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Differentiating rapid- and slow-growing mycobacteria by difference in time to growth detection in liquid media☆,☆☆,★

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ABSTRACT

Nontuberculous mycobacteria (NTM) are classified into 2 categories: slow-growing mycobacteria (SGM) and rapid-growing mycobacteria (RGM), based on interval to colony formation by subculture on solid media. However, little is known about the growth rate of NTM in liquid broth media. We evaluated the differences in time to growth detection (TGD) of RGM and SGM in liquid broth media according to acid-fast stain. Among the 696 NTM isolates, 201 were RGM and 495 were SGM. In acid-fast bacilli (AFB)–negative specimens, the mean TGD was 133 h for RGM and 269 h for SGM ($P < 0.001$). In AFB-positive specimens, the mean TGD was 112 ± 37 h for RGM and 155 ± 125 h for SGM ($P = 0.063$). In the AFB-negative group, a cut-off value of 6 days was most effective for distinguishing SGM from RGM; however, in the AFB-positive group, an appropriate cut-off value was hard to define with TGD only.

1. Introduction

Nontuberculous mycobacteria (NTM) are mycobacteria species other than *Mycobacterium tuberculosis* complex or *M. leprae*. NTM species are traditionally categorized into rapid- and slow-growing mycobacteria (RGM and SGM, respectively) based on their growth rates (Runyon, 1965). Species that form visible colonies on a subculture plate in 3–7 days are classified as RGM, while species that take more than 7 days to form a visible colony are classified as SGM. Early determination of whether the causative organism is RGM or SGM is useful for choosing appropriate empirical antibiotics, since antibiotic regimens that are effective for RGM may not be effective for SGM and vice versa (Thomson and Yew, 2009). Therefore, current guidelines recommend that the time (in days) of NTM growth to visible colonies should be stated on the laboratory report to help clinicians differentiate between RGM and SGM (Griffith et al., 2007).

Time to growth detection (TGD) of NTM varies widely among clinical specimens, mainly due to variations in the number of organisms in the specimen (Ogwang et al., 2009). Recently, liquid-based culture systems are widely used for higher yields and rapid detection of mycobacterium in specimens (Chew et al., 1998; Chien et al., 2000; Lee et al., 2003; Sorlozano et al., 2009); however, there are limited data regarding whether TGD can differentiate between RGM and SGM in clinical specimens, especially in the liquid-based culture systems. This study investigated the use of TGD in liquid broth media to distinguish RGM and SGM according to acid-fast bacilli (AFB) staining results, which reflect the NTM burden in clinical specimens.

2. Materials and methods

2.1. Specimens, culture, and identification

We identified all clinical specimens with NTM from January 2009 to April 2011 at the Seoul National University Hospital, a 1600-bed, university-affiliated, tertiary-care teaching hospital in South Korea, retrospectively. The BACTEC MGIT 960 system (BD Diagnostics, Sparks, MD, USA) was used to detect mycobacteria in clinical specimens. Prior to inoculation, nonsterile specimens were decontaminated with 4% NaOH, homogenized, and concentrated by centrifugation ($3000 \times g$, 20 min). The pellet was resuspended in phosphate buffered saline and inoculated into an MGIT 7-mL tube supplemented with PANTA (BD Diagnostics) and growth factors. Further identification of growing NTM isolates at the species level was performed using *rpoB* polymerase chain reaction restriction fragment length polymorphism analysis or *16S rRNA* sequencing analysis (Hong et al., 2011).

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2.2. Definition and classification of NTM

The growing microorganisms were classified as RGM or SGM as described previously (Petrini, 2006). *M. abscessus, M. fortuitum*, and *M. conceptionense* were classified as RGM, and *M. avium, M intracellulare, M. kansasii, M. lentiflavium*, *M. peregrinum*, and *M. terrae* were classified as SGM. Because some reports have classified *M. gordonae* as intermediate-growing mycobacteria (Collins, 1966), we excluded it from analysis.

Specimens were classified into 2 groups, AFB negative and AFB positive, according to their AFB staining results. The AFB staining results were interpreted as proposed by the Centers for Disease Control and Prevention, and specimens with trace results (<1 per 100 fields at 1000×) on the AFB smear were classified as AFB negative (Forbes, 2008).

The specimens for culture were also classified as either respiratory or nonrespiratory: sputum, endotracheal aspirate, bronchial washings, and bronchoalveolar lavage specimens were classified as respiratory specimens, and other specimens were classified as nonrespiratory specimens.

Clinically significant isolates were defined as 1) from sterile sites (e.g., tissue biopsy, pleural effusion, or joint aspiration fluid) or 2) associated with pulmonary NTM diseases as defined by the American Thoracic Society (Griffith et al., 2007). In brief, the diagnostic criteria for pulmonary NTM diseases were 1) positive culture from at least 2 separate samples or 1 sterile sample, 2) pulmonary symptoms, and 3) nodular or cavitary opacities on chest X-ray or multifocal bronchiectasis with multiple small nodules on high-resolution computed tomography. Culture-positive specimens other than from clinically significant isolates were considered to be colonizers. The TGD was defined as the number of hours elapsed from inoculation of a specimen in liquid medium to the detection of growth using an alarm.

2.3. Analysis

TGD values were compared using a linear mixed model with a random patient effect that took into account a possible clustering effect of multiple culture specimens from the same patient. The correlation of the AFB smear grade and TGD was evaluated by using 1-way analysis of variance and contrast test. To test the diagnostic accuracy of potential TGD cutoff values, receiver operating characteristic (ROC) curves were plotted and the areas under the curves (AUCs) were calculated. All significance tests were 2-sided, and a P value of <0.05 was considered to be statistically significant. Statistical analyses were performed using SPSS software (v. 19.0; SPSS, Chicago, IL, USA). The study protocol was approved by the Institutional Review Board of Seoul National University Hospital.

3. Results

3.1. General findings

Of 1213 NTM isolates from 520 patients during the study period, 422 (34.8%) isolates were not identified at the species level and12 (1.0%) isolates of *M. gordonae* were excluded from the analysis. Among the 779 remaining isolates, 696 isolates from 173 patients were clinically significant isolates; the others were considered to be colonization. Most of the samples were respiratory specimens (n = 681, 97.8%), mainly from sputum (n = 639, 91.8%); 15 (2.2%) of the clinical isolates were from tissue or joint aspiration fluid.

There were 201 (28.9%) isolates classified as RGM and 495 (71.1%) isolates classified as SGM (Table 1). The RGM group included *M. abscessus* (199 isolates), *M. fortuitum* (1 isolate), and *M. conceptionense* (1 isolate). The SGM group included *M. avium* (261 isolates), *M. intracellulare* (219 isolates), *M. kansasii* (8 isolates), *M. lentiflavium* (3 isolates), *M. terrae* (1 isolate), and *M. peregrinum* (1 isolate).

Table 1
Characteristics of patients and clinical specimens.

	RGM	SGM	Total
General characteristics			
Isolates	201	495	696
Patients	50	123	173
Age in years ± SD	59.3 ± 13.0	62.0 ± 12.0	61.2 ± 12.3
Sex (male/female)	18/32	46/77	64/109
Specimens			
Respiratory	190	491	681
Sputum	183	456	639
Bronchial washing	6	23	29
BAL	1	2	3
Endotracheal aspiration	0	10	10
Nonrespiratory	11	4	15
Abscess/pus	8	0	8
Tissue	3	4	7
AFB staining results			
+++ to ++++	11	47	58
++	9	29	38
+	14	54	68
– or ±	167	364	531

RGM = Rapid-growing mycobacteria; SGM = slow-growing mycobacteria; BAL = bronchoalveolar lavage; AFB = acid-fast bacilli.
Data are shown as number unless otherwise indicated.

3.2. Time to growth detection

There were statistically significant differences in the mean TGD between the RGM and SGM groups. The mean TGD was 130 ± 53 h in the RGM group and 239 ± 163 h in the SGM group (P < 0.001). The difference in mean TGD between the RGM and SGM groups remained significant for AFB-negative specimens. In AFB-negative specimens, the mean TGD was 133 ± 55 h for RGM (n = 167) and 269 ± 165 h for SGM (n = 364) (P < 0.001). In AFB-positive specimens, the mean TGD was 112 ± 37 h for RGM (n = 34) and 155 ± 125 h for SGM (n = 130) (P = 0.063) and the correlation of the AFB smear grade and TGD was not statistically significant in the RGM or SGM group (P = 0.896 [RGM] and P = 0.188 [SGM], respectively). Fig. 1 depicts the mean value and distribution of TGD for each group of species.

In subgroup analysis with sputum specimens, the results were similar to those with all specimens. In AFB-negative specimens, the mean TGD was 134 ± 56 h for RGM (n = 149) and 274 ± 170 h for SGM (n = 329) (P < 0.001). In AFB-positive specimens, the mean TGD was 112 ± 37 h for RGM (n = 34) and 149 ± 110 h for SGM (n = 126) (P = 0.064). In subgroup analysis with the specimens other than sputum, the mean TGD was 124 ± 42 h for RGM (n = 18) and 215 ± 101 h for SGM in the AFB-negative group (n = 35) (P = 0.001). In the AFB-positive group, the mean TGD was 305 ± 372 h for SGM (n = 4), but no specimens were RGM in the group.

3.3. ROC Curves

We drew ROC curves of TGD to differentiate between RGM and SGM in the AFB-negative and -positive groups, respectively (Fig. 2). In the AFB-negative group, a TGD value of 6 days was most effective for distinguishing SGM from RGM with 81.0% sensitivity and 71.3% specificity (Table 2). The AUC was 0.837 (P < 0.001). In the AFB-positive group, a suitable TGD value was hard to define and the AUC was 0.629 (P = 0.02).

4. Discussion

In this study, we compared the mean TGD values for RGM and SGM in liquid broth media for clinically significant specimens. Although several previous studies reported the TGD of NTM in diverse culture systems (Alcaide et al., 2000; Chew et al., 1998; Hanna et al., 1999; Kanchana et al., 2000; Leitritz et al., 2001; Sharp et al., 1997; Sorlozano