Mycobacterium tuberculosis Bacteremia Detected by the Isolator Lysis-Centrifugation Blood Culture System

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***Mycobacterium tuberculosis* was detected by the Isolator lysis-centrifugation blood culture system from the blood of a patient with tuberculosis of the breast. The organism also grew on conventional laboratory media inoculated with pleural fluid from the patient.**

We have shown that bacteremia due to *Mycobacterium avium* complex can readily be detected by the Isolator lysis-centrifugation blood culture system in patients with disseminated infection (1a, 5). The Isolator system provided an efficient and accurate technique for determining the number of organisms per milliliter of blood. In one patient with high-grade mycobacteremia we demonstrated that at least 90% of total colonies recovered by the Isolator system were in the sediment. Many of the mycobacteria circulated intracellularly, and the colony counts from the Isolator system were two- to fivefold higher than those from a nonlytic system because of release of organisms from the leukocytes by the Isolator lytic agent saponin. These findings of increased colony counts were confirmed when desoxycholate was used in a second lytic system.

Culture-positive mycobacteremia due to *Mycobacterium tuberculosis* was reported in the 1930s (1, 2); however, blood cultures for this organism have not become a routine laboratory test. Our experience with the Isolator system with *M. avium* complex led us to attempt to culture *M. tuberculosis* from the blood of a patient with severe *M. tuberculosis* infection. This paper reports the recovery of *M. tuberculosis* from a patient with tuberculosis of the lungs, pleural cavity, and breast.

Case report. A 78 year-old woman was admitted to Memorial Sloan-Kettering Cancer Center on 27 March 1984 for evaluation of a mass in the left breast. Past medical history was remarkable for hypertension, treated with hydrochlorothiazide, and right bundle branch block. She had taken erythromycin for the previous 6 weeks because of the breast mass from which a physician had aspirated creamy pus. She complained of diarrhea.

On physical examination, the temperature was 36°C, pulse rate was 100/min and regular, respiratory rate was 16/min, and blood pressure was 105/65 mmHg (1 mmHG = 133.3 Pa). There were dullness to percussion and decreased breath sounds at the right lower lung field. A large nodular mass with a sinus tract was present in the upper outer quadrant of the left breast.

The hemoglobin was 12.7 g/dl, hematocrit was 39%, and the leukocyte count was 12,200/μl (90% polymorphonuclear leukocytes, 2% bands, 3% lymphocytes). The chest X-ray revealed a large right pleural effusion which failed to layer. The left lung appeared normal. A sonogram of the chest confirmed the presence of numerous loculated fluid collec-

tions. The patient failed to respond to intradermal challenge with *Trichophyton* spp., *Candida* spp., and PPD-T (1 and 5 TU).

On 29 March, a wide excision of the left breast mass showed an abscess with acute and chronic inflammation. Granulomas and numerous acid-fast bacteria (Ziehl-Neelsen stain) were seen microscopically in the pathology laboratory; however, the specimen was not submitted for culture. On 5 April, a sonographically guided thoracentesis yielded 60 ml of thick, creamy, nonfoul-smelling pus. This material was sent to the microbiology laboratory and processed for stain and culture in both the bacteriology and mycobacteriology sections of the laboratory. Many acid-fast bacilli were seen on an Auramine O fluorochrome stain, and a Gram stain revealed many gram-positive, beaded bacilli. For bacteriology culture, pleural fluid was inoculated directly to chocolate agar, MacConkey agar, and colistin-nalidixic acid agar with 5% sheep blood (CNA agar) for aerobic incubation and Columbia base 5% sheep blood agar and enriched thioglycolate medium for anaerobic incubation. All media were incubated for 21 days since the Gram stain results suggested the presence of *Nocardia* or *Actinomyces* species.

Because of the patient's confusion and withdrawn, unco-operative behavior, a lumbar puncture and computerized tomography scan were performed. The lumbar puncture was normal, and the tomography scan showed cortical atrophy. Infection with *M. tuberculosis* was suspected, and the patient was treated with isoniazid (300 mg), rifampin (600 mg), and ethambutol (900 mg).

On 16 April, 9 ml of blood was collected in an Isolater lysis-centrifugation tube for mycobacterial culture. The tube was delivered to the laboratory and processed according to the instructions of the manufacturer except that 1.5 ml of sediment was divided equally and inoculated onto four Middlebrook 7H11 agar plates. Plates were incubated at 35°C in 5% CO_2 in air and examined for visible colonies twice each week.

The patient was transferred to a hospital near her family. During the next 5 months the pleural effusion gradually cleared while she received treatment with INH and rifampin.

Organism growth. On 12 April, after 7 days of culture, acid-fast organisms from the pleural fluid were seen visibly as pinpoint colonies on Middlebrook 7H11 agar plates in the mycobacteriology laboratory and on chocolate, Mac-Conkey, and CNA agars in the bacteriology laboratory. On 2 May, after 36 days of culture, two colonies of acid-fast bacilli (0.2 CFU/ml) were observed on 7H11 agar plates from

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the lysis-centrifugation blood culture. All isolates were identified as *M. tuberculosis* with the following results: niacin, nitrate, and cording on 7H11 agar were positive; and semiquantitative and 68° catalase, aryl sulfatase, Tween hydrolysis, and photochromogenicity tests were negative. The organism was inhibited by the Bactec NAP reagent (p-nitro-α-acetyl-amino-β-hydroxy-propiophenone) as expected for the *M. tuberculosis* complex.

Susceptibility studies. The organism, when tested for antimicrobial susceptibility by a conventional agar dilution method (3), was susceptible to the following compounds and concentrations (in micrograms per milliliter): ansamycin (2.0), clofazimine (1.0), cycloserine (30.0 and 60.0), ethambutol (5.0, 7.5, and 15.0), ethionamide (10.0 and 15.0), isoniazid (0.2, 1.0, and 5.0), rifampin (1.0, 5.0, and 10.0), and streptomycin (2.0 and 10.0). It was resistant to 5.0 μg of ethionamide per ml.

This case illustrates two important microbiological points. Although mycobacteriology laboratories have used broth media such as Dubos or Middlebrook 7H9 for culture of sterile body fluids (3) or tissues (4), blood is not routinely cultured for *M. tuberculosis*. We were unable to find a well-documented case of culture-proven *M. tuberculosis* bacteremia published since the 1930s. Using the lysis-centrifugation system and culturing for *M. tuberculosis*, we were able to detect *M. tuberculosis* bacteremia. Microbiology laboratories may now be able to determine how frequently mycobacteremia occurs in patients with different forms of tuberculosis and different degrees of severity.

This case also shows that pinpoint colonies of *M. tuberculosis* can be found on media routinely used for culture of bacteria other than mycobacteria. Thus, care should be taken when this media is examined for growth of organisms. On subculture, the isolate did not have growth or identifying

characteristics that differed from other subcultures of *M. tuberculosis* in our laboratory. After 14 days of incubation, a subculture of the organism demonstrated good growth on Lowenstein-Jensen and 7H11 agars, very scant growth on blood and chocolate agars, and no growth on MacConkey agar. Thus, it appears that the pleural fluid that was distributed onto the primary agar media acted as a source of nutrients for growth of the numerous organisms present in the original specimen.

LITERATURE CITED

1. **Jensen, K. A.** 1935. Report on investigations regarding the Loewenstein technique of cultivating tubercle bacilli from blood. Acta Tuberc. Scand. **9:**253–277.
1a.**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.
2. **Shapiro, L.** 1932. The frequency of bacillaemia in tuberculosis. Am. Rev. Tuberc. **26:**418–423.
3. **Vestal, A. L.** 1975. Procedures for the isolation and identification of mycobacteria. Department of Health, Education and Welfare publication no. (CDC)75-8230. Department of Health, Education and Welfare, Washington, D.C.
4. **Wichelhausen, R. H., R. L. McLean, and F. B. Lowrey.** 1966. Reinforcement of diagnostic value of pleural biopsies by culture in liquid medium. Am. Rev. Respir. Dis. **93:**288–290.
5. **Wong, B., F. F. Edwards, T. E. Kiehn, E. Whimbey, H. Donnelly, E. M. Bernard, J. W. M. Gold, and D. Armstrong.** 1985. Continuous high-grade *Mycobacterium avium-intracellulare* bacteremia in patients with the acquired immune deficiency syndrome. Am. J. Med. **78:**35–40.