

Mycobacteria in Stool Specimens: the Nonvalue of Smears for Predicting Culture Results

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Received 16 November 1992/Accepted 23 February 1993

A previous recommendation suggests that stool be cultured for mycobacteria only if the smear is positive. We have correlated smear and culture results of 2,176 stool specimens submitted for mycobacterial culture. The sensitivity, specificity, and positive and negative predictive values for smears to predict culture results are 34, 99, 90, and 87%, respectively. We recommend that the stool smear not be used as a screening technique to decide which specimens from at-risk patients should be cultured because it lacks the necessary sensitivity.

It is thought that *Mycobacterium avium* complex (MAC) is acquired from the environment and colonizes either the respiratory or gastrointestinal tract before disseminating in human immunodeficiency virus (HIV)-infected patients (4, 6). Disseminated MAC is associated with significant morbidity and shortened survival times (4, 6, 7, 10). Although it is controversial (4, 5, 16, 17), data suggest that therapy for disseminated MAC improves patient symptoms (2, 8, 11, 18) and survival rates (6). These findings have prompted suggestions that prophylaxis, which helps in other AIDS-related opportunistic infections, may prevent or delay disseminated MAC infection (4, 7, 18). Whether the issue is the study of the natural history of MAC acquisition and dissemination, the response to therapy, or the success of prophylaxis, laboratory methods of detection need to be adequately sensitive.

The *Manual of Clinical Microbiology* recommends that if acid-fast bacilli are not observed in unprocessed stool, then the specimen need not be cultured (15). This suggestion is based on the data of Kiehn et al. (13). In that study, direct smears with acid-fast staining were performed on 17 specimens. All 12 smear-positive stools grew MAC; the 5 smear-negative stools were not cultured. More-recent data from the same group showed that 21 of 31 MAC culture-positive stool specimens (68%) were smear negative (12). We are unaware of data on a large number of stool specimens which have had both smears and cultures for mycobacteria performed. We have, therefore, analyzed our data to find out how sensitive the acid-fast stain is in predicting stool mycobacterial culture results.

Data from three institutions on three continents which perform smears and cultures on all stool specimens submitted for mycobacterial culture were analyzed. Duke University Medical Center (DUMC), Durham, N.C., is a tertiary care university medical center serving an HIV-AIDS clinic caring for approximately 1,100 patients. The National Center for Mycobacteria (NCM), Zurich, Switzerland, is a refer-

ence laboratory and also serves an AIDS clinic at the University Hospital. Results for 1991 were analyzed for both of these laboratories. Data from Fairfield Institute for Infectious Diseases (FIID), Fairfield, Victoria, Australia, cover fecal specimens submitted from 511 patients with HIV infection or AIDS who were receiving care there from January 1985 to January 1992, inclusive. In all three laboratories, smears were made from the decontaminated and centrifuged fecal slurry. Decontamination methods were different at each institution. At DUMC, 2% NaOH-N-acetyl-L-cysteine was used, with 30 min of exposure; at NCM, 1% NaOH-3.16% sodium dodecyl sulfate was used, with 10 min of exposure; and at FIID, 4% NaOH was used, with 15 min of exposure. At DUMC and NCM, the auramine-rhodamine stain was used. The Ziehl-Neelsen stain was used at FIID. At DUMC, a BACTEC 12B vial and a Middlebrook 7H10-Middlebrook 7H11S biplate were inoculated and kept for 6 and 8 weeks, respectively, at 37°C. At NCM, a BACTEC 12B vial, a Middlebrook 7H10-Middlebrook 7H11S biplate, and a Lowenstein-Jensen agar slant were inoculated and incubated for 6, 6, and 8 weeks, respectively, at 37°C. At FIID, specimens were inoculated onto Middlebrook 7H10 medium containing vancomycin, cycloheximide, aztreonam, ticarcillin, and amphotericin B and incubated for 6 weeks at 37°C or for 12 weeks at 31°C. At DUMC and NCM, smear and culture results were quantified. Culture results were not quantified at FIID.

A total of 2,176 stool specimens, almost all from HIV-infected or AIDS patients, had both acid-fast smears and mycobacterial cultures performed. Results are shown in Table 1. There were 393 culture-positive specimens, yielding 396 isolates. Most isolates, 358 (90%), were MAC. Remaining isolates were the following: 10 *M. tuberculosis*, 10 *M. gordonae*, 4 *M. kansasii*, 2 each of *M. fortuitum* and *M. simiae*, and one each of *M. xenopi*, *M. terrae*, and *M. chelonae*. Seven isolates could not be identified by conventional methods, but on the basis of 16S rRNA sequencing three of them were identified as "*M. genavense*" (1). If only MAC and *M. tuberculosis* isolates are considered, the sensitivity of the fecal smear to detect mycobacteria increases from 34% to 36%. The relationship between the number of organisms seen on smears and the amount of growth at

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TABLE 1. Smear and culture results on stool specimens submitted for mycobacterial culture

| Smear result | No. of specimens positive or negative by culture ^a | | Total no. |
|--------------|---|----------|-----------|
| | Positive | Negative | |
| Positive | 132 | 16 | 148 |
| Negative | 261 | 1,767 | 2,028 |
| Total no. | 393 | 1,783 | 2,176 |

^a Prevalence, 18.1%; sensitivity, 33.6%; specificity, 99.1%; positive predictive value, 89.2%; and negative predictive value, 87.1%.

DUMC and NCM is shown in Table 2. Forty of 65 (62%) culture-positive specimens had a negative smear, 9 (14%) grew in BACTEC 12B broth only, suggesting low numbers, and another 16 (25%) had fewer than 11 colonies, but 4 smear-negative specimens grew more than 100 colonies of MAC. Seventeen of the 30 specimens (57%) with positive smears grew more than 100 colonies of either MAC or *M. tuberculosis*. Similar results were observed at FIID, where only 221 of 1,552 specimens (14%) with a negative smear yielded mycobacteria versus 107 of 118 specimens (91%) with a positive smear.

The question remains as to the clinical usefulness of performing smears for acid-fast bacilli in fecal specimens from HIV-infected or AIDS patients. At FIID, MAC was cultured from smear-negative stools of 27 patients on one or more occasions prior to its recovery from one or more smear-positive fecal specimens. On the other hand, in 13 patients the first smear-positive, MAC-positive fecal specimen was not preceded by a smear-negative, MAC culture-positive specimen. Although the routine examination of fecal smears for acid-fast bacilli may have clinical utility, such utility was not examined in this study. There are few data on the frequency and quantity of mycobacteria in stools from healthy populations. Mycobacteria have been isolated in low numbers (one to three colonies per processed stool specimen) from the stools of approximately 50% of healthy individuals (14). Twenty percent of isolates were MAC, but it is not known whether these are *M. avium* or *M. intracellulare* (14). At FIID, however, only 20 of 1,100 refugees (2%) screened by the same methods used for HIV-infected or AIDS patients had mycobacteria recovered from their stools (3). Twenty-nine of the 65 culture-positive patients (45%) who had growth quantified had either ≤ 10 colonies or growth in only the BACTEC system. The relevance of such numbers is unclear and needs prospective study.

The presence of acid-fast bacilli in the fecal smear and the

TABLE 2. Quantitative smear and culture results of stools submitted for mycobacterial culture

| Smear result | No. of specimens with the following culture result | | | | | Growth in BACTEC 12B broth only | Total no. |
|--------------|--|----------|---------------|-----------------|---------------|---------------------------------|-----------|
| | No growth | 1 colony | 2-10 colonies | 11-100 colonies | >100 colonies | | |
| Negative | 436 | 4 | 12 | 11 | 4 | 9 | 476 |
| Suspicious | 1 | | | | | | 1 |
| Rare | | | 1 | | 4 | | 5 |
| Few | 3 | 1 | 1 | 3 | 6 | | 14 |
| Numerous | 1 | 1 | 1 | 1 | 7 | | 10 |
| Total no. | 441 | 5 | 15 | 15 | 21 | 9 | 506 |

inability of conventional solid media to support their recovery after a reasonable period of incubation may suggest infection with a *Mycobacterium* sp. that requires a different incubation temperature or infection with a fastidious mycobacterium that requires enrichment and prolonged incubation, for example, "*M. genavense*" (1, 9).

Our data are almost identical to those of Kiehn and Cammarata (12). In their series of 31 MAC culture-positive stool specimens, the smear had a sensitivity of 32%; our result was 34%. These data suggest that the fecal smear result should not determine whether a culture for mycobacteria should be performed.

We thank Pascale Kissling and Janis Matthews for help in identifying the isolates.

REFERENCES

- Bottger, E. C., A. Teske, P. Kirschner, S. Bost, H. R. Chang, V. Beer, and B. Hirschel. 1992. Disseminated "*Mycobacterium genavense*" infection in patients with AIDS. *Lancet* 340:76-80.
- Dautzenberg, B., C. Truffot, S. Legris, M.-C. Meyohas, H. C. Berlie, A. Mercat, S. Chevret, and J. Grosset. 1991. Activity of clarithromycin against *Mycobacterium avium* infection in patients with the acquired immune deficiency syndrome: a controlled clinical trial. *Am. Rev. Respir. Dis.* 144:564-569.
- Dwyer, B. Unpublished data.
- Ellner, J. J., M. J. Goldberger, and D. M. Parenti. 1991. *Mycobacterium avium* infection and AIDS: a therapeutic dilemma in rapid evolution. *J. Infect. Dis.* 163:1326-1335.
- Heifets, L. B., and M. D. Iseman. 1991. Individualized therapy versus standard regimens in the treatment of *Mycobacterium avium* infections. *Am. Rev. Respir. Dis.* 144:1-2.
- Horsburgh, C. R., Jr. 1991. *Mycobacterium avium* complex infection in the acquired immunodeficiency syndrome. *N. Engl. J. Med.* 324:1332-1338.
- Horsburgh, C. R., Jr., J. A. Havlik, D. A. Ellis, E. Kennedy, S. A. Fann, R. E. DuBois, and S. E. Thompson. 1991. Survival of patients with acquired immune deficiency syndrome and disseminated *Mycobacterium avium* complex infection with and without antimycobacterial chemotherapy. *Am. Rev. Respir. Dis.* 144:557-559.
- Hoy, J., A. Mijch, M. Sandland, L. Grayson, R. Lucas, and B. Dwyer. 1990. Quadruple-drug therapy for *Mycobacterium avium-intracellulare* bacteremia in AIDS patients. *J. Infect. Dis.* 161:801-805.
- Jackson, K., A. Sievers, B. C. Ross, and B. Dwyer. 1992. Isolation of a fastidious *Mycobacterium* species from two AIDS patients. *J. Clin. Microbiol.* 30:2934-2937.
- Jacobson, M. A., P. C. Hopewell, D. M. Yajko, W. K. Hadley, E. Lazarus, P. K. Mohanty, G. W. Modin, D. W. Feigal, P. S. Cusick, and M. A. Sande. 1991. Natural history of disseminated *Mycobacterium avium* complex infection in AIDS. *J. Infect. Dis.* 164:994-998.
- Kemper, C. A., T.-C. Meng, J. Nussbaum, J. Chiu, D. F. Feigal, A. E. Bartok, J. M. Leedom, P. G. Tilles, S. C. Deresinski, J. A. McCutchan, and the California Collaborative Treatment Group. 1992. Treatment of *Mycobacterium avium* complex bacteremia in AIDS with a four-drug oral regimen: rifampicin, ethambutol, clofazimine, ciprofloxacin. *Ann. Intern. Med.* 116:466-472.
- Kiehn, T. E., and R. Cammarata. 1986. Laboratory diagnosis of mycobacterial infections in patients with acquired immunodeficiency syndrome. *J. Clin. Microbiol.* 24:708-711.
- Kiehn, T. E., F. F. Edwards, P. Brannon, A. Y. Tsang, M. Maio, J. W. M. Gold, E. Whimbey, B. Wong, J. K. McClatchy, and D. Armstrong. 1985. Infections caused by *Mycobacterium avium* complex in immunocompromised patients: diagnosis by blood culture and fecal examination, antimicrobial susceptibility tests, and morphological and seroagglutination characteristics. *J. Clin. Microbiol.* 21:168-173.
- Portaels, F., L. Larsson, and P. Smeets. 1988. Isolation of mycobacteria from healthy persons' stool. *Int. J. Lepr.* 56:468-471.

15. **Roberts, G. D., E. W. Koneman, and Y. Y. Kim.** 1991. Procedure for decontamination of fecal material, p. 310. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.
16. **Wallace, R. J., J. Glassroth, and R. O'Brien.** 1991. A plea for clinical trials to resolve the issue of optimal therapy in the treatment of *Mycobacterium avium* infection. *Am. Rev. Respir. Dis.* **144**:3-4.
17. **Wallace, R. J., R. O'Brien, J. Glassroth, J. Raleigh, and A. Dutt.** 1990. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. American Thoracic Society statement. *Am. Rev. Respir. Dis.* **142**:940-953.
18. **Young, L. S., L. Wiviott, M. Wu, P. Kolonski, R. Bolan, and C. B. Inderlied.** 1991. Azithromycin for treatment of *Mycobacterium avium-intracellulare* complex infection in patients with AIDS. *Lancet* **338**:1107-1109.