Impact of the Human Immunodeficiency Virus Epidemic on Detection of Mycobacterium Isolates in a General Hospital

EMILIO BOUZA,1,* JAVIER ALBA DALEJO,1 EMILIA CERCENADO,1 M. JESÚS RUIZ SERRANO,1 TERESA VICENTE,1 AND ARTURO ORTEGÁ1

Servicio de Microbiología, Hospital General Universitario “Gregorio Marañón,” Madrid, Spain.

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The number of human samples processed in our mycobacteriology laboratory ranged from 148 per 1,000 admissions in 1988 to 263 per 1,000 admissions in 1995 (5.2% were positive). The human immunodeficiency virus (HIV)-positive population contributed 33.3% of all samples, 43.3% of all isolates, and 36% of all new patients. Given that the ratios of the total number of samples to the number of Mycobacterium-positive samples were 18.3:1 in HIV-positive patients and 28:1 in HIV-negative patients, efforts to reduce the laboratory workload should begin with the HIV-negative population.

Information regarding the mycobacteriology laboratory workload of general hospitals in the AIDS era is scarce. During an 8-year period (January 1988 to December 1995), we evaluated the requests for mycobacterial isolation, the diagnostic yields of different laboratory procedures, and the species of mycobacteria isolated from human immunodeficiency virus (HIV)-positive and -negative patients at our institution.

Our center is a teaching and reference hospital serving a population of approximately 650,000 inhabitants of the city of Madrid, Spain. All human samples received at the mycobacteriology laboratory were processed as follows. Samples contaminated by normal flora were decontaminated by using a volume of 2% NaOH solution equal to that of the sample, left at room temperature for 15 min, and completely mixed for neutralization with 1.25 N HCl with a pH indicator (Karlson’s method) (4). Concentration was done by centrifugation at 3,800×g for 15 min, and samples were inoculated in Lowenstein-Jensen medium and Coletos medium (an egg-based enrichment medium with glutamate and pyruvate). Normally, sterile fluid samples were not decontaminated and were cultured in Lowenstein-Jensen, Coletos, and liquid enrichment (Middlebrook 7H9 media). Cultures were incubated for 8 weeks at 37°C. Skin samples were incubated at 30 and 37°C. A smear was also made for staining with auramine-rhodamine (4, 9). Weekly readings were taken, and macroscopic evidence of growth was confirmed by Ziehl-Neelsen staining. Mycobacterium tuberculosis, M. avium, and M. kansasi were identified by using DNA probes (Accu-Probe; Movaco, San Diego, Calif.). Other mycobacteria, including M. gordonei, were identified by standard tests (4, 9). We looked for M. haemophilum only in those clinical situations in which it was specifically requested.

Blood cultures were processed by using BACTEC 6A bottles (Becton Dickinson, Cockeysville, Md.). After regular incubation for 5 days at 37°C for recovery of other blood pathogens, we added 5 ml of Middlebrook 7H9 to the bottles, which were then incubated for a further 7 days. After this second incubation period, 10 ml of the blood broth culture was transferred into a conical tube with 0.5 ml of sodium deoxycholate (0.1 g/ml) and the samples were then centrifuged at 3,800×g for 15 min. A sample of the leukocyte layer was taken for inoculation into Lowenstein-Jensen and Coletos media and for smear preparation for auramine-rhodamine staining. All tubes, including the conical ones, were incubated at 37°C for 8 weeks and checked weekly for macroscopic and microscopic growth.

To estimate the number of samples being tested from HIV-positive patients compared with the number of samples from HIV-negative patients, a random 12-day study was carried out in each of the last 3 years of the study. All of the data were entered into a database (Microsoft Access 2.0). Statistical analysis of the results was done with the program WinSTAT 3.0. For qualitative variables, the χ² test with a 95% level of significance was used.

The number of admissions during the study period remained stable (a total of 378,931 and a mean of 47,368 patients per year). We received 105,433 samples for mycobacterial culture, of which 93,302 were fully processed (12,131 [12%] were rejected, and 7,463 [7%] were contaminated). The number of samples processed for mycobacteria per 1,000 admissions increased significantly (P < 0.05) throughout the study period and ranged from 148 in 1988 to 263 in 1995, with a maximum of 334 samples in 1994 (mean, 246.3) (P < 0.05). Thirty-three percent of all mycobacteriology laboratory workload came from HIV-positive patients, and that percentage remained stable during the last 3 years. Among the 93,302 samples processed, 4,603 (5.2%) were positive. The distribution of positive samples according to the type of specimen is shown in Fig. 1. Of all the positive samples, 43.3% were from HIV-positive patients. The yields of mycobacteria from blood cultures and other sterile fluids from HIV-positive patients (9 and 40%, respectively) were markedly and significantly different from those of the HIV-negative population (1.5 and 19.8%, respectively) (P < 0.05). Forty percent of all positive samples were initially detected by auramine-rhodamine staining, whereas the remaining 60% of positive samples were detected only after culture (this proportion remained stable throughout the study period). Overall, 3,929 isolates (85.4%) were identified as M. tuberculosis and 674 (14.6%) were identified as other mycobacteria. The recovery of these other mycobacteria increased over the study period from 4.6% in 1988 to 24.1% in 1995 (P < 0.05), with a noticeable increase in M. avium complex infection among HIV-positive patients, from 3.2% in 1988 to 31.2% in 1995 (P < 0.05). The number of samples processed per positive sample was 18.3 in the HIV-
positive population and 28 in the HIV-negative population ($P < 0.05$).

When we considered patients instead of samples, the overall number of new patients diagnosed with tuberculosis or other mycobacteria at our institution (2,094) remained stable throughout the study period (mean, 262) (Table 1) with an incidence of 5.5 cases per 1,000 admissions. However, proportion of all new cases that were HIV positive increased (Table 1) from 22.3% in 1988 to 37.2% in 1995 ($P < 0.05$%). Figure 2 shows the evolution of the number of patients infected with different species of mycobacteria according to their serologic status. Of all of the new patients, 686 (32.8%) showed evidence by culture of extrapulmonary involvement and 1,048 (67.2%) had exclusively pulmonary forms. This high percentage in extrapulmonary forms was observed mainly in HIV-positive patients.

The World Health Organization’s aim of eradicating tuberculosis by the year 2010 has been cut short by the development of this disease over the last decade, since the incidence of tuberculosis is no longer falling regularly (7). We emphasize the number of samples processed per new patient diagnosed, which ranged from 27 in 1988 to 45 in 1995 ($P < 0.05$), with a maximum of 66 samples per infected patient in 1993. We also draw attention to the disproportionate suspicion of tuberculosis in the HIV-negative population, probably induced as a reflection of the situation in the HIV-positive population. The limitation of laboratory resources demands rational use of mycobacteriology laboratories which, according to our data, should begin with physicians caring for HIV-negative patients.

In the United States, it has been calculated that 12% of patients with tuberculosis are HIV positive (3), and in another study (7), this figure was 16%. Our data show that in our environment, 32.5% of patients with $M. tuberculosis$, 63.7% of patients with $M. avium$ complex, and 28.3% of patients with other mycobacteria were HIV positive. The increase in infections due to

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of samples processed</th>
<th>No. processed/1,000 admissions</th>
<th>No. positive</th>
<th>% Positive</th>
<th>No. of new patients</th>
<th>No. of new HIV-positive patients</th>
<th>No. of new HIV-negative patients</th>
<th>No. of new patients/1,000 admissions</th>
</tr>
</thead>
<tbody>
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<td>83</td>
<td>173</td>
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<tr>
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<tr>
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<td>4,603</td>
<td>5.2</td>
<td>2,094</td>
<td>754</td>
<td>1,340</td>
<td>5.5</td>
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</table>

FIG. 1. Total numbers of processed and positive samples according to origin. [ ], processed samples; ■, positive samples. The percentage of positive samples was determined by using the following equation: Percent positive = Number of positive samples/Number of processed samples × 100. The percentages of positive samples were as follows: sputum, 6.9%; urine, 2.2%; blood cultures, 5.4%; CSF, 4.1%; other respiratory samples, 5.4%; other samples, 7.2%; all samples 5.2%.

FIG. 2. Number of patients infected mycobacteria according to serologic status (serostatus). ■, $M. tuberculosis$; □, $M. avium$ complex; ○, other mycobacteria; ■, patients with mycobacterial disease.
the *M. avium* complex has been fully recognized both in the United States and in other countries (1, 2, 5, 6, 8), but it is surprising that this problem has been slow to appear among HIV-positive patients in our environment.

Our data suggest an excessive mycobacteriology laboratory workload which is attributable not only to the HIV-positive population but also to a disproportionate demand from HIV-negative patients. Efforts to cope with an increasing and reasonable demand from HIV-positive patients should be accompanied by efforts to reduce the disproportionate workload created by an excessive demand from the HIV-negative population.

REFERENCES