Serial sputum induction in nontuberculous mycobacterial pulmonary disease

To the Editor:

The recommended procedure for diagnosis of nontuberculous mycobacterial pulmonary disease (NTM-PD) is collection of three sputum samples on separate days [1]. Sputum induction is suggested for patients unable to expectorate spontaneously. These recommendations align with evidence-based guidelines for pulmonary tuberculosis (TB) [2] but literature supporting their applicability to NTM-PD is lacking. In addition, the clinical utility of colony counts on solid media requires clarification. Early changes in semiquantitative colony counts are predictive of clinical and radiological improvement and treatment success [3] but the relationships of colony count with disease markers, such as smear status, have not been reported.

Standard evaluation of patients in the Division of Mycobacterial and Respiratory Infections at National Jewish Health (NJH) in Denver, CO, USA, includes three sputum inductions, generally by administration of nebulised 3–10% hypertonic saline *via* Aerobika oscillating positive expiratory pressure device. One spontaneously expectorated sputum sample is usually requested pre-admission. Microbiological analysis includes smear examination, culture in Mycobacteria Growth Indicator Tube (MGIT) and on Löwenstein-Jensen (LJ) slant and agar plate, and quantitation of colonies on solid media. The present study constituted a retrospective audit of this evaluation protocol with the following goals: 1) describe the yield of serial induced sputum samples for NTM and variation of yield with induction methodology; 2) compare the yield of induced and spontaneously expectorated samples; and 3) elucidate the relationship between colony count and smear status.

Diagnostic yield per sputum sample, as reported in the TB literature, translates imperfectly to NTM-PD. Whereas TB is defined by a single positive culture, NTM-PD diagnosis requires at least two positive sputum cultures with appropriate clinical and radiological findings [1]. Furthermore, patients at referral institutions commonly present with a history of positive cultures and isolation of multiple species. We therefore studied "detection yield", defined as culture of any potentially pathogenic NTM.

Inclusion criteria were age >18 years, induction of three sputum samples with hypertonic saline during hospital admission, and culture of NTM other than *M. gordonae* from at least one of these samples. Electronic medical records for subjects identified by a search of the NJH Research Database were reviewed in reverse chronological order to apply the inclusion criteria and extract data. In total, 416 records were reviewed to obtain an *a priori*-defined sample of 200 patients, who were admitted between November 2016 and October 2018. Statistical analysis was performed in R, version 3.5.1. Continuous variables were compared with permutation analysis and proportions with Fisher's exact test.

Studied patients included 178 (89%) females and were of mean age 66 (95% CI 65–67) years and mean BMI 21.8 (95% CI 21.3–22.3) $kg \cdot m^{-2}$. Cough was reported by 152 (76%) subjects and haemoptysis within the preceding 6 months by 34 (17%). Reported computed tomography pulmonary findings included bronchiectasis in 188 (94%) patients, nodules in 161 (80%) and cavities in 51 (26%). NTM had previously been cultured from at least two sputum samples or at least one bronchoscopic fluid sample in 195 (98%) patients. 57 (28%) patients were receiving anti-NTM antibiotics at the time of admission and a further 61 (30%) had previously been treated.

Induced sputum samples were collected over a median 3 (range 3–10) days. At least two samples were culture-positive in 156 (78%) cases, 93% having repeated isolation of the same species. *Mycobacterium*

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Incremental detection yields of serial induced sputum samples support collection of three samples for detection of nontuberculous mycobacteria. In addition, higher colony counts on solid media are associated with markers of disease severity. http://bit.ly/2Tn14Qa

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avium complex and *M. abscessus* were isolated from 154 (77%) and 53 (27%) patients, respectively. More than one species was isolated in 27 (14%) patients.

The proportion of positive cultures differed with induced sputum number (p=0.03) and was lowest for third inductions (table 1). Bronchodilator pre-treatment and airway clearance device use were documented more frequently for first inductions, whereas administered hypertonic saline concentrations did not differ. Binary logistic regression analysis, including patient identifier as a random effects term, identified only sputum number as having a statistically significant influence on the likelihood of culture positivity (linear term OR 0.69, 95% CI 0.48–1.0; p=0.048). This diminishing likelihood of culture positivity with successive sputum inductions possibly reflects interventions initiated during admission, such as aggressive airway clearance. Absence of a statistically significant effect of saline concentration is consistent with a meta-analysis of sputum induction for TB diagnosis [4], although a weakness of the present study is paucity of patients receiving concentrations under 7%. In addition, it is possible that unrecorded bronchodilator pre-treatment or airway clearance device use confounded the lack of observed effect of these interventions.

Incremental detection yields for the first, second and third induced samples were 80%, 14% and 6%, respectively (table 1). These results align closely with diagnostic yields of serial smears and sputum cultures for TB [5–7]. Cumulative detection yield of the second sample from patients with pulmonary cavitation was 100%. In contrast, there was a trend towards detection yields being less efficient in patients using inhaled corticosteroids, potentially representing an effect of the medication or underlying pulmonary disease. Detection yields did not vary with treatment status (likely reflecting referral bias toward treatment-refractory patients), forced expiratory volume in 1 s, haemoptysis or serum C-reactive protein (CRP) level. Additional comorbid diagnoses were not specifically studied due to marked predominance of bronchiectasis and diagnostic uncertainty inherent in the retrospective study design.

TABLE 1 Serial induced sputum sampling methodologies and results

Parameter	Induced sputum number		
	1	2	3
Microbiological results			
Positive smear	12 (6%)	10 (5%)	7 (4%)
Positive culture[#]	160 (80%)	163 (82%)	142 (71%)
Colony count	20 (3–100)	20 (2–100)	18 (2–100)
Induction methodology			
Bronchodilator pre-treatment[¶]	34 (17%)	24 (12%)	19 (10%)
Airway clearance device[+]	198 (99%)	158 (79%)	160 (80%)
Hypertonic saline concentration			
3%	10 (5%)	6 (3%)	8 (4%)
7%	131 (66%)	126 (63%)	116 (58%)
10%	59 (30%)	68 (34%)	76 (38%)
Incremental detection yields and selected sub-group comparisons			
All patients	160 (80%)	27 (14%)	13 (6%)
Single species (n=173)	135 (78%)	25 (14%)	13 (8%)
Pulmonary cavitation[§]			
Present (n=51)	47 (92%)	4 (8%)	N/A
Absent (n=149)	113 (76%)	23 (15%)	13 (9%)
Current treatment			
Present (n=57)	45 (79%)	8 (14%)	4 (7%)
Absent (n=143)	115 (80%)	19 (13%)	9 (6%)
Inhaled corticosteroids[f]			
Present (n=44)	31 (70%)	7 (16%)	6 (14%)
Absent (n=156)	129 (83%)	20 (13%)	7 (4%)

Data are presented as n (%) or median (interquartile range). [#]: rates of culture positivity differed with sputum number (p=0.03); [¶]: there was a trend towards differing of rates of bronchodilator pre-treatment with sputum number (p=0.08); [+]: rates of airway clearance device (Aerobika or vest) use during induction differed with sputum number (p<0.001); [§]: detection yields differed with present/absent cavitation (p=0.02); [f]: there was a trend towards differing of detection yields with present/absent inhaled corticosteroid use (p=0.07).

Pre-admission spontaneously expectorated samples were submitted by 93 patients whose treatment status did not change between collection of pre-admission and first induced samples, a median interval of 42 (range 5–185) days. The rates of culture positivity were similar between pre-admission and first induced samples (81% *versus* 78%; p=0.86) and there was concordance of positive/negative results in 67 (72%) cases. Species identification concurred in 55 of 61 (90%) cases with concordant positive results. Similarity of detection yields between spontaneously expectorated and induced samples was also reported in a prospective study of diagnostic yields for TB in patients who were able to expectorate spontaneously and were not pre-screened by previous negative sputum smear/culture [8].

Colony counts for culture-positive samples were recorded from agar plate culture reports or designated as zero for growth only in MGIT and/or on LJ slant. Colony counts of 29 smear-positive samples were statistically significantly greater than 436 smear-negative samples (median 200 (interquartile range (IQR) 100–200) *versus* median 17 (IQR 2–100); p<0.01). The optimal cut-point for predicting smear positivity on receiver operating characteristic curve analysis was 100 colonies (sensitivity 79%, specificity 73%, area under the curve 0.79). Patients with colony counts ⩾100 on at least one culture exhibited greater serum CRP concentrations (1.5, 95% CI 1.0–2.3, *versus* 0.6, 95% CI 0.5–0.8 $mg{\cdot}dL^{-1}$; p<0.001) and frequencies of pulmonary cavitation (27/72, 38% *versus* 24/128, 19%; p<0.01) than those without. Smear positivity in NTM-PD is associated with reduced treatment response, cavitation and increased disease progression and mortality [9–11]. Further evaluation of the clinical significance of colony counts and their utility for treatment decision-making, especially in smear-negative patients, is warranted.

In conclusion, incremental detection yields of serial induced sputum samples, observed in a cohort of patients with predominantly pre-existing diagnoses of NTM-PD, supported the practice of collecting three samples to detect NTM. Although the detection yields of single spontaneous and induced sputa were comparable, the present study did not compare serial collections and was unable to evaluate diagnostic utility of induction. Furthermore, the study design precluded determination of sensitivity of induced sputum culture for NTM. A threshold of 100 colonies on agar plate was modestly sensitive and specific for smear positivity and associated with greater serum CRP concentration and frequency of pulmonary cavitation. Limitations of the present study are its retrospective design and, due to being performed at a referral institution, potential for selection bias and limited generalisability. For example, collection of sputum samples on three successive days may be difficult in an outpatient setting. Prospective study of optimal induction methodology, detection yields and colony count correlates at the point of NTM-PD diagnosis is required.

Michael R. Holt [1,2], Shannon H. Kasperbauer[1,2] and Charles L. Daley[1,2]
[1]Division of Mycobacterial and Respiratory Infections, Dept of Medicine, National Jewish Health, Denver, CO, USA. [2]Dept of Medicine, University of Colorado Denver, Aurora, CO, USA.

Correspondence: Michael R. Holt. E-mail: michaelrholt@outlook.com

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