Diagnosis of Mycobacterium Bacteremia in Patients with Acquired Immunodeficiency Syndrome by Direct Examination of Blood Films

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Received 20 September 1988/Accepted 27 December 1988

Thirty acquired immunodeficiency syndrome patients with mycobacterial bacteremia documented by Du Pont Isolator (Du Pont Co., Wilmington, Del.) blood cultures underwent microscopic examination of buffy coat blood smears. Of 30 patients, 14 were culture positive for Mycobacterium avium-Mycobacterium intracellulare complex and 1 was positive for M. tuberculosis. Of 15 culture-positive patients, 13 had identifiable organisms on Kinyoun- or auramine-stained direct blood smears.

Patients with human immunodeficiency virus infection are rendered susceptible to a variety of opportunistic infections, including other viruses, fungi, bacteria, and mycobacteria, as well as to noninfectious conditions, such as lymphoma and Kaposi's sarcoma. Because of the complex nature of this syndrome, it is often difficult to identify and therefore to treat the multiple opportunistic microbial agents that infect these patients. The diagnostic procedures are often laborious and require long hospitalization stays which impose financial burdens on the patients and on the institutions caring for the patients. We describe here a rapid diagnostic method for detecting bacteremia caused by Mycobacterium avium-Mycobacterium intracellulare complex, the second most common opportunistic pathogen found in patients with acquired immunodeficiency syndrome (AIDS) after Pneumocystis carinii (3, 5, 9).

Patient population. Patients with documented AIDS or suspected of having AIDS had blood cultures processed with Du Pont Isolators (Du Pont Co., Wilmington, Del.) as part of diagnostic care. When the microbiology laboratory received a Du Pont Isolator culture, the hematology laboratory was notified and blood buffy coat films were prepared from the next specimens submitted from these patients for blood counts.

Culture method. Du Pont Isolators were centrifuged, and the pellets from the lysates were used to inoculate two Lowenstein Jensen agar slants (BBL Microbiology Systems, Cockeysville, Md.) for mycobacterial culture. The inoculated slants were incubated in a 5 to 10% CO2 atmosphere at 35°C and monitored for growth for up to 8 weeks.

Direct smear examination. Blood films were prepared after centrifugation of blood collected in EDTA-containing tubes. For each sample, the interface between the plasma layer and the erythrocyte layer, which contained a high density of leukocytes, was aspirated by using disposable transfer pipettes, and 1 drop was placed onto each of three glass microscope slides (the third slide was an extra). The drop was spread on the slide surface with the same transfer pipette to cover 75% of the available surface. The film was air dried and fixed in 100% methanol. One slide was stained by the Kinyoun carbolfuchsin-malachite green method (7) and examined microscopically at a magnification of ×1,000. The second buffy coat blood film was stained by the auramine method of Truant et al. (12). This film was screened by using an epifluorescence microscope at a magnification of ×400 for fluorescent-staining material. Presence of fluorescent rod-shaped microorganisms was confirmed by examination at a magnification of ×1,000.

Of the 30 specimens processed for mycobacterial culture and for direct examination by light and epifluorescence microscopic examination, 14 grew M. avium-M. intracellulare and 1 grew Mycobacterium tuberculosis (Table 1). No concurrent bacteremia with organisms other than mycobacteria occurred in the study patients. One mycobacterium culture-negative patient had fungemia with Cryptococcus neoformans. The mycobacterial cultures showed growth for M. avium-M. intracellulare between days 21 and 30 on a weekly examination. The culture which yielded M. tuberculosis had growth evident by 28 days of incubation. Tentative identifications utilizing niacin and nitrate tests were performed to identify the organisms as M. tuberculosis or mycobacteria other than tubercle bacilli (MOTT). Final identifications were performed at the Veterans Administration Reference Laboratory for Mycobacteria, West Haven, Conn.

All patients had blood films prepared and examined within 1 week of the submission of the Du Pont Isolator culture. The light microscopic examination required an average of 10 to 15 min of examination prior to concluding that no acid-fast organisms were present. The numbers of acid-fast organisms present varied. In five patients, acid-fast organisms were seen at a magnification of ×1,000 when the first two to three high-power fields were examined. In six patients, acid-fast organisms were seen only after examining more than 20 fields at a magnification of ×1,000. The minimum number of organisms seen per slide was 5 and the maximum was more than 100. Many of the organisms were intracellular. Despite a suggestion from published information that intracellular organisms may be difficult to detect or may stain poorly (8), all organisms detected intracellularly were well stained. Of 15 culture-positive patients, 13 had identifiable acid-fast organisms on the blood films. One case of positive culture with M. avium-M. intracellulare and one case of positive culture with M. tuberculosis had no organisms seen on the blood films examined.
Fluorescently stained smears required less examination time. At a magnification of ×400, reading of the smear could be confidently completed in 2 min, with suspicious areas of the smear being examined in detail at a magnification of ×1,000 (Fig. 1). Results were identical to those found in the buffy coat smears processed with the Kinyoun stain.

The importance of mycobacterial infections in patients with AIDS is well documented (4, 5, 9). The early diagnosis of this infection is important to both the quality of life of the patient and to the cost-effective management of patients in any medical facility. Recently, much effort has been devoted to the diagnosis of mycobacterial infection. Rapid culture methods using 7H10 biphasic media have reduced culture time from 30 to 7 days (2). Similarly, radiometric culture systems have reduced culture time dramatically (10). DNA probes are currently being evaluated for practicality in identifying the mycobacterial species (R. Lu, E. Peterson, C. Floyd, A. Nakasone, G. Friedly, and L. M. de la Maza, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, C21, p. 335) and have even been used to directly detect mycobacteria in clinical specimens (K. D. Eisenach, G. H. Mazurek, J. T. Crawford, and J. H. Bates, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, U72, p. 144).

The approach presented by us is simple. Most clinical laboratories already have facilities to prepare blood films and to perform carbol fuchsin or fluorescent stains. Direct immunofluorescence microscopy is available in many microbiology laboratories, especially those involved in processing specimens for mycobacterial culture. Therefore, the addition of a direct examination to the list of diagnostic tests already performed requires only additional technologist time. In view of the high diagnostic yield and the large time savings involved for the diagnosis of mycobacterial bacteraemia, such a direct examination appears to produce reliable results.

*M. avium-M. intracellulare* does not always produce bacteraemia in AIDS patients (3), but in those cases with bacteraemia, it is unclear whether direct examination of blood film is more or less sensitive method than the DNA probe methods currently being developed; however, it is certainly less expensive. Surprisingly, one case of *M. tuberculosis* infection had the organism grown from the blood culture. Unfortunately, no organisms were detected on the blood smear, as was also the case for one patient with *M. avium-M. intracellulare* bacteraemia. Our results support the findings of others (1, 11; J. P. Manzella, J. Kellogg, and J. F. Sanzsteach, Letter, J. Am. Med. Assoc. 254:2741, 1985) that *M. tuberculosis* can cause detectable bacteraemia. We chose to use the buffy coat from the blood for examination, but for most patients examined the leukocyte layer cannot be visualized after centrifugation due to the patients' neutropenia. However, despite the neutropenia, organisms were detected without difficulty. Our results support evidence from other laboratories on the degrees of bacteraemia in patients with *M. avium-M. intracellulare*. In fact, one case has already been reported of mycobacterial bacteraemia diagnosed by direct examination of blood (6). We have extended the observation to 30 patients and have shown that for AIDS patients, buffy coat examination is both a worthwhile and reliable procedure for the diagnosis of mycobacterial bacteraemia.

This work was in part supported by the Veterans Administration Merit Review Program.

**LITERATURE CITED**


