Peer Review File
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Reviewer A

Comment 1: However, in my opinion, the usefulness of nasal breathing and controlling maxillary sinusitis (eosinophilic or dental) should never be emphasized too much in this context.
Reply: We thank the Reviewer for bringing up these topics but apologize because we are not quite sure how to answer this comment. As written, it suggests we should not emphasize nasal breathing and controlling maxillary sinusitis. If so, we never discussed these two entities. But if Reviewer A means "…can never be emphasized too much…" which we take to mean that these two entities should be discussed, we have included a sentence in the Methods section that other entities such as sinusitis, sleep, and improving mental health can also help impact well-being of patients with NTM-LD but their discussion is beyond the scope of this review.

Reviewer B

Comment 2: About methods: It is not written the years considered in the review.
Reply: We have added the following text to the Methods section: *"While there was limitation of the publication year, citations from 2000 to 2022 were preferentially referenced."*

Comment 3: In section VII, page 21, lines 555 to 559. The paragraph is not clear in the context.
Reply: We agree that this paragraph did not provide a proper context of novel compounds that might protect against aminoglycoside-induced ototoxicity. We have moved the relevant text to the Treatment section of Part VII (MINIMIZATION of VESTIBULAR AND COCHLEAR DYSFUNCTION ASSOCIATED with SOME ANTI-NTM DRUGS) to put it in better context of the discussion. We have also revised the paragraph to the following in Section 5, Treatment of vestibular dysfunction: *"Once it is determined that a medication is causing vestibular or cochlear dysfunction, it should be discontinued promptly, as the nerve damage may become irreversible with continued use. While such discontinuation may deprive individuals of an effective antibiotic against the offending NTM, there is optimism that aminoglycoside-induced ototoxicity may be prevented pharmacologically. This possibility is based on screening of thousands of chemical compounds in the zebrafish larvae model revealing several that protect the neuromasts from aminoglycoside-induced cell death (110-114). The mechanism of protection appears to be competitive inhibition of aminoglycoside for the mechanoelectrical transducer channel of the hair cells, preventing entry of aminoglycosides (113)."*

Comment 4: At table 4, in "Healthy fats", "herring" is written twice.
Reply: Corrected.

Comment 5: There could be a paragraph discussing limitations of data available about the subject and quality of articles reviewed.
Reply: We agree. We have added the following sentence to the Methods section: *"Because most of the references on treatments such as hypertonic saline and nutrition pertained to bronchiectasis in general or to those who are malnourished, respectively, and not necessarily to individuals with NTM-associated bronchiectasis, we have discussed and such papers but noted the limitations."*

Reviewer C

Comment 6: Very comprehensive, but recommend that they review closely and remove

duplication and reduce redundancy.
Reply: We agree and have revised the paper for greater clarity and succinctness. For example, two sections of Part VII (MINIMIZATION of VESTIBULAR AND COCHLEAR DYSFUNCTION ASSOCIATED with SOME ANTI-NTM DRUGS) have been removed. But we had to expand certain sections based on other Reviewers' comments.

Reviewer D

Comment 7: Overall, I think the manuscript is too descriptive and requires extensive summarization with more focus on available evidence in the context of NTM-LD.
Reply: We agree and have removed two entire sections of Part VII (MINIMIZATION of VESTIBULAR AND COCHLEAR DYSFUNCTION ASSOCIATED with SOME ANTI-NTM DRUGS). We have added the following sentence to the Methods section: *"Because most of the references on treatments such as hypertonic saline and nutrition pertained to bronchiectasis in general or to those who are malnourished, respectively, and not necessarily to individuals with NTM-associated bronchiectasis, we have discussed such papers but noted the limitations."*

Comment 8: Line 127- Figure 1, not figure 1A
Reply: Corrected.

Comment 9: LINE 142- specify the rate in patients with NTM-LD
Reply: This text has been revised to (Part III, paragraph 3): *"However, a study of ~1,800 bronchiectatic patients from the U.S. Bronchiectasis and Non-TB Mycobacteria Research Registry showed that only 56% of the subjects were employing non-pharmacologic bronchial hygiene. Those with NTM-LD had greater use of bronchial hygiene (59% vs 50%), chest percussion (19% vs 12%), and use of flutter or PEP valve (52% vs 40%) than those without NTM-LD, with all individual comparisons being statistically significant (7)."*

Comment 10: LINE 146/147- By what percentage does the QOL improve and what percentage does the exacerbations reduce? Quantify the improvement and effect of therapy? Is there evidence for this from outside the US? References should be cited in order (7,8). Has an effect on improving outcomes on bronchiectasis associated with NTM-LD (either as improved response rates, or reduced relapse rates) been shown? Comment on this as your review is on NTM – LD
Reply: We are grateful for Reviewer D bringing this issue up because with more careful reading of the original paper and corresponding with the author of one of the paper, we realized that we had *misinterpreted* the relevant data. Contrary to what we originally wrote, use of ACT (airway clearance therapy) was actually associated with worse QoL-bronchiectasis Treatment Burden domain (spent more time with treatment) and with worse QoL-bronchiectasis Respiratory Symptoms (more problematic respiratory symptoms) domain.

This text has been revised to (Part III, paragraph 3): *"In a retrospective, medical record analysis at enrollment of the U.S. Bronchiectasis and Non-TB Mycobacteria Research Registry and at one-year follow-up, ACT use at baseline and follow-up was associated with greater odds of experiencing exacerbations at follow-up compared to those who did not use ACT; the authors interpreted this counterintuitive finding to mean that use of ACT perhaps identified a more ill bronchiectasis population (8). Similarly, a prospective study of bronchiectasis patients showed that adherence to ACT was associated with lower (i.e., worse) scores in both the QoL-bronchiectasis Treatment Burden (i.e., spending longer time on treatment) and QoL-bronchiectasis Respiratory Symptoms domains (i.e., having more problematic respiratory symptoms) with a highly statistically significant negative regression co-efficient in both (9)."*

Comment 11: I would summarize the details of the breathing and cough techniques with appropriate references. The review should be on the evidence that these are useful or not in

NTM-LD, rather than on the technique itself. Reference this section so that readers who want details of the techniques can find them if needed.
Reply: To the best of our knowledge, there breathing and coughing techniques are based on bronchiectasis in general and has been applied to NTM-LD, which is dominated by bronchiectasis. We believe this is valid since there are papers showing that in some patients with relatively mild NTM-LD, culture conversion of their NTM may occur in 30-50% with airway clearance alone. This last point has now been added and referenced on Part III, paragraph 3.

Comment 12: Pharmacologic agents – I would highlight the rate of improvement seen with these measures, rather than describing the measures themselves and comment on whether evidence for NTM-LD is available or not.
Reply: This is a very good suggestion. The one area that really needed clarification is the use of hypertonic saline. Thus, we have revised this section extensively taking into account Reviewer D's suggestions.

The text has been revised extensively to (Part III, Section 4.1, paragraphs 1 and 2):
"While hypertonic saline is commonly used in patients with cystic fibrosis and non-cystic fibrosis bronchiectasis including those with NTM-LD (15), the available studies are relatively small and most often compared to isotonic (0.9%) saline. Nebulized hypertonic saline modestly improves lung function in bronchiectasis associated with cystic fibrosis (FEV1 increased by 3-4% at four weeks of treatment but not at 48 weeks), reduces the frequency of pulmonary exacerbations in adults requiring antibiotics by 0.5 exacerbations per person, and enhances sputum clearance (16).

"There have been four studies on the use of hypertonic saline in non-cystic fibrosis subjects and one specifically with NTM-LD. In a four-week trial of 24 subjects with non-cystic fibrosis bronchiectasis of isotonic vs. 7% saline – both of which included nebulized terbutaline and ABCT – use of hypertonic saline increased sputum weight and ease of expectoration, and decreased viscosity compared to isotonic saline use (17). In a follow-up, randomized cross-over study of 30 non-cystic fibrosis bronchiectasis patients of daily isotonic saline vs. 7% saline for three months for each of the two phases, 7% saline was superior to isotonic saline with regards to increase in forced expiratory volume in the first second (FEV1) and ease of expectoration as well as significant decrease in respiratory symptoms, antibiotic usage, and emergency room visits (18). In contrast to these salubrious effects of hypertonic saline, another study of 40 subjects with non-CF bronchiectasis showed no difference in exacerbation frequency, QoL parameters, sputum colonization, or FEV1 after one year of treatment when randomized to either isotonic saline or 6% saline (19). Similarly, in 22 patients with primary ciliary dyskinesia who underwent a randomized, cross-over study of isotonic saline vs. 7% saline (12 weeks for each of the two phases), there were no differences in the FEV1, forced vital capacity (FVC), St George's Respiratory Questionnaire score, number of exacerbations, or inflammatory markers (20). Only the QoL-bronchiectasis Health Perception score improved more with hypertonic saline than isotonic saline (20). In a retrospective study of 25 NTM-LD patients (mostly with nodular-bronchiectasis) who were treated for at least three months with hypertonic saline without antibiotics, 36% reported symptomatic improvement, 28% reported stability, and 20% noted deterioration (2). There was no change in lung function measurements with the hypertonic saline. Of the 12 patients who continued the hypertonic saline without antibiotics beyond three months, six converted their respiratory culture to negative. While this study showed hypertonic saline is promising in bronchiectatic patients with NTM-LD, the lack of a control arm limited more definitive interpretation (2)."

Comment 13: Physical and pulmonary rehabilitation- rather than detail the measures, what evidence is available for improvement of function in either bronchiectasis/ lung disease or NTM-LD? While it can be assumed that these measures are helpful, is there objective evidence for this?
Reply: We agree. We have now discussed in a new first paragraph that there are no studies that have examined the impact of pulmonary rehabilitation in patients with NTM-LD. But we also

cited one study and one review showing that pulmonary rehabilitation does help patients with bronchiectasis, an entity closely associated with NTM-LD. This paragraph in Part IV, paragraph 1 now reads: *"Pulmonary rehabilitation has been studied considerably in chronic obstructive pulmonary disease and to a lesser extent in bronchiectasis, known risk factors for NTM-LD (32). However, to the best of our knowledge, there has been no systematic studies of pulmonary rehabilitation in patients specifically with NTM-LD. However, it is not unreasonable to extrapolate data on pulmonary rehabilitation in bronchiectasis since bronchiectasis is so pervasive in NTM-LD. A retrospective study of 108 patients with non-cystic fibrosis bronchiectasis showed that three weeks of pulmonary rehabilitation significantly improved 6MWT distance by ~36 meters ($p<0.0001$), dyspnea (a change in the Baseline Dyspnea Index of ≥ 1 unit in 90%, and HR-QoL ($p<0.0001$) (33). Furthermore, those with airflow limitation showed an even greater improvement in 6MWT distance and HR-QoL with pulmonary rehabilitation (33). A review of four studies (164 participants) demonstrated that eight weeks of outpatient pulmonary rehabilitation or exercise training in patients with non-cystic fibrosis bronchiectasis significantly increased walk distance by ~67 meters (95% CI: 52-82 meters) and improved HR-QoL for up to six months (34)."*

Comment 14: Nutrition – line 396 to 402- this paragraph does not make much sense as a whole. Three different concepts seem to be included. What is the real point the authors are trying to get across?
Reply: We agree that this paragraph is confusing. In part, this is because it does not belong in Section 1 of Nutrition. We have revised this paragraph and placed it in a new Section 4 titled *"Albumin is not an accurate biomarker of nutritional status."* The paragraph has been revised to: *"Since plasma albumin level decreases in the setting of inflammation, it is not a reliable biomarker for malnutrition (77). Furthermore, a normal albumin does not necessarily indicate adequate nutrition as evinced by the fact that individuals with anorexia nervosa often have adequate levels (78). Thus, plasma albumin level does not reliably correlate with weight loss, calorie restriction, or nitrogen balance. The implication is that achieving a normal albumin requires both optimizing caloric intake and controlling inflammation; in the context of NTM infection, it would also entail reducing NTM load with antibiotics and effective airway clearance. Compared to albumin, prealbumin appears to have greater validity as a measure of nutritional status in individuals with acute, chronic, or critical illnesses, due, in part, to its shorter half-life than albumin (2 days vs. 20 days), less affected by liver disease, and not affected by hydration (79). While prealbumin may be a better biomarker of nutritional status than albumin, its level has also been found to be inversely related to acute inflammation associated with critical illness (80); thus, its use as a marker of malnutrition may be limited. Although preoperative prealbumin level <20 mg/dL is a risk factor for post-operative infection after elective spinal surgery (81) and longer mechanical ventilation after cardiac surgery (82), comparable data are unavailable for surgical lung resection for NTM-LD."*

Comment 15: Optimizing nutrition – has optimizing nutrition been shown to help in patients with NTM-LD in general or just in patients who have an underlying eating disorder? Be specific about the groups in which there is evidence and where there is no evidence. While generally good nutrition will help, you must specify where evidence for weight gain is available and where it is not.
Reply: We have clarified the text in Nutrition (Section 1) to: *"While optimizing nutrition improves outcomes in individuals with caloric-restrictive eating disorders (67), it is not known whether active weight gain improves NTM-LD outcomes due to a lack of prospective studies. Nevertheless, until such data are available, it is prudent to optimize nutrition."*

Comment 16: Swallowing dysfunction and GERD -what is the prevalence in patients with NTM-LD? Is there evidence that intervention for these has a positive effect on the disease?
Reply: With regards to prevalence, the following text has been added to Part VI (DIAGNOSIS and MITIGATION OF SWALLOWING DYSFUNCTION and of GASTROESOPHAGEAL REFLUX DISEASE (GERD), 1st paragraph): *"While the prevalence of swallowing dysfunction has not been specifically studied in patients with NTM-LD, based on several studies the prevalence of dysphagia in those over 50 years is estimated to be 15-22%, higher than the 6-9% prevalence for the general population (84). In three separate studies, GER was present in*

26–44% of NTM-LD subjects and 12–28% in non-NTM infected controls (85-87). One important caveat is that individuals may not experience typical reflux symptoms. Whether mitigation of swallowing dysfunction or of GER impacts outcomes of NTM-LD has not been specifically studied. Nevertheless, based on collective clinical experience and the fact that either gastrointestinal disorders can cause overt or occult aspiration that can then result in recurrent lung inflammation and infection, it seems prudent to evaluate for the possibility of both, particularly in those with suspicious symptoms and/or recalcitrant NTM-LD."

Comment 17: Hypothesizing about antacids preventing suppression of NTM growth in the stomach is not relevant here. The stomach does not present a suitable environment for NTM growth. In vivo and in vitro growth are very different. Management options do not need details. Only if there is evidence that such interventions are of benefit to the lung disease.
Reply: We have reduced the discussion on evaluation of swallowing dysfunction and GERD. But we also believe that some details are necessary to help clinicians understand what these tests entail to better inform the patient of what they will undergo and why.

While we acknowledge that further studies need to be performed, viable NTM has been isolated from gastric juices and possibly related to the use of antacids. The following text has been added to Part VI, Section 2, Paragraph 3: *"Antacid medications such as proton pump inhibitors or H2 blockers are used mainly to control the heartburn symptoms of reflux and to prevent esophagitis (93). However, in the context of NTM-LD, acid reduction may prevent the suppression of NTM growth in the stomach as the optimal growth pH is ~6.0 for slow-growing NTM and Mycobacterium chelonae and is ~7-7.4 for other rapidly growing mycobacteria (97-99). This pH hypothesis is supported by a study that analyzed for the presence of NTM in both the gastric juice and gastrostomy tubes in 16 cystic fibrosis patients who were receiving gastric tube feedings (100). In 7 of 16 patients, live NTM (M. abscessus complex) were isolated in the gastric juices in three subjects and in the gastrostomy tube in two individuals (100). Interestingly, all 5 patients in whom NTM was isolated from gastric juice or gastrostomy tube had gastric pH ≥ 3 (range: 3 to 6) with a mean pH of 5.0; in other words, all with NTM isolated had gastric pH greater than the normal gastric pH of 0.3-2.9 (101). Furthermore, of the 11 patients without NTM isolated from either the gastric juice or gastrostomy tube, five had gastric pH of 2 and six had gastric pH ≥ 3 with a mean pH of 3.4 (100). While not definitive, these findings bolster the notion that a higher gastric pH may be more likely to support NTM viability in the upper gastrointestinal tract."*

Comment 18: Details of cochlear and vestibular dysfunction are not required as this is basic. What is the incidence of toxicity in NTM-LD? Details of interventions are again not needed.
Reply: We entirely agree. There are some data on the incidence of ototoxicity with the use of aminoglycosides in the setting of NTM-LD. We have added the following text with a relevant citation to Part VII, Section 1, paragraph 1: *"Ototoxicity was observed in 42% of patients treated with streptomycin and 27% of patients treated with amikacin for macrolide-resistant Mycobacterium avium complex pulmonary disease (105)."*

In addition to making the discussion in Part VII (on ototoxicity and treatment of vestibular dysfunction) more succinct, we have deleted two sections (*Vestibular contributions to gaze stability and postural control* and *Types of vestibular dysfunction*) based on the Reviewer's recommendations. Hence, we have also deleted the previous Figure 8.