Culture Result Discrepancy Between Laboratories for Nontuberculous Mycobacteria in Patients with Cystic Fibrosis

Mariana Bedoya[1], Max Salfinger[2], Alejandra Weisman[3], and Andrew Colin[3]

[1]Monroe Carell Junior Children's Hospital at Vanderbilt
[2]University of South Florida Morsani College of Medicine
[3]University of Miami School of Medicine

August 17, 2022

Abstract

NTM-infected CF patients may be at risk of being underdiagnosed or inappropriately treated when relying on culture and susceptibility results from non-specialized laboratories. Since this was a small study with convenience samples, a larger study needs to be carried out. If our findings are confirmed, the drivers should be elucidated for the discrepant results. Given the increasing prevalence of NTM in the population at large and not in the CF community alone[[13]](#ref-0013), elucidating any differences in testing to ensure the correct identification, including subspeciation and antimicrobial susceptibilities should be paramount

To the Editor:

Culture Result Discrepancy Between Laboratories for Nontuberculous Mycobacteria in Patients with Cystic Fibrosis

Mariana Bedoya, MD1, Max Salfinger, MD2, Alejandra Weisman 3, Andrew Colin, MD3

1 Division of Allergy, Immunology, Pulmonary and Sleep Medicine, Monroe Carell Jr. Children's Hospital at Vanderbilt, Nashville, TN 2. University of South Florida, College of Public Health & Morsani College of Medicine, Tampa, FL 3. Division of Pediatric Pulmonology, Miller School of Medicine, University of Miami, Miami, FL

Nontuberculous mycobacteria (NTM) are ubiquitous organisms that can cause severe chronic pulmonary infection, particularly in patients with cystic fibrosis (CF), and have become a rising concern worldwide.[1] The predominant NTM species found among people with CF include*Mycobacterium avium* and *Mycobacterium abscessus* .[2] The prevalence of NTM based on large studies in adult and pediatric patients with CF in the Americas, Europe, and Australia varies between 6-13%.[3-6] In a large study in the United States (US), NTM prevalence was shown to vary significantly among patients with CF by geographic area, an observation attributed to environmental factors.[7] Another US study identified significant spatial clustering of NTM in 8 states, including Florida.[8]

The 2018 CF patient registry reported an annual prevalence of 13.6% of positive cultures of one or more mycobacterial species isolated from those who had a mycobacterial culture [9], and the longitudinal prevalence (years 2010-2016) of one or more NTM species isolated was 19%.[10] We led a Florida-based, two-center prevalence review in conjunction with University of Florida in 2012, and found 8 out of 85 patients (9.4%) under 18 years of age had at least one positive culture of NTM among the two CF centers.[11] Our pediatric practice in Miami, FL, has been experiencing a dramatic increase in the number of CF patients with at least one culture positive for NTM; our prevalence in 2017 was 31.8%.

NTM is essentially undiagnosable from conventional throat cultures.[11] NTM require adequate samples of sputum or bronchoalveolar lavage (BAL) fluid. Specialty laboratories are key for reliable culture results. It is crucial to identify the sample as deriving from a CF patient for the laboratory, especially from those who harbor *Pseudomonas* species, since sample decontamination is essential to prevent overgrowth of bacteria that are omnipresent in CF. These bacteria could interfere with the growth of NTM, resulting in false negative results.[12] Subsequent to growth, identification to species and subspecies level and antimicrobial susceptibility testing, including drug resistance markers, is also crucial. These tests will determine whether patients will be placed on prolonged, complex, and onerous antimicrobial regimens. Currently, at our institution, acid-fast bacilli (AFB) sputum cultures are sent to commercial laboratories when obtained in our outpatient practice. The commercial laboratory is dictated by insurance, without flexibility in the choice of specialty laboratories. Inpatient samples (sputum and BAL) are analyzed in our teaching hospital laboratory, where a positive AFB culture will trigger PCR for *Mycobacterium tuberculosis.* If negative for*Mycobacterium tuberculosis* , the sample is forwarded to a national reference laboratory for culture, speciation and antimicrobial susceptibility testing.

The ominous reality of the tripling prevalence of NTM in our pediatric CF center was particularly concerning for several patients who presented with deteriorating health, deemed disproportionate to their known infectious etiologies and in whom we excluded other specific potential comorbidities such as allergic bronchopulmonary aspergillosis, malnutrition, and CF related diabetes. While following guidelines for annual AFB cultures, we suspected false negative AFB cultures or possibly reporting an incorrect identification or antimicrobial susceptibility results, all of which can affect diagnosis and outcome.

Our goal was to ascertain the reliability of diagnostic tests in our teaching hospital laboratory (A) and referral commercial laboratories (B) and compare their reported results to a specialized laboratory for NTM (C).

We obtained 19 samples (BAL and/or sputum) for AFB from patients with suspected or known NTM. Identical samples were contemporaneously sent to laboratory A and/or B, and all were sent to laboratory C (Table 1). All cultures were followed for up to 56 days. One sample was lost in shipping to laboratory C and removed from analysis. Eight of 18 samples grew NTM in at least one laboratory. Fifty percent of the positive samples for NTM had discrepancies. Two samples (11.1% of total) sent to laboratory A or laboratory B failed to grow NTM when compared to laboratory C, and 2 samples (11.1% of total) had growth at both laboratory B and laboratory C but revealed different species. Additionally, laboratory B failed to report antimicrobial susceptibility testing results in three out of four of its positive samples**.**Overall, we found discrepancies in 22.2% of AFB culture results from CF patients from duplicate BAL or sputum samples between laboratory A and/or laboratory B compared to the specialized laboratory for NTM (C).

We conclude that NTM-infected CF patients may be at risk of being underdiagnosed or inappropriately treated when relying on culture and susceptibility results from non-specialized laboratories. Since this was a small study with convenience samples, a larger study needs to be carried out. If our findings are confirmed, the drivers should be elucidated for the discrepant results. Given the increasing prevalence of NTM in the population at large and not in the CF community alone[13], elucidating any differences in testing to ensure the correct identification, including subspeciation and antimicrobial susceptibilities should be paramount.

Table 1

	Patient Initials	**Date**	**Sample**	**A**	**B**	**C**
1	**JD**	2/2/2017	Sputum	NT	*M. avium-intracellulare* complex	*M. abscessus* subsp. *m*
2	**LP**	2/2/2017	Sputum	NT	No growth	*M. abscessus* subsp. *m*
3	**AZ**	2/22/2017	Sputum	No growth	NT	No growth
4	**DL**	5/4/2017	BAL	No growth	NT	No growth
5	**DP**	11/4/2017	Sputum	NT	*M. chelonae-abscessus*	*M. abscessus* subsp. *m*
6	**DC**	4/17/2017	BAL	No growth	NT	No growth
7	**BB**	5/15/2017	BAL	No growth	NT	No growth

	Patient Initials	Date	Sample	A	B	C
8	**LP**	5/24/2017	Sputum	NT	No growth	No growth
9	**NG**	12/15/2017	Sputum	*M. abscessus*	NT	*M. abscessus* subsp. *m*
10	**DL**	7/24/2018	BAL	No growth	NT	No growth
11	**DP**	7/16/2018	Sputum	NT	*M. avium-intracellulare* complex	*M. abscessus* subsp. *a*
12	**NG**	2/8/2018	Sputum	No growth	NT	No growth
13	**JD**	9/8/2018	BAL	No growth	NT	No growth
14	**MR**	8/16/2018	BAL	No growth	No growth	No growth
15	**MK**	8/16/2020	Sputum	NT	*M. abscessus*	*M. abscessus* subsp. *m*
16	**NG**	1/16/2019	BAL	No growth	No growth	Lost sample
17	**DL**	8/2/2019	Sputum	No growth	NT	*M. abscessus* subsp. *m*
18	**DC**	4/3/2020	BAL	No growth	NT	No growth
19	**MK**	6/23/2020	BAL	*M. abscessus*	NT	*M. abscessus* subsp. *m*

A: Teaching hospital laboratory; B: Referral commercial laboratories; C: Specialized laboratory for nontuberculous mycobacteria; BAL: Bronchoalveolar lavage; M.: *Mycobacterium;* subsp.: subspecies; NT: Not tested

References

1. Skolnik, K., G. Kirkpatrick, and B.S. Quon, *Nontuberculous Mycobacteria in Cystic Fibrosis.* Curr Treat Options Infect Dis, 2016.**8** (4): p. 259-274.

2. Floto, R.A., et al., *US Cystic Fibrosis Foundation and European Cystic Fibrosis Society consensus recommendations for the management of non-tuberculous mycobacteria in individuals with cystic fibrosis.*Thorax, 2016. **71 Suppl 1** : p. i1-22.

3. Adjemian, J., K.N. Olivier, and D.R. Prevots, *Epidemiology of Pulmonary Nontuberculous Mycobacterial Sputum Positivity in Patients with Cystic Fibrosis in the United States, 2010-2014.* Ann Am Thorac Soc, 2018. **15** (7): p. 817-826.

4. Aiello, T.B., et al., *Prevalence and clinical outcomes of nontuberculous mycobacteria in a Brazilian cystic fibrosis reference center.* Pathog Dis, 2018. **76** (5).

5. Gardner, A.I., 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. **68** (5): p. 731-737.

6. Roux, A.L., et al., *Multicenter study of prevalence of nontuberculous mycobacteria in patients with cystic fibrosis in france.*J Clin Microbiol, 2009. **47** (12): p. 4124-8.

7. Adjemian, J., K.N. Olivier, and D.R. Prevots, *Nontuberculous mycobacteria among patients with cystic fibrosis in the United States: screening practices and environmental risk.* Am J Respir Crit Care Med, 2014. **190** (5): p. 581-6.

8. Adjemian, J., et al., *Spatial clusters of nontuberculous mycobacterial lung disease in the United States.* Am J Respir Crit Care Med, 2012. **186** (6): p. 553-8.

9. *Cystic Fibrosis Foundation Patient Registry Annual Data Report.* 2018.

10. Cystic-Fibrosis-Foundation-Patient-Registry. *Cystic Fibrosis Foundation Patient Registry Annual Data Report* . 2017.

11. Yousef S, S.P., Baker D, Della Volpe A, Colin A, Quizon A,*Prevalence of non-tuberculous mycobacteria (NTM) in pediatric patients with cystic fibrosis (CF) in Florida – A two center study.*Paediatr Respir Rev, 2013. **14** (Supp 2): p. S72.

12. Griffith, D.E., et al., *An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases.* Am J Respir Crit Care Med, 2007. **175** (4): p. 367-416.

13. Ratnatunga, C.N., et al., *The Rise of Non-Tuberculosis Mycobacterial Lung Disease.* Frontiers in Immunology, 2020. **11** .