF worse n=12 (48%)
LF unknown n=6 (24%)
Unclassified
n=6 (10%)
LF stable/improved n=3 (50%)
LF worse n=2 (33%)
LF unknown n=1 (17%)

SUPPLEMENTARY MATERIAL

Experience of treating nontuberculous mycobacteria infection in children with cystic fibrosis: a multicentre retrospective study

Gemma L Saint[1,2*], Matthew F Thomas[3,4*], Noreen Zainal Abidin[3,4], Ross J Langley[5], Malcolm Brodlie[3,4#], Paul S McNamara[1,2#^] and NTM Collaborators Group[6]

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[5]Paediatric Respiratory and Sleep Medicine, Royal Hospital for Children, NHS Greater Glasgow and Clyde, Glasgow, UK

[6]See below for full list of collaborators

*Joint first author

#Joint senior author

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CF centre	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	TOTAL
Birmingham			1	1					1	3/***1***	4		10/***1***
Cardiff			1						1				2
Leicester							2	2	***2***	3/***1***	2		9/***3***
Liverpool							3	1	1	1/***1***			6/***1***
Manchester					5	1			1	4	1		12
Newcastle	1			1		***1***	2	1	***1***	1			6/***2***
Nottingham				1	2		1			2		1	7
Sheffield									1	1			2
Stoke						***1***	1						1/***1***
Glasgow						1		3		1			5
Edinburgh										***2***			***2***
TOTAL	1	0	2	3	7	2/***2***	9	7	5/***3***	16/***5***	7	1	60/***10***

Supplementary Table 1: Number of patients at each centre by year diagnosed with *Mycobacterium abscessus* complex (black) and *Mycobacterium avium* complex (*bold italics*)

Characteristics	Median (IQR) or n (%)	
	Fulfilled NTMPD diagnostic criteria	***Did not fulfil NTMPD diagnostic criteria***
Total number of patients	51	19
Age (years)	10 (8-12)	13 (11.5-16)
Sex (Female)	26 (51)	14 (73.7)
BMI (kg/m^2)	16.1 (14.9-17.8)	17.6 (15.9-21.3)
NTM species isolated		
MABSC	47 (92.2)	13 (68.4)
MAC	4 (7.8)	6 (31.6)
Microbiology in the year prior to NTM diagnosis		
Pseudomonas aeruginosa	23 (45.1)	10 (52.6)
Staphylococcus aureus	23 (45.1)	10 (52.6)
Aspergillus sp.	21 (41.2)	6 (31.6)
Stenotrophomonas maltophilia	2 (3.9)	1 (5.3)
Haemophilus influenzae	2 (3.9)	1 (5.3)
Burkholderia cepacia	0 (0)	1 (5.3)
Comorbidities		
Allergic bronchopulmonary aspergillosis	9 (17.6)	3 (15.8)
Pancreatic insufficiency	49 (96.1)	17 (89.5)
CF-related diabetes	5 (9.8)	3 (15.8)
CF-related liver disease	9 (17.6)	8 (42.1)
Medications received in the year prior to NTM diagnosis		
Oral proton pump inhibitor	21 (41.2)	10 (52.6)
Oral corticosteroids	10 (19.6)	6 (31.6)
Macrolide	18 (35.3)	12 (41.4)

Supplementary Table 2: Characteristics of patients that fulfilled NTMPD diagnostic criteria and those who did not.

Abbreviations: IQR: interquartile range; NTMPD: Nontuberculous mycobacteria pulmonary disease; BMI: body mass index; NTM: Nontuberculous mycobacteria; MABSC: *Mycobacterium abscessus* complex; MAC: *Mycobacterium avium* complex; CF: Cystic fibrosis

DRUG	Adverse event 1	Adverse event 2	Adverse event 3	Adverse event 4	TOTAL RECEIVING DRUG
Amikacin IV	hearing loss (3) 6% *1 changed to neb therapy*	Nausea and loss of appetite (2) 4%	headache (1) *dose reduced 2%*	-	54
Amikacin neb	hearing loss (1) 2%	tinnitus (1) 2%	hoarseness (1) 2%	irritating cough (1) 2%	46
Azithromycin PO	-	-	-	-	13
Cefoxitin IV	deranged renal function (2) 17% *1 stopped*	deranged LFTs (3) 25% *2 stopped early*	rash (4) 33% *All stopped early*	nausea/vomiting (2) 17%	12
Cefoxitin IV + linezolid IV	diarrhoea, deranged LFTs, neutropenia and thrombocytopenia, erythema multiforme (1)				1
Ciprofloxacin PO	vomiting (1) 7% *preparation changed*	-	-	-	14
Clarithromycin PO	nausea/vomiting (1) 2%	deranged LFTs (2) 4%		-	46
Doxycycline PO	nausea/vomiting (1) 33% *Stopped*	-		-	3
Ethambutol PO	nausea/diarrhoea (3) 18% *1 stopped*	hearing loss (1) 6%		-	17
Gamma interferon	severe foot, hip and hand pains (1) 50%	-		-	2
Imipenem IV	nausea/vomiting (8) 27% *1 stopped*	lethargy (1) 3% *Stopped*		-	29
Linezolid PO	nausea/vomiting (2) 33%	low platelets and Hb (1) 16% *Stopped*	diarrhoea (1) 16% *Stopped*	-	6
Meropenem IV	rash (1) 4% *Changed to imipenem*	nausea and loss of appetite (1) (4%)		-	23
Meropenem neb	-	-	-	-	5
Minocyline PO	rash (1) 20%	-		-	5
Moxifloxacin PO	-	-	-	-	19
Rifampacin	rash (3) 27%	diarrhoea (2) 18%	hair loss (1) 9%	-	11
Tigacyline IV	nausea/vomiting (4) 29% *2 stopped*		-	-	14
Tobramycin IV	hearing loss (1) 11%	-	-	-	9
Tobramycin neb	systemic absorption and raised levels (1) 25% *reduced dose*	-	-	-	4

Supplementary Table 3: Adverse events associated with drugs, given orally or intravenously. The side effects are shown with the number of patients affected and what percentage of patients who took the drug were affected (total events=70). Abbreviations: PO oral; IV intravenous.

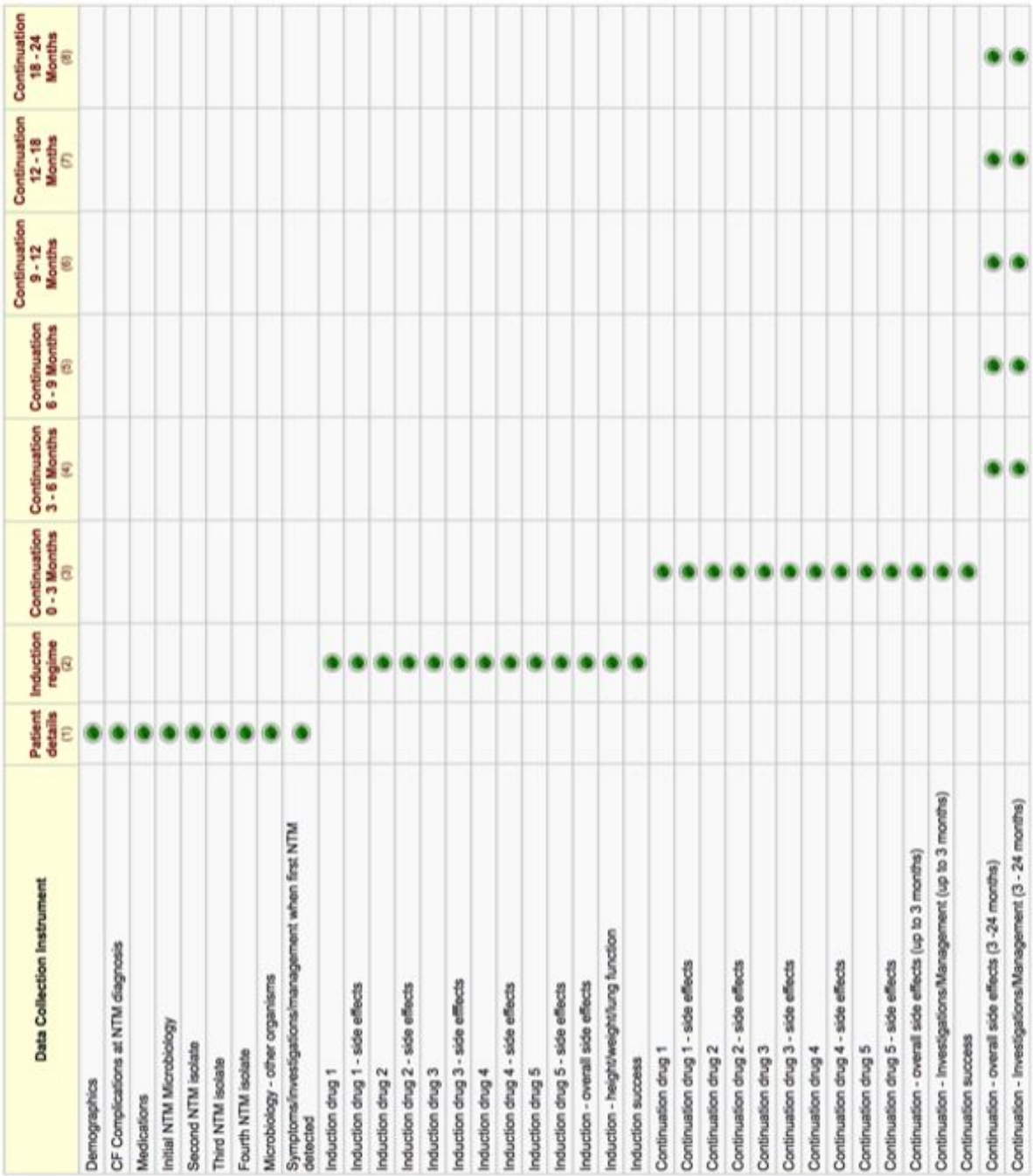
Characteristics	Median (IQR) or n (%)	
	Fulfilled NTMPD diagnostic criteria	***Did not fulfil NTMPD diagnostic criteria***
Total number of patients	47	13
Age (years)	10 (8-12)	14 (12-16)
Sex (Female)	24 (51)	11 (85)
BMI (kg/m^2)	16 (15-18)	19 (16-23)
Microbiology in the year prior to NTM diagnosis		
Pseudomonas aeruginosa	23 (49)	8 (62)
Staphylococcus aureus	20 (43)	6 (46)
Aspergillus sp.	20 (43)	5 (39)
Stenotrophomonas maltophilia	1 (2)	0 (0)
Haemophilus influenzae	1 (2)	0 (0)
Comorbidities		
Allergic bronchopulmonary aspergillosis	8 (17)	2 (15)
Pancreatic insufficiency	45 (96)	12 (92)
CF-related diabetes	4 (9)	2 (15)
CF-related liver disease	8 (17)	6 (46)
Medications received in the year prior to NTM diagnosis		
Oral proton pump inhibitor	20 (43)	7 (54)
Oral corticosteroids	9 (19)	4 (31)
Macrolide	16 (34)	8 (62)
Induction regime received		
Guideline-directed	22 (47)	8 (62)
Not guideline-directed	23 (49)	4 (31)
Unknown	2 (4)	1 (8)
NTM status on follow-up		
Eradicated	21 (45)	8 (62)
Not eradicated	20 (43)	4 (31)
Unknown	2 (4)	1 (8)

Supplementary Table 4: Characteristics of children treated for *Mycobacterium abscessus* complex according to whether or not NTMPD diagnostic criteria were met.

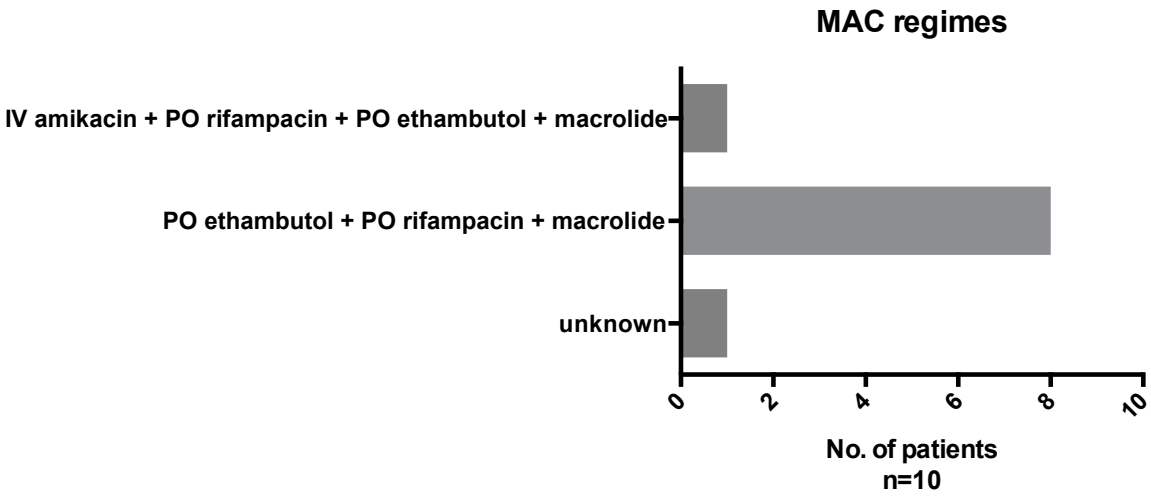
Abbreviations: IQR: interquartile range; NTMPD: Nontuberculous mycobacteria pulmonary disease; BMI: body mass index; NTM: Nontuberculous mycobacteria; CF: Cystic fibrosis

Characteristics	Median (IQR) or n (%)	
	Fulfilled NTMPD diagnostic criteria	***Did not fulfil NTMPD diagnostic criteria***
Total number of patients	4	6
Age (years)	9 (7-11)	13 (10-13)
Sex (Female)	2 (50)	2 (50)
BMI (kg/m^2)	15 (14-16)	16 (15-18)
Microbiology in the year prior to NTM diagnosis		
Pseudomonas aeruginosa	0 (0)	2 (33)
Staphylococcus aureus	3 (75)	4 (67)
Aspergillus sp.	1 (25)	1 (17)
Stenotrophomonas maltophilia	1 (25)	1 (17)
Haemophilus influenzae	1 (25)	1 (17)
Comorbidities		
Allergic bronchopulmonary aspergillosis	1 (25)	1 (17)
Pancreatic insufficiency	4 (100)	5 (83)
CF-related diabetes	1 (25)	1 (17)
CF-related liver disease	1 (25)	2 (33)
Medications received in the year prior to NTM diagnosis		
Oral proton pump inhibitor	1 (25)	3 (50)
Oral corticosteroids	1 (25)	2 (33)
Macrolide	2 (50)	4 (67)
Induction regime received		
Guideline-directed	3 (75)	6 (100)
Not guideline-directed	0 (0)	0 (0)
Unknown	1 (25)	0 (0)
NTM status on follow-up		
Eradicated	2 (50)	6 (100)
Not eradicated	1 (25)	0 (0)
Unknown	1 (25)	0 (0)

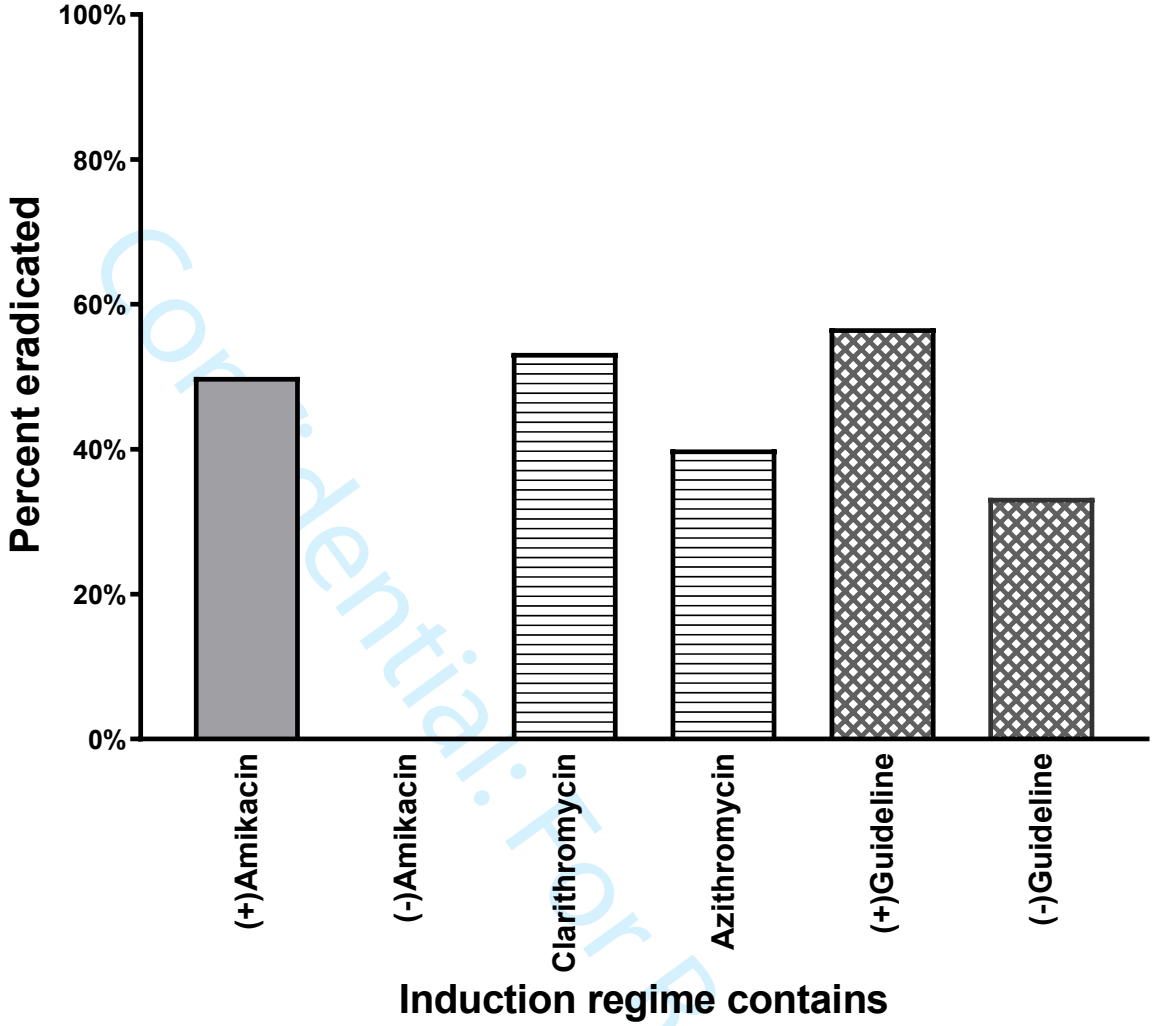
Supplementary Table 5: Characteristics of children treated for *Mycobacterium avium* complex according to whether or not NTMPD diagnostic criteria were met. Abbreviations: IQR: interquartile range; NTMPD: Nontuberculous mycobacteria pulmonary disease; BMI: body mass index; NTM: Nontuberculous mycobacteria; CF: Cystic fibrosis



Supplementary Figure 1: List of question domains in the on-line database tool, with number of fields within each domain.



Supplementary Figure 2: Different *Mycobacterium avium* complex regimes used. Abbreviations: MAC: *Mycobacterium avium* complex; IV: intravenous; PO: oral.



Supplementary Figure 3: Percentage of patients that eradicated and did not eradicate *Mycobacterium abscessus* complex according to induction regimes used.

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29 September 2021

Dear Nick and Colin,

Thank you for inviting us to submit a revised version of our manuscript 'Treating nontuberculous mycobacteria in children with cystic fibrosis: a multicentre retrospective study'. We are grateful for the comments raised at peer review and have responded fully to these in the point-by-point response below. The revised manuscript has been strengthened in our opinion by taking these comments on board.

Please do not hesitate to contact me should you have any questions or queries.

Yours sincerely

Malcolm Brodlie BSc (Hons), MB ChB, PhD, FRCPCH

Reviewer 1

1. This is a nicely written paper describing a multi-site audit of across 11 CF centres describing their experience of treating non-tuberculous mycobacteria in children. All the centres have used different treatment regimens, the supplemental material shows the multitude of different regimes used, the authors have done well in collecting this retrospective data.

   Thank you for these positive comments. No response required.

2. However, there appear to be very low levels of NTM cases reported in this for such a large time period. Twhat happened to the rest? 11 years of data collection. The 11 centres will have 2000 patients per year under their care. There are 141 children in the registry in 2019 reported as having an NTM growth within the last 2 years, the centres represent 45% of the UK paediatric population so the small number in this paper suggests they may be missing quite a few. This is quite a weakness of the study, and should be discussed in more detail. Or the authors may want to report the total number of cases detected and not treated if this makes it look like they have collected more extensive data and just not reported the actual prevalence of NTM in their clinics.

The aim of this pragmatic study was to retrospectively collect data on the treatment strategies used by the paediatric CF centres involved. It only included children who had received treatment for NTM over the time period (2006 to 2017) and focused on regimes used, their relative clinical success and any associated adverse effects. On this basis the dataset represents the largest in the literature that we are aware of in the paediatric age group.

We fully acknowledge that there are inherent limitations to this methodology and that not all cases will have been captured and that these results should generate further studies to collect accurate prospective data, including children who isolate NTM but are not treated. Most clinicians would also agree that the clinical threshold for treating children with NTM lowered during the census period in the UK.

Furthermore, there has been a trend of increased numbers of children isolating NTM in the UK CF Registry over the last decade (Gardner *et al*. Epidemiology of Nontuberculous Mycobacteria Infection in Children and Young People With Cystic Fibrosis: Analysis of UK Cystic Fibrosis Registry. Clin Infect Dis. 2019 Feb 15;68(5):731-737. doi: 10.1093/cid/ciy531. PMID: 29982302. and Abidin *et al.* Trends in nontuberculous mycobacteria infection in children and young people with cystic fibrosis. J Cyst Fibros. 2020 Sep 16:S1569-1993(20)30872-9. doi: 10.1016/j.jcf.2020.09.007. PMID: 32950411).

These limitations have now been discussed more fully in the final paragraph of the discussion.

3. They report a drop in lung function from previous best with the presentation of NTM which is useful information, especially as this appeared to persist rather than return to baseline.

   We agree. No response required.

4. The authors do not make it clear if the ABPA was only in the year before diagnosis of NTM or at the time or during. page 10 line 13, although it appears in the table.

   Thank you for raising this point. We have now clarified in the manuscript (in the first subsection of Results and in the Table 1 legend) that we asked about ABPA in the year preceding isolation of NTM.

5. It is not clear from the text what criteria were used for treating NTM, p10 "NTM Isolated and Treated" suggests that some cases may have been treated with only one positive growth. The authors need to clarify whether this is the case. If so, patients have not met the ATS guidelines for treatment, although I note in the next section the authors state 92% did meet guidelines. They then list the numbers that don't meet the criteria, which appear to add up to more than the 8% suggested in the next paragraph on page 11. Some clarity might be needed on this. If patients do not meet the criteria to treat, claiming treatment eradicates might be over-stating the case, it is recognised that NTM may appear transiently in a patients sputum, rather than be there causing disease. Low numbers of CT scans also does not fit with meeting ATS criteria.This confusion about whether they are using NTM-PD or ATS criteria and absolute numbers meeting the criteria needs to be clarified throughout the text.

   Thank you for this comment. We have attempted to make this clearer in the revised manuscript. This was an observational retrospective study where treatment decisions were made by the clinical teams involved.

   We have gone back through the raw data and have now rewritten this section as follows: "*Most patients who were commenced on treatment met ATS diagnostic*

criteria. Overall, 51 (73%) patients met ATS diagnostic criteria and 19 (27%) patients did not. Of those who met ATS diagnostic criteria, 47 (92%) and four (8%) were treated for MABSC and MAC respectively. Of those who did not meet criteria, 13 were treated for MABSC and 6 were treated for MAC. Of those not meeting criteria, ten (53%) had no radiological changes identified, four (21%) had only one sputum sample in which NTM was detected (rather than two) and five (26%) had both no radiological changes and NTM detected in only one sputum sample."

6. MABSC. 42% failed eradication. 29 (48%) eradicated. A statement on how many were eradicated that had met criteria for treatment would be useful.

   We agree this is an important point. We have now included in the results section: "*Out of the 29 that eradicated MABSC, 21 (72%) patients had met ATS diagnostic criteria."*

7. Culture conversion is stated as pragmatic can the authors state how many had samples taken and the average number of samples taken in first 6 months and during treatment?

   Unfortunately, we do not have these data from centres. Practice undoubtedly varied at each centre and also depended on the clinical situation.

8. Page 12 line 40 states 17 received nebulised amikacin, fig 3 seems to show 45 received nebulised Amikacin can the authors clarify the correct number?

   We apologise if this sentence is unclear. It states that *"17 patients received nebulised amikacin, a macrolide plus two or more additional antibiotics."* This ties in with the numbers in Figure 3 if one adds up the number of cases in the section labelled 'guideline-directed' you get 17. The phrase *"(in accordance with guidelines)"* has now been added to clarify.

9. Page 15 para beginning line 25. Can the authors state the absolute numbers here rather than %.

   We have now also included the absolute numbers.

10. Table 2 in Supplement should also have the total numbers stated in the headings for both columns, likewise table 4.

    We have now added a row to Supplementary Tables 2, 4 and 5 called 'total number of patients', thank you for highlighting this.

11. Again this does not feel like it agrees with the text for meeting guidelines.

    We have now clarified the text as per response to point 5. The revised text now tallies correctly with the data.

12. Page 31 figure refers to MAB rather than MABSC as used in text.

    This has now been changed.

Reviewer 2

Thank you for the positive comments. No response required.

SUPPLEMENTARY MATERIAL

Experience of treating nontuberculous mycobacteria infection in children with cystic fibrosis: a multicentre retrospective study

Gemma L Saint[1,2*], Matthew F Thomas[3,4*], Noreen Zainal Abidin[3,4], Ross J Langley[5], Malcolm Brodlie[3,4#], Paul S McNamara[1,2#^] and NTM Collaborators Group[6]

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*Joint first author

#Joint senior author

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CF centre	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	TOTAL
Birmingham			1	1					1	3/***1***	4		10/***1***
Cardiff			1						1				2
Leicester							2	2	***2***	3/***1***	2		9/***3***
Liverpool							3	1	1	1/***1***			6/***1***
Manchester					5	1			1	4	1		12
Newcastle	1			1		***1***	2	1	***1***	1			6/***2***
Nottingham				1	2		1			2		1	7
Sheffield									1	1			2
Stoke						***1***	1						1/***1***
Glasgow						1		3		1			5
Edinburgh										***2***			***2***
TOTAL	1	0	2	3	7	2/***2***	9	7	5/***3***	16/***5***	7	1	60/***10***

Supplementary Table 1: Number of patients at each centre by year diagnosed with *Mycobacterium abscessus* complex (black) and *Mycobacterium avium* complex (*bold italics*)

Characteristics	Median (IQR) or n (%)	
	Fulfilled NTMPD diagnostic criteria	***Did not fulfil NTMPD diagnostic criteria***
Total number of patients	51	19
Age (years)	10 (8-12)	13 (11.5-16)
Sex (Female)	26 (51)	14 (73.7)
BMI (kg/m^2)	16.1 (14.9-17.8)	17.6 (15.9-21.3)
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Haemophilus influenzae	2 (3.9)	1 (5.3)
Burkholderia cepacia	0 (0)	1 (5.3)
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Allergic bronchopulmonary aspergillosis	9 (17.6)	3 (15.8)
Pancreatic insufficiency	49 (96.1)	17 (89.5)
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Medications received in the year prior to NTM diagnosis		
Oral proton pump inhibitor	21 (41.2)	10 (52.6)
Oral corticosteroids	10 (19.6)	6 (31.6)
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Supplementary Table 2: Characteristics of patients that fulfilled NTMPD diagnostic criteria and those who did not.

Abbreviations: IQR: interquartile range; NTMPD: Nontuberculous mycobacteria pulmonary disease; BMI: body mass index; NTM: Nontuberculous mycobacteria; MABSC: *Mycobacterium abscessus* complex; MAC: *Mycobacterium avium* complex; CF: Cystic fibrosis

DRUG	Adverse event 1	Adverse event 2	Adverse event 3	Adverse event 4	TOTAL RECEIVING DRUG
Amikacin IV	hearing loss (3) 6% *1 changed to neb therapy*	Nausea and loss of appetite (2) 4%	headache (1) *dose reduced 2%*	-	54
Amikacin neb	hearing loss (1) 2%	tinnitus (1) 2%	hoarseness (1) 2%	irritating cough (1) 2%	46
Azithromycin PO	-	-	-	-	13
Cefoxitin IV	deranged renal function (2) 17% *1 stopped*	deranged LFTs (3) 25% *2 stopped early*	rash (4) 33% *All stopped early*	nausea/vomiting (2) 17%	12
Cefoxitin IV + linezolid IV	diarrhoea, deranged LFTs, neutropenia and thrombocytopenia, erythema multiforme (1)				1
Ciprofloxacin PO	vomiting (1) 7% *preparation changed*	-	-	-	14
Clarithromycin PO	nausea/vomiting (1) 2%	deranged LFTs (2) 4%		-	46
Doxycycline PO	nausea/vomiting (1) 33% *Stopped*	-		-	3
Ethambutol PO	nausea/diarrhoea (3) 18% *1 stopped*	hearing loss (1) 6%		-	17
Gamma interferon	severe foot, hip and hand pains (1) 50%	-		-	2
Imipenem IV	nausea/vomiting (8) 27% *1 stopped*	lethargy (1) 3% *Stopped*		-	29
Linezolid PO	nausea/vomiting (2) 33%	low platelets and Hb (1) 16% *Stopped*	diarrhoea (1) 16% *Stopped*	-	6
Meropenem IV	rash (1) 4% *Changed to imipenem*	nausea and loss of appetite (1) (4%)		-	23
Meropenem neb	-	-	-	-	5
Minocyline PO	rash (1) 20%	-		-	5
Moxifloxacin PO	-	-	-	-	19
Rifampacin	rash (3) 27%	diarrhoea (2) 18%	hair loss (1) 9%	-	11
Tigacyline IV	nausea/vomiting (4) 29% *2 stopped*		-	-	14
Tobramycin IV	hearing loss (1) 11%	-	-	-	9
Tobramycin neb	systemic absorption and raised levels (1) 25% *reduced dose*	-	-	-	4

Supplementary Table 3: Adverse events associated with drugs, given orally or intravenously. The side effects are shown with the number of patients affected and what percentage of patients who took the drug were affected (total events=70). Abbreviations: PO oral; IV intravenous.

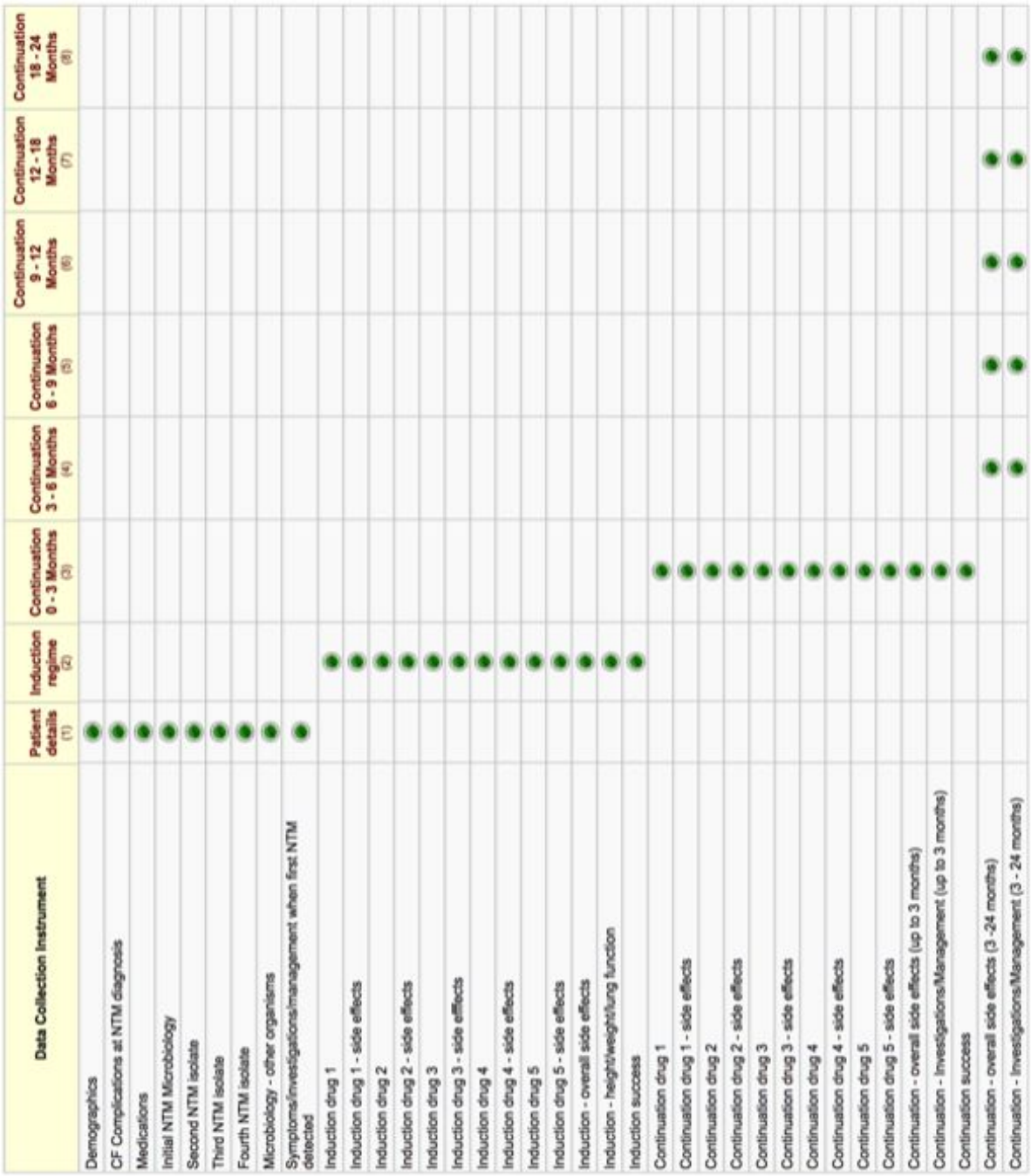
Characteristics	Median (IQR) or n (%)	
	Fulfilled NTMPD diagnostic criteria	***Did not fulfil NTMPD diagnostic criteria***
Total number of patients	47	13
Age (years)	10 (8-12)	14 (12-16)
Sex (Female)	24 (51)	11 (85)
BMI (kg/m^2)	16 (15-18)	19 (16-23)
Microbiology in the year prior to NTM diagnosis		
Pseudomonas aeruginosa	23 (49)	8 (62)
Staphylococcus aureus	20 (43)	6 (46)
Aspergillus sp.	20 (43)	5 (39)
Stenotrophomonas maltophilia	1 (2)	0 (0)
Haemophilus influenzae	1 (2)	0 (0)
Comorbidities		
Allergic bronchopulmonary aspergillosis	8 (17)	2 (15)
Pancreatic insufficiency	45 (96)	12 (92)
CF-related diabetes	4 (9)	2 (15)
CF-related liver disease	8 (17)	6 (46)
Medications received in the year prior to NTM diagnosis		
Oral proton pump inhibitor	20 (43)	7 (54)
Oral corticosteroids	9 (19)	4 (31)
Macrolide	16 (34)	8 (62)
Induction regime received		
Guideline-directed	22 (47)	8 (62)
Not guideline-directed	23 (49)	4 (31)
Unknown	2 (4)	1 (8)
NTM status on follow-up		
Eradicated	21 (45)	8 (62)
Not eradicated	20 (43)	4 (31)
Unknown	2 (4)	1 (8)

Supplementary Table 4: Characteristics of children treated for *Mycobacterium abscessus* complex according to whether or not NTMPD diagnostic criteria were met.

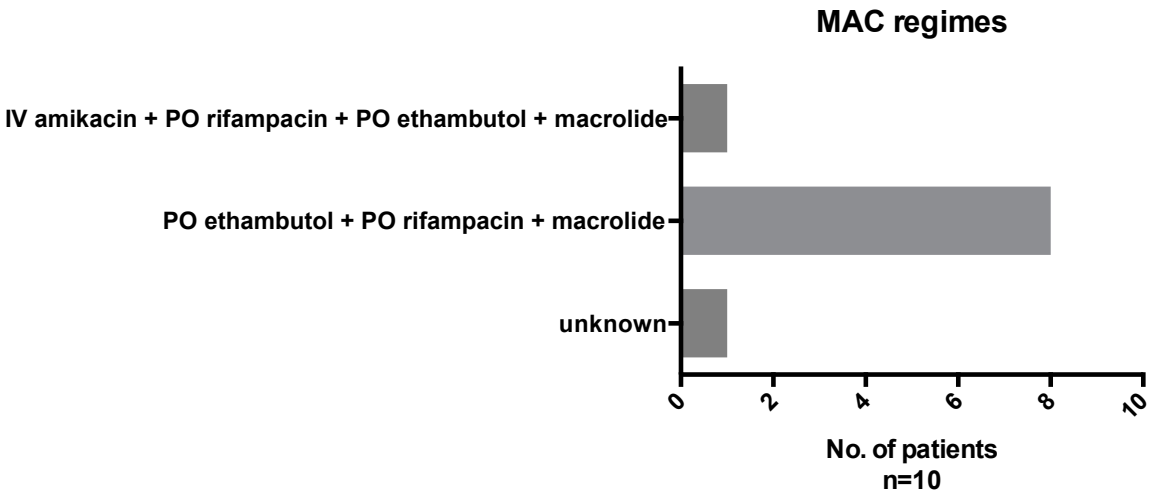
Abbreviations: IQR: interquartile range; NTMPD: Nontuberculous mycobacteria pulmonary disease; BMI: body mass index; NTM: Nontuberculous mycobacteria; CF: Cystic fibrosis

Characteristics	Median (IQR) or n (%)	
	Fulfilled NTMPD diagnostic criteria	***Did not fulfil NTMPD diagnostic criteria***
Total number of patients	4	6
Age (years)	9 (7-11)	13 (10-13)
Sex (Female)	2 (50)	2 (50)
BMI (kg/m^2)	15 (14-16)	16 (15-18)
Microbiology in the year prior to NTM diagnosis		
Pseudomonas aeruginosa	0 (0)	2 (33)
Staphylococcus aureus	3 (75)	4 (67)
Aspergillus sp.	1 (25)	1 (17)
Stenotrophomonas maltophilia	1 (25)	1 (17)
Haemophilus influenzae	1 (25)	1 (17)
Comorbidities		
Allergic bronchopulmonary aspergillosis	1 (25)	1 (17)
Pancreatic insufficiency	4 (100)	5 (83)
CF-related diabetes	1 (25)	1 (17)
CF-related liver disease	1 (25)	2 (33)
Medications received in the year prior to NTM diagnosis		
Oral proton pump inhibitor	1 (25)	3 (50)
Oral corticosteroids	1 (25)	2 (33)
Macrolide	2 (50)	4 (67)
Induction regime received		
Guideline-directed	3 (75)	6 (100)
Not guideline-directed	0 (0)	0 (0)
Unknown	1 (25)	0 (0)
NTM status on follow-up		
Eradicated	2 (50)	6 (100)
Not eradicated	1 (25)	0 (0)
Unknown	1 (25)	0 (0)

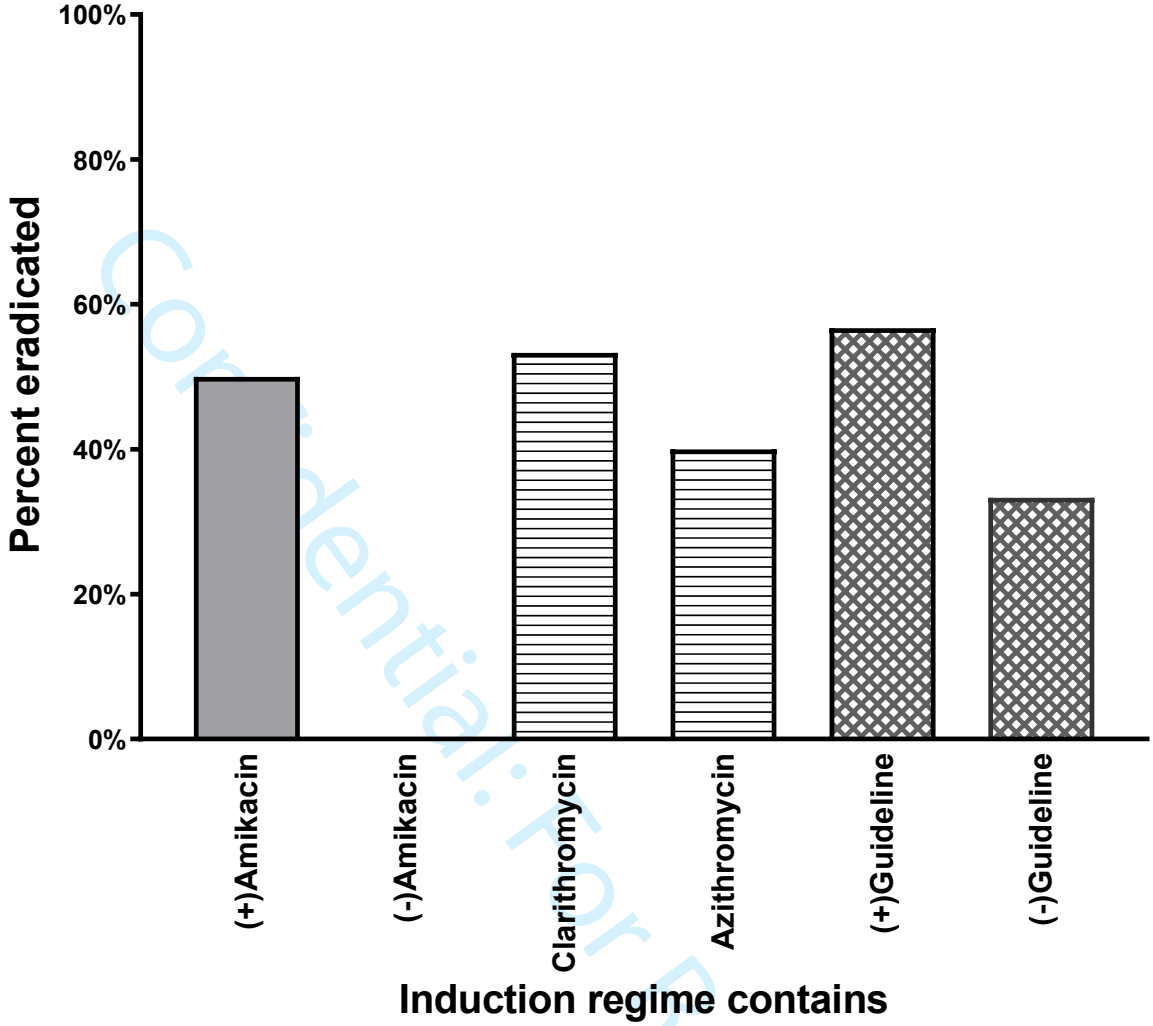
Supplementary Table 5: Characteristics of children treated for *Mycobacterium avium* complex according to whether or not NTMPD diagnostic criteria were met. Abbreviations: IQR: interquartile range; NTMPD: Nontuberculous mycobacteria pulmonary disease; BMI: body mass index; NTM: Nontuberculous mycobacteria; CF: Cystic fibrosis



Supplementary Figure 1: List of question domains in the on-line database tool, with number of fields within each domain.



Supplementary Figure 2: Different *Mycobacterium avium* complex regimes used. Abbreviations: MAC: *Mycobacterium avium* complex; IV: intravenous; PO: oral.



Supplementary Figure 3: Percentage of patients that eradicated and did not eradicate *Mycobacterium abscessus* complex according to induction regimes used.