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Sensitivity of Fluorochrome Microscopy for Detection of *Mycobacterium tuberculosis* versus Nontuberculous Mycobacteria

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The results for 6,532 consecutive mycobacterial respiratory specimens collected from 1,040 patients from 1993 to 1995 in a Texas hospital were studied to determine the sensitivity of fluorescence microscopy for detection of *Mycobacterium tuberculosis* and nontuberculous mycobacteria (NTM). Smears were positive for acid-fast bacilli (AFB) in 63% (677 of 1,082) of specimens growing *M. tuberculosis* and 56% (638 of 1,148) of specimens growing the four most common species of NTM. Smear positivity by species was 58% (446 of 776) for *M. avium* complex, 51% (154 of 300) for rapidly growing mycobacteria (98% were *M. abscessus*), 78% (29 of 37) for *M. kansasii*, and 26% (9 of 35) for *M. gordonae*. Definite or probable disease by clinical criteria was present in 79% of patients with *M. avium* complex, 93% of patients with rapidly growing mycobacteria, 100% of patients with *M. kansasii*, and 0% of patients with *M. gordonae*. Patients with *M. avium* complex had a low incidence of AIDS (7%), and approximately 50% of non-AIDS patients had upper-lobe cavitary disease and 50% had nodular bronchiectasis. Only 23 of 6,532 (0.35%) of AFB smears were positive with a negative culture excluding patients on therapy for established mycobacterial disease. These studies suggest that NTM are as likely as *M. tuberculosis* to be detected by fluorescent microscopy in specimens from patients from areas endemic for NTM lung disease and at low risk for AIDS.

Tuberculosis, infection caused primarily by *Mycobacterium tuberculosis* and less commonly by *M. africanum* or *M. bovis*, is responsible for 3 million deaths each year, which is more than one-quarter of avoidable deaths due to an infectious disease. In the United States, *M. avium* complex is probably the most common cause of mycobacterial infection, exceeding *M. tuberculosis* in most areas of the country (2, 6, 18, 25). While advances in tuberculosis therapy continue to be made, patients remain undiagnosed and thus untreated. Advances in techniques for the diagnosis of tuberculosis are also being made in an attempt to address this problem. These advances include radiometric cultures, detection of tuberculostearic acid (gas chromatography-mass spectrometry) and mycobacterial antigens (enzyme-linked immunosorbent assays), DNA probes, and nucleic acid amplification systems such as PCR. The use of stained-sputum microscopy (Ziehl-Neelsen, Kinyoun, or fluorochrome) for acid-fast bacilli (AFB), however, still remains the most available, easy to perform, inexpensive, and rapid diagnostic test for tuberculosis (7). This is especially true for laboratories in developing countries, where limited resources often do not allow even culture isolation as a diagnostic option (4).

The greatest difficulty in diagnosing tuberculosis and other mycobacterial infections by sputum microscopy is this test's lack of sensitivity and specificity (23). The sensitivity of this test has improved considerably with improved techniques and standardization of sputum preparation. These include (i) liquefaction with *N*-acetyl-L-cysteine and 2% sodium hydroxide, (ii) concentration of the sputum by centrifugation, and (iii) promotion of the use of auramine-rhodamine with the fluorochrome method instead of the classic acid-fast stains of Ziehl-Neelsen and Kinyoun, which use carbol-fuchsin (25).

A recent study surveying laboratories participating in the College of American Pathologists Mycobacteriology E survey (in 1993) reports the increased usage of the fluorochrome stain (57% in 1992; 61% in 1993) instead of conventional (carbolfuchsin) acid-fast stains and also the increased use of DNA probes (30% in 1992; 51% in 1993) and radiometric cultures (34% in 1992; 38% in 1993) (26). While the sensitivity of sputum specimen microscopy for the diagnosis of tuberculosis has improved with these new techniques, its diagnostic reliability is still uncertain, especially in select populations such as the AIDS population and the population at low risk for human immunodeficiency virus (HIV) with a high prevalence of infection with *M. avium* complex and other nontuberculous mycobacteria (NTM).

This study examines the sensitivity of fluorochrome stain microscopy of respiratory specimens for the detection of tuberculosis and NTM, including *M. avium* complex, *M. kansasii*, and *M. abscessus*. Some previous studies (3, 9, 27) have suggested that AFB smear positivity usually indicates tuberculosis rather than NTM infection and that smear positivity occurs infrequently with NTM (especially *M. avium* complex) (27). This study was done to assess these concepts in a referral hospital in Texas that frequently finds NTM lung disease in HIV-negative hosts.

MATERIALS AND METHODS

Consecutive sputum and bronchial wash specimens submitted for acid-fast stain and culture to the Microbiology Laboratory at the University of Texas Health Center at Tyler from February 1993 to February 1995 were analyzed. Data collection was performed for all NTM for which more than 20 isolations

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from at least five patients were available. The University of Texas Health Center at Tyler is a referral center for patients with proven or suspected tuberculosis or NTM disease.

The collection of sputum sediments was monitored by a trained nurse to ensure optimal samples for analysis. This nurse was responsible for all sputum collection, and this was her full-time assignment. Every morning she would visually examine the patients' sputum specimens to determine their acceptability. If the specimen appeared acceptable (grossly purulent, thick, and not watery like saliva), she would submit it to the lab. If the specimen did not appear acceptable, she would discard it and collect another one after giving more patient education. If this failed, she would request respiratory therapy assistance with either ultrasonic nebulization with hypertonic saline or chest percussion or both.

Most sputum specimens were expectorated, with only approximately 10% being induced. These specimens included sputum samples from all patients tested for mycobacterial disease, without regard to treatment, HIV infection, or other diagnostic status.

The respiratory specimens were liquefied and decontaminated with *N*-acetyl-L-cysteine, 2% NaOH, and 1.45% sodium citrate. Following vortexing, the specimens were concentrated by centrifugation at 3,000 × *g* for 15 min. Three drops of sediment from a glass pipette (approximately 0.15 ml) were placed on a slide and heat fixed on a slide warmer at 75°C for approximately 2 h. The slide was stained with Truant's stain (auramine-rhodamine stain) and examined with an American Optical Microscope with a 20× objective under a standard fluorescence UV filter. Oil immersion (100× objective) microscopy was used when confirmation of morphology was required. Each slide was examined for the presence of fluorescent bacilli by making three to four passes along the long axis of the slide.

The processed specimens were plated on Löwenstein-Jensen medium (0.1 to 0.2 ml of sediment) and incubated for 6 weeks, on Middlebrook 7H10 medium (0.35 to 0.4 ml) and incubated for 3 weeks, and in BACTEC 12B broth (0.5 ml) and incubated for 6 weeks (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.).

AFB-positive cultures were processed in accordance with standard procedures (7, 25). *M. avium* complex and *M. tuberculosis* were identified by using a commercial DNA probe (ACCUPROBE; GEN-PROBE, San Diego, Calif.), while isolates of *M. kansasii* and *M. gordonae* were identified by biochemical methodology as presented by Kent and Kubica (7). Isolates which gave unusual or unexpected results were subjected to high-performance liquid chromatography.

Rapidly growing mycobacteria were identified as part of the *M. fortuitum* complex on the basis of mature growth in less than 7 days, a positive 3-day arylsulfatase reaction, absence of pigment production, and typical acid-fast staining (19). Species identification utilized nitrate reduction (19) combined with antimicrobial susceptibility patterns to agents including polymyxin B (24), ciprofloxacin (20), cefoxitin (22, 25), amikacin (22, 25), and tobramycin (22).

Chart reviews including chest radiograph results were performed for the patients with isolates other than *M. tuberculosis* to determine their clinical significance. The diagnostic criteria of the American Thoracic Society (ATS) published in 1990 and revised in 1997 were utilized (25). A category of "possible disease" was added for patients who met the clinical criteria, including a typical chest X-ray, but who had insufficient numbers of positive cultures to meet the diagnostic criteria (25).

RESULTS

A total of 6,532 respiratory specimens were collected from 1,040 patients over the 2-year study period. *M. tuberculosis* was isolated from one or more of the culture media in 1,082 (16.6%) of the 6,532 sputum specimens, with 677 (62.6%) of these 1,082 culture-positive specimens having AFB detected by fluorochrome microscopy (Table 1). There were 217 bronchoalveolar lavage specimens analyzed during the 2-year period (2% of all the respiratory samples), of which only 8 (3.7%) grew *M. tuberculosis*. None of these eight specimens were AFB smear positive.

AFB smear and culture data were summarized for four NTM species for which at least 20 culture-positive specimens from at least five patients were identified. One of these NTM was isolated from 1,148 specimens, of which 638 (55.6%) were AFB smear positive (Table 1). Thus, almost equal numbers of cultures and smears positive for *M. tuberculosis* and for NTM were seen during the study period. The NTM species included *M. avium* complex, rapidly growing mycobacteria, *M. kansasii*, and *M. gordonae.*

M. avium complex was recovered from 776 specimens from 156 patients and was observed on fluorochrome smears of 446 (57.5%) of these 776 specimens. Case reviews revealed that 11 of 156 patients (7.1%) had AIDS at the time of specimen collection, with no additional cases being diagnosed in the study population in the 2 years since the data collection ended. A review of the clinical records and chest radiographs of the 145 non-AIDS patients revealed that 102 (70.3%) of the patients met the ATS clinical and microbiologic criteria for NTM lung disease (25), 13 (9.0%) of the patients had possible disease, and 29 (20%) of the patients did not meet the criteria. Clinical records for one patient could not be located. Chest radiograph review for 100 of the 102 patients with definite disease revealed that 53% had nodular bronchiectasis and 47% had cavitary upper-lobe disease.

TABLE 1. Fluorochrome AFB smear positivity in respiratory specimens yielding mycobacteria on culture

Organism cultured	No. (%) of specimens		
	Culture positive	Smear negative	Smear positive (%)
NTM			
M. avium complex	776	330	446 (57.5)
Rapidly growing mycobacteria (*M. abscessus*)[a]	300	146	154 (51.3)
M. kansasii	37	8	29 (78.4)
M. gordonae	35	26	9 (25.7)
Total	1,148	510	638 (55.6)
M. tuberculosis complex			
M. tuberculosis	1,082	405	677 (62.6)

[a] Five of the 300 isolates belonged to other species (four were *M. fortuitum* and one was *M. chelonae*). Two isolates were identified only as *M. chelonae-M. abscessus* group.

Three hundred rapidly growing mycobacteria were isolated from 29 patients, of which 154 (51.3%) culture-positive specimens were positive by microscopy. Isolates from two patients (one each) were identified only as *M. chelonae-M. abscessus* group. Of the remaining 27 patients, one had *M. chelonae*, three had *M. fortuitum*, and 23 (85%) had *M. abscessus.*

Clinical review of the 29 patients with respiratory isolates of rapidly growing mycobacteria revealed that 2 patients with *M. fortuitum* (one isolate each) were not considered clinically significant (25). Of the remaining 27 patients, 26 had multiple positive cultures and definite clinical disease and one patient with a single positive specimen (only one was ordered) had possible disease (underlying bronchiectasis). Of the 298 isolates from these patients identified to the species level, 293 isolates (98.3%) were *M. abscessus*, 4 isolates were *M. fortuitum* (1.3%), and 1 isolate was *M. chelonae* (0.3%).

Twenty-nine (78.4%) of 37 specimens culture positive for *M. kansasii* were AFB smear positive on fluorochrome microscopy. These were recovered from eight patients, all of whom had multiple positive specimens and met ATS clinical and microbiologic criteria for disease (25).

M. gordonae was recovered from 35 cultured specimens from 31 patients, of which 9 (25.7%) were AFB positive. Three patients (all with culture-proven *M. tuberculosis* and more than 20 specimens submitted) had multiple positive cultures for *M. gordonae* (three, two, and two positive cultures, respectively). The remainder were single positive isolates from different patients. None of the cases met ATS criteria for disease (25).

One hundred ninety-two smear-positive specimens failed to produce mycobacterial growth in any of the three culture media. One-hundred sixty-nine of these specimens were from patients with multiple other culture-positive specimens whose

TABLE 2. AFB smear positivity results for specimens culture positive for tuberculosis and NTM respiratory disease

Reference	Smear method	Total no. of respiratory specimens	No. of specimens smear positive/no. of specimens (%) culture positive				
			M. tuberculosis	*M. avium* complex	Rapidly growing species	*M. kansasii*	All NTM
This study	Fluorochrome	6,532	677/1,082 (62.6)	446/776 (57.5)	154/300 (51.3)	29/37 (78.4)	638/1,148 (55.6)
Anargyros et al. (1)	Fluorochrome	2,563	46/162 (28.4)				35/100 (35.0)
Lipsky et al. (10)	Ziehl-Neelsen	3,207	57/95 (60.0)				1/81[a] (1.2)
Levy et al. (9)	Ziehl-Neelsen	2,560	71/107 (66.3)				0/7 (0)
Strumpf et al. (21)	Fluorochrome	1,769	70/106 (66.0)				0/10 (0)
Gordin and Slutkin (5)	Fluorochrome	2,956	137/264 (51.9)				5/61 (8.2)
Yajko et al. (27)	Fluorochrome	19,343	248/450 (55.1)	22/314 (7.0)			33/404 (8.2)
Badak et al. (2)	Fluorochrome	16,336	97/157[b] (62.5)	255/686 (37.1)	43/73 (58.9)		379/759 (49.9)
Murray et al. (15)	Fluorochrome	10,468[c]	(46)				(32)

[a] Two of the 81 specimens were indeterminate.
[b] Numbers estimated from percentages given in abstract (2).
[c] For all mycobacteria, 158 of 406 (38.9%) were fluorochrome positive.

smear-positive, culture-negative specimens were obtained while the patients were on drug therapy. One hundred thirty of the 169 specimens were collected from tuberculosis patients on therapy, 34 were from patients on *M. avium* complex therapy, and 5 were from patients being treated for *M. abscessus* infection. Hence, the organisms were considered to be present but nonviable. The remaining 23 smear-positive, culture-negative sputum specimens represented false positives since none of the patients had other positive mycobacterial cultures and none were on antimicrobial therapy at the time. Of the 23 false positives, two specimens grew *Rhodococcus equi*. The remainder had no growth. The overall incidence of the true false positives was 0.35% (23 of 6,532 specimens).

DISCUSSION

Table 2 presents AFB smear positivity results for both *M. tuberculosis* and NTM culture-positive specimens from the current study and eight previously published studies (1, 2, 5, 9, 10, 15, 21, 27). Our current *M. tuberculosis* smear positivity rate by AFB sputum microscopy of 63% falls within the range of 28 to 66% found in these prior studies. These rates of smear positivity are considerably higher than those obtained with direct sputum AFB smears in which the specimen is smeared without concentration or sedimentation (13). The direct (unconcentrated) AFB smear positivity rate ranges from 8.8 to 46.4% depending upon the location and technical resources of the laboratory (4, 12). While earlier studies indicated greater sensitivity with fluorescent microscopy, this was not found in the two studies using light microscopy as compared with the six studies using fluorescence microscopy (Table 2) (1, 2, 5, 9, 10, 15, 21, 27).

Our rate of AFB smear positivity (with fluorescent microscopy) for *M. tuberculosis* (62.6%) was not very different than that for NTM (56%). Surprisingly few other published reports have compared the sensitivity of fluorochrome microscopy in diagnosis of tuberculosis with its sensitivity in diagnosis of NTM pulmonary disease (Table 2). Two such studies which were done showed a much lower rate of smear positivity with NTM than with *M. tuberculosis*. In a pre-AIDS population (1977 to 1982), Gordin and Slutkin (5) found that only 5 of 61 (8%) NTM culture-positive sputum specimens had positive fluorescent microscopy results. Yajko and colleagues (27) observed that only 22 of 314 (7.0%) specimens culture positive for *M. avium* complex were AFB smear positive in a population with a high incidence of AIDS. They concluded that in their setting (i.e., high incidence of AIDS and low incidence of non-AIDS NTM lung disease), rates of NTM smear positivity were so low that in spite of the high prevalence of *M. avium* complex, a positive AFB smear predicted *M. tuberculosis* infection 92% of the time.

Not all studies have shown such a low incidence of NTM positivity, however. At the University of North Carolina hospitals, where there is a high incidence of isolation of *M. avium* complex, a low incidence of recovery of *M. tuberculosis*, and presumably a low incidence of AIDS, Badak et al. (2) reported that a positive AFB smear at their institution was as likely to be *M. avium* complex as *M. tuberculosis*. In this study of 16,336 specimens primarily (83%) from respiratory sources (2), they reported that for specimens that were culture positive for mycobacteria, AFB smear positivities of 62.5% for *M. tuberculosis*, 37.1% for *M. avium* complex, and 59.4% for *M. abscessus* were observed. Our study produced similar results to this latter study for a similar patient population (i.e., with a low incidence of AIDS and a high incidence of non-AIDS NTM lung disease).

One reason for these observed differences in NTM smear positivity rates is the variety of clinical disease present with *M. avium* complex in the test population. Three major disease states with *M. avium* complex involvement of the lung have been described previously (6, 11, 14, 17, 18, 25). One has been observed in the setting of advanced HIV disease (11). Chest X-rays are typically normal or only minimally abnormal, and the lung infection is usually a manifestation of disseminated disease. Colony counts of *M. avium* complex are usually low, and a low frequency of positive AFB smears is expected (such as was observed in the study of Yajko et al. [27]). A second disease state is the classic fibrocavitary upper-lobe disease found predominantly in middle-aged alcoholic male smokers (11, 25). Cavitary disease is invariably present, and colony counts are high, with high smear positivity expected. The third disease state is the recently described syndrome of nodular bronchiectasis, usually found in elderly women, with low colony counts and low smear positivity expected (6, 14, 25). Only 7% of our patients had diagnosed AIDS at the time of their positive cultures and during a 2-year follow-up. Approximately 50% of our *M. avium* complex patients had nodular-bronchiectasis disease, while the remaining 50% had fibrocavitary disease. The relatively high incidence of *M. avium* complex smear positivity observed (58%) seems reasonable for the study population. Data as to the type of pulmonary disease (other than HIV status) based on current diagnostic criteria (25) have not been given in most of the prior studies (2).

Studies support the concept that sputum specimens with low

colony counts of mycobacteria are less likely to be detected by smear microscopy (8, 16). In a study by Pollock and Wieman (16), only 14 of 178 (7.9%) specimens with <25 colonies on solid medium were smear positive. The difference also could relate in part to the technical capabilities of the laboratory. For example, *M. avium* complex isolates may appear on a smear as coccobacilli and can be easily missed with screening at low power. In our study, the AFB smears were read almost entirely by two microbiologists with more than 30 years of experience in the detection of mycobacteria.

The use of mycobacterial smears of sputum specimens is a rapid, specific, and reasonably sensitive tool in the diagnosis of AFB disease for both *M. tuberculosis* and NTM, including *M. avium* complex, *M. abscessus*, and *M. kansasii*. However, because NTM and *M. tuberculosis* are both readily detected by fluorescence microscopy and are difficult to distinguish by smear alone, a diagnosis of both tuberculosis and infection with NTM should be considered until a more definite diagnosis can be accomplished, especially in the setting of a patient population at low risk for HIV.

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