

BACTERIAL AGENTS ISOLATED FROM CEREBROSPINAL FLUID OF PATIENTS WITH ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS) AND NEUROLOGICAL COMPLICATIONS

Ilka Maria LANDGRAF (1), Moisés PALACI (1), Maria de Fátima Paiva VIEIRA (1), Sueli Yoko Mizuka UEKI (1), Maria Conceição MARTINS (1), Daisy Nakamura SATO (1), Lucilaine FERRAZOLI (1), Maria Alice Silva TELLES (1), Maria Cecília Outeiro GORLA (1), Tania Mara Ibelli VAZ (1), Rosemarie LORENÇO (2) & Lilian Regina Macelloni MARQUES (1)

SUMMARY

Cerebrospinal fluid (CSF) samples from 2083 patients with acquired immunodeficiency syndrome (AIDS) and neurological complications were bacteriologically examined during a period of 7 years (1984-1990). The percentage of patients who had at least one bacterial agent cultured from the CSF was 6.2%. *Mycobacterium tuberculosis* was the most frequently isolated agent (4.3%), followed by *Mycobacterium avium* complex or MAC (0.7%), *Pseudomonas spp* (0.5%), *Enterobacter spp* (0.4%), and *Staphylococcus aureus* (0.3%). Among 130 culture positive patients, 89 (68.5%) had *M. tuberculosis* and 15 (11.6%) had MAC. The frequency of bacterial isolations increased from 1988 (5.2%) to 1990 (7.2%), partly due to the increase in MAC isolations. Bacterial agents were more frequently isolated from patients in the age group 21-30 years and from women ($p < 0.05$).

KEYWORDS: Acquired Immunodeficiency Syndrome; Cerebrospinal fluid; Bacterial agents.

INTRODUCTION

Central nervous system (CNS) complications are a major clinical problem at all stages of human immunodeficiency virus (HIV) infection, and may occur as a result of direct HIV infection, secondary opportunistic infections or neoplasms^{2, 12, 20}.

Reports on bacterial opportunistic infections of CNS in patients with acquired immunodeficiency syndrome (AIDS) are relatively scarce. Meningeal infections by *Neisseria meningitidis* serogroup A²², *Listeria monocytogenes*^{11, 31}, group G *Streptococcus*²⁴, *Streptococcus milleri*³¹ and *Escherichia coli*¹² have been referred only in case reports or short series of patients.

More numerous and extensive reports are available for *Mycobacterium tuberculosis* infections^{5, 6, 23, 25, 28}. The nontuberculous mycobacteria included in the *Mycobacterium avium* complex (MAC) have been cultured from the cerebrospinal fluid (CSF) of a few patients with AIDS^{16, 17, 21, 30}.

The frequency of *M. tuberculosis* meningeal infections in HIV-infected patients has been determined only among patients with tuberculosis. In a study of 199 patients with pulmonary and/or extrapulmonary tuberculosis in the United States, 10% showed meningeal involvement²⁵. The same frequency was detected

(1) Seção de Bacteriologia, Instituto Adolfo Lutz, Av. Dr. Arnaldo 355, 01246-902 São Paulo, SP, Brasil.

(2) Instituto de Infectologia Emílio Ribas, Av. Dr. Arnaldo, 165, 01245-901 São Paulo, SP, Brasil.

Correspondence to: Ilka Maria Landgraf, Seção de Bacteriologia, Instituto Adolfo Lutz, Av. Dr. Arnaldo 355, 01246-902 São Paulo, SP, Brasil Fax: 055-011-851-3505

in another study of 455 patients with tuberculosis in Spain ⁵.

To our knowledge, an extensive search for different bacterial agents in CSF samples of patients with AIDS and neurological complications has not been described. We have performed such search at the "Instituto Adolfo Lutz", a Public Health Central Laboratory of São Paulo State and the National Reference Center for Meningitis in Brazil, during a period of 7 years. The purpose of the present report is to show the results obtained from 2083 patients studied during this period.

MATERIALS AND METHODS

Patients belonging to the group IV of the AIDS classification made by the Centers for Disease Control and Prevention, Atlanta, U. S. A. ⁷ and having any neurological symptoms (headache, drowsiness, mental confusion, vomiting, paresis, paresthesia) with or without fever had a CSF sample collected as part of a routine procedure at the "Instituto de Infectologia Emílio Ribas", a Public Health Hospital. During a period of 7 years (1984-1990), a total of 2083 patients (1888 men and 195 women, with age ranging from one month to 70 years) was submitted to this procedure.

CSF samples from all 2083 patients were examined by Gram, Ziehl-Neelsen, and auramine staining procedures and cultured at the "Instituto Adolfo Lutz".

Chocolate agar (Mueller-Hinton base) and Löwenstein-Jensen (L-J) slants were used as primary culture media and incubated at 37°C for 24-48 h (5-10% CO₂ atmosphere) and 30-60 days, respectively.

Gram-negative rods with a growth that resembled enterobacteria or non-fermenters were subsequently inoculated onto MacConkey agar to verify if there was lactose fermentation and in standard media for biochemical identification ^{9, 14}.

Gram-positive and non-spore forming rods were examined for a characteristic umbrella-like motility in a semi-solid medium and further speciation was performed according to standardized procedures ¹.

Gram-positive cocci were submitted to the catalase test and the complete identification was performed according to a previously described methodology ^{10, 18}.

After isolation in L-J medium, mycobacteria were identified according to the criteria described by VESTAL ²⁹ and by DAVID et al ⁸.

The culture results were statistically analyzed by the chi-square test (Mantel-Haenszel) with regard to the age and sex of the patients.

RESULTS

Among the 2083 AIDS patients, 130 (6.2%) had at least one bacterial agent isolated from their CSF

TABLE I

Frequency of isolation of bacterial agents from cerebrospinal fluid of patients with acquired immunodeficiency syndrome (1984-1990)

| Bacterial Agent | Number of Patients | Percentage among Patients | |
|---|--------------------|---------------------------|----------------|
| | | Culture Positive (n=130) | Total (n=2083) |
| <i>Mycobacterium tuberculosis</i> | 89 | 68.5 | 4.3 |
| <i>Mycobacterium avium</i> complex | 14 | 10.8 | 0.7 |
| <i>Mycobacterium avium</i> complex+ <i>Streptococcus pneumoniae</i> | 1 | 0.8 | 0.05 |
| <i>Pseudomonas spp*</i> | 5 | 3.8 | 0.3 |
| <i>Pseudomonas spp*+ Enterobacter spp**</i> | 4 | 3.0 | 0.2 |
| <i>Enterobacter spp**</i> | 4 | 3.0 | 0.2 |
| <i>Staphylococcus aureus</i> | 7 | 5.3 | 0.3 |
| <i>Streptococcus pneumoniae</i> | 1 | 0.8 | 0.05 |
| <i>Streptococcus spp</i> | 1 | 0.8 | 0.05 |
| <i>Listeria monocytogenes</i> | 1 | 0.8 | 0.05 |
| <i>Escherichia coli</i> | 1 | 0.8 | 0.05 |
| <i>Klebsiella pneumoniae</i> | 1 | 0.8 | 0.05 |
| <i>Proteus mirabilis</i> | 1 | 0.8 | 0.05 |

* *P. aeruginosa* and *P. putida*.

** *E. cloacae* and *E. agglomerans*.

samples. Four patients had positive smears (one with Gram-positive diplococcus and three with Gram-negative rods), but negative cultures.

Table 1 shows the frequency of isolation of the different bacterial agents among the patients that were culture positive and among all patients studied. The most commonly isolated agent was *M. tuberculosis*, which was isolated from 4.3% of all patients and 68.5% of the culture positive patients. The second most frequently isolated agent was MAC (0.75% of all patients and 11.6% of the culture positive patients), followed by *Pseudomonas spp* (0.5% of all patients), *Enterobacter spp* (0.4%), and *Staphylococcus aureus* (0.3%). Among *Pseudomonas spp* strains, some were *P. aeruginosa* and others *P. putida* and among *Enterobacter spp* strains, *E. cloacae* and *E. agglomerans* were identified. Five patients had more than one bacterial agent isolated from their CSF.

Acid-fast bacilli were detected in the CSF smears from only one of the 89 patients culture for *M. tuber-*

culosis and none of the 15 patients with MAC. CSF smears stained by the Gram procedure were positive for 5 of the 27 culture positive patients.

As can be seen in Fig. 1, the frequency of isolation of bacteria from CSF of AIDS patients increased from 1988 (5.2%) to 1990 (7.2%). Among mycobacteria, isolation of MAC but not *M. tuberculosis* increased during this period. As a matter of fact, the frequency of *M. tuberculosis* isolation increased considerably until 1988, but remained steady during the following 2 years.

The distribution of AIDS patients with bacterial agents in CSF according to age and sex is shown in Table 2. Bacterial agents were isolated significantly more from patients belonging to the 21-30 year age group ($p < 0.05$; OR = 1.85 [1.28-2.69]) and from women ($p < 0.05$; OR = 1.85 [1.08-3.12]). The same age group ($p < 0.05$; OR = 2.09 [1.33-3.27]) and sex ($p < 0.05$; OR = 2.04 [1.10-3.74]) showed the highest frequency of *M. tuberculosis* isolation. MAC was isolated only from patients over 21 years of age and there were no

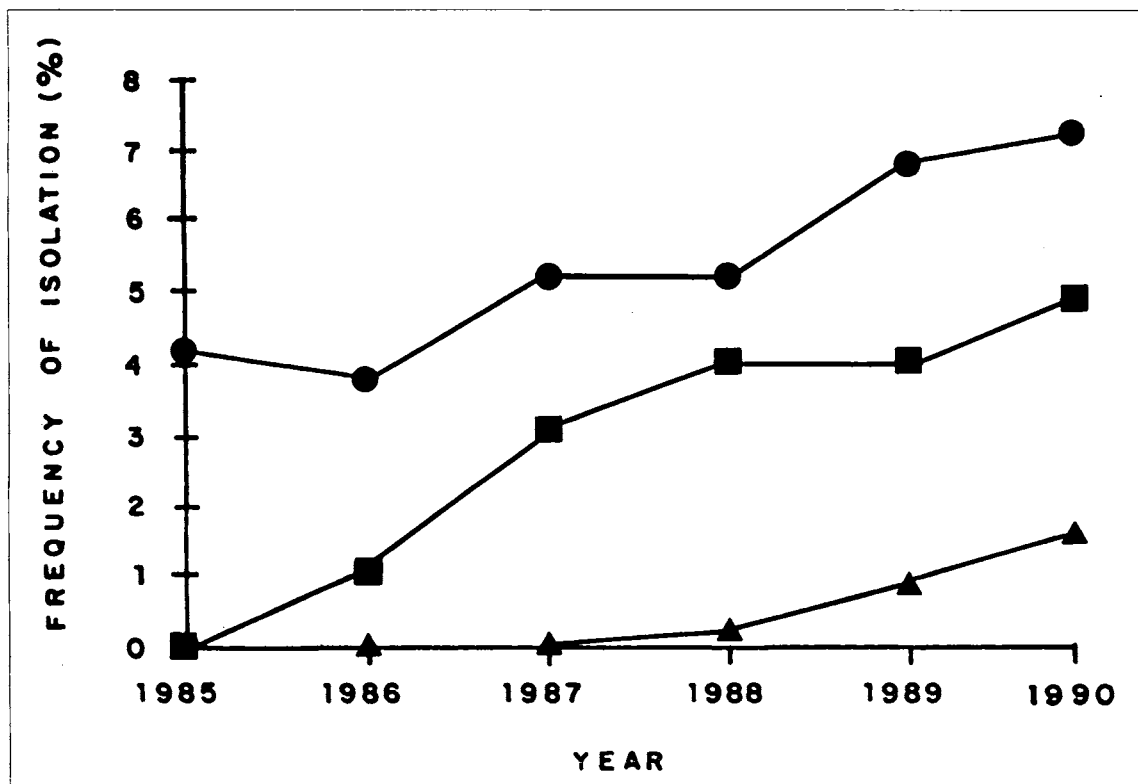


Fig. 1 - Annual frequency of isolation of bacterial agents from cerebrospinal fluid of patients with acquired immunodeficiency syndrome (1985-1990). * In 1984 only two CSF samples were examined and both were positive for *Mycobacterium tuberculosis*.

+ Symbols: ● = all bacteria; ■ = *M. tuberculosis*; ▲ = *Mycobacterium avium* complex.

TABLE 2

Distribution of patients with acquired immunodeficiency syndrome and bacteria in the cerebrospinal fluid according to age and sex.

| Patients | Total Number | Number of Patients (%) with: | | |
|------------|--------------|------------------------------|-----------------------------------|----------|
| | | Bacteria | <i>Mycobacterium tuberculosis</i> | MAC* |
| Age | | | | |
| (years) | | | | |
| 0-10 | 54 | 2 (3.7) | 0 (0.0) | 0 (0.0) |
| 11-20 | 143 | 6 (4.2) | 4 (2.8) | 0 (0.0) |
| 21-30 | 809 | 69 (8.5)** | 50 (6.2)** | 5 (0.6) |
| 31-40 | 758 | 40 (5.3) | 26 (3.4) | 7 (0.9) |
| > 41 | 319 | 13 (4.1) | 9 (2.8) | 3 (0.9) |
| Sex | | | | |
| Males | 1888 | 110 (5.8) | 74 (3.9) | 14 (0.7) |
| Females | 195 | 20 (10.3)** | 15 (7.7)** | 1 (0.5) |

* *Mycobacterium avium* complex

** p<0.05 by the Mantel-Haenszel chi-square test

significant differences in the frequency of isolation among the different age groups or the sex of the patients.

DISCUSSION

Bacterial agents were detected in the CSF from 6.2% of the 2083 patients with AIDS and neurological complications examined during a 7-year period (1984-1990). A much higher frequency of bacterial isolation from CSF samples (17%) was obtained in a previous study performed at the "Instituto Adolfo Lutz" employing the same methodology but including patients without AIDS ²⁶.

M. tuberculosis was the most commonly isolated bacterial agent from CSF samples of the AIDS patients with neurological symptoms. To our knowledge, there are no published data on the frequency of *M. tuberculosis* isolation from CSF of such patients. The reports on tuberculous meningitis from the United States ²⁵ and Spain ⁵ included only HIV-infected patients selected for having tuberculosis.

Extrapulmonary tuberculosis occur more frequently in patients with AIDS than in patients with less advanced HIV infection or persons not infected by HIV ³. This clinical feature can be clearly evidenced when the frequency of tuberculous meningeal infection detected in the present study of AIDS patients (4.3%) is compared with the one obtained in the previous study of non-AIDS patients (0.8%)²⁶.

MAC organisms were the second most frequently

isolated bacterial agent in the present study. These organisms are ubiquitous in nature and appear to have a particular predilection for infecting and disseminating within HIV-infected patients ¹⁵. Disseminated MAC infections are more frequent in AIDS patients with high level of immune dysfunction and can involve almost any organ system ^{4, 15}.

The clinical significance of MAC detection in the CSF of 15 AIDS patients could not be determined in the present study. The fact that 5 patients had more than one CSF sample culture positive for MAC (data not shown) suggests infection of the CNS by these organisms, but histopathological evidence of meningeal involvement was not pursued. Reviewing the records of CSF samples positive for nontuberculous mycobacteria filed in a hospital of the United States, JACOB et al. ¹⁶ also identified 15 patients with AIDS or at least one risk factor for AIDS and CSF culture-positive for MAC. Three patients had autopsy data recorded which included CNS lesions.

Recently, BENSON & ELLNER ⁴ stated that isolation of MAC organisms from normally sterile tissues should be interpreted as indicative of disseminated disease. In the present study, 3 patients with CSF culture positive for MAC also had these organisms isolated from blood and/or bone marrow aspirate (manuscript in preparation). All patients described by JACOB et al. ¹⁶ had widespread MAC dissemination.

In the present study, several agents other than mycobacteria were cultured from CSF samples of few or single patients, including *Pseudomonas spp.*, mem-

bers of the family *Enterobacteriaceae* (*Enterobacter*, *Escherichia coli*, *Klebsiella* and *Proteus*), *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus spp*, and *Listeria monocytogenes*. Among these agents, only *Escherichia coli*¹², streptococci other than *Streptococcus pneumoniae*^{24,31}, and *Listeria monocytogenes*^{11,31} have been referred in the AIDS literature.

It is important to point out that *Streptococcus pneumoniae*, which has been identified as one of the three leading causes of bacterial meningitis in non-AIDS patients examined in São Paulo City^{19,26}, was cultured from the CSF of only two AIDS patients. The other two leading agents, i.e., *Neisseria meningitidis* and *Haemophilus influenzae*, were not found among these patients.

The increased frequency of bacterial isolation from CSF observed between 1988 and 1990 may be partly accounted for the increase in MAC isolations. According to HORBSBURGH¹³, MAC infections in AIDS patients have been detected more frequently because of a more intense surveillance and longer survival of patients with high level of immune dysfunction obtained through better diagnosis, prophylaxis, and treatment of other opportunistic diseases.

Bacterial agents were isolated more frequently from CSF samples of patients 21-30 years of age and from females. This result was particularly true with the *M. tuberculosis* infections, but not for MAC isolations. In a recent analysis of AIDS surveillance information on extrapulmonary tuberculosis in U.S.A., SLUTSKER et al.²⁷ found that few cases were reported in patients under 20 years of age and that women were significantly more likely to have extrapulmonary tuberculosis than were men.

Although this study aimed to examine only bacterial isolates from CSF, *Cryptococcus spp* was cultured and identified in samples of 7.0% of all patients. Among the patients that had bacterial agents, only two had also *Cryptococcus spp*. One of these patients had *M. tuberculosis* and one had *Pseudomonas spp* isolated from their CSF samples.

The present extensive study showed that meningeal infection by bacterial agents other than *M. tuberculosis* in AIDS patients with neurological symptoms is not frequent and indicated that further studies have to be conducted to determine the significance of MAC isolation from CSF of these patients.

RESUMO

Agentes bacterianos isolados de líquido cefalorraquidiano de pacientes com Síndrome de Imunodeficiência Adquirida (SIDA) e complicações neurológicas

Amostras de líquido cefalorraquidiano (LCR) de 2083 pacientes com Síndrome de Imunodeficiência Adquirida (SIDA) e complicações neurológicas foram examinados durante um período de 7 anos (1984-1990). A porcentagem de pacientes que tiveram pelo menos um agente bacteriano cultivado do LCR foi de 6,2%. *Mycobacterium tuberculosis* foi o mais frequentemente isolado (4,3%), seguido do complexo *Mycobacterium avium* ou MAC (0,7%), de *Pseudomonas spp* (0,5%), *Enterobacter spp* (0,4%), e *Staphylococcus aureus* (0,3%). Entre 130 pacientes com cultura positiva, de 89 (68,5%) foi isolado *M. tuberculosis* e de 15 (11,6%) MAC. A frequência de isolamentos bacterianos aumentou de 1988 (5,2%) a 1990 (7,2%), particularmente devido ao maior isolamento de MAC. Os agentes bacterianos foram mais frequentemente isolados de pacientes na faixa etária de 21-30 anos e de mulheres ($p < 0.05$).

REFERENCES

1. ALBRITTON, W. L.; WIGGINS, G. L.; DeWITT, W. E. & FEELEY, J. C. - *Listeria monocytogenes*. In: LENNETTE, E. H.; BALLOWS, A.; HAUSLER Jr., W. J. & TRUANT, J. P., ed. *Manual of clinical microbiology*. Washington, American Society for Microbiology, 1980. p. 139-142.
2. ATWOOD, W. J.; BERGER, J. R.; KADERMAN, R.; TORNATORE, C. S. & MAJOR, E. O. - Human immunodeficiency virus type 1 infection of the brain. *Clin. Microbiol. Rev.*, 6: 339-366, 1993.
3. BARNES, P. F.; BLOCH, A. B.; DAVIDSON, P. T. & SNIDER Jr., D. E. - Tuberculosis in patients with human immunodeficiency virus infection. *New Engl. J. Med.*, 324: 1644-1650, 1991.
4. BENSON, C. A. & ELLNER, J. J. - *Mycobacterium avium* complex infection and AIDS: advances in theory and practice. *Clin. infect. Dis.*, 17: 7-20, 1993.
5. BERENQUER, J.; MORENO, S.; LAGUNA, F. et al. - Tuberculous meningitis in patients infected with the human immunodeficiency virus. *New Engl. J. Med.*, 326: 668-672, 1992.
6. BISHBURG, E.; SUNDERAM, G.; REICHMAN, L. B. & KAPILA, R. - Central nervous system tuberculosis with the acquired immunodeficiency syndrome and its related complex. *Ann. intern. Med.*, 105: 210-213, 1986.
7. CENTERS FOR DISEASE CONTROL - Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. *M. M. W. R.*, 36 (suppl.): 1S-15S, 1987.

8. DAVID, H.; LEVY-FREBAULT, V. & PAPA, F. - *Méthodes de laboratoire pour mycobactériologie clinique*. Paris, Institut Pasteur, 1986.
9. EDWARDS, P. R. & EWING, W. H. - *Identification of Enterobacteriaceae*. Minneapolis, Burgess Publishing, 1972.
10. FACKLAM, R. R. - *Streptococci and Aerococci*. In: LENNETTE, E. H.; BALOWS, A.; HAUSLER Jr., W. J. & TRUANT, J. P., ed. *Manual of clinical microbiology*. Washington, American Society for Microbiology, 1980. p. 88-110.
11. GRADON, J. D.; TIMPONE, J. G. & SCHNITTMAN, S. M. - Emergence of unusual opportunistic pathogens in AIDS: a review. **Clin. infect. Dis.**, 15: 134-157, 1992.
12. GRAY, F.; GHERARDI, R. & SCARAVILLI, F. - The neuropathology of the acquired immune deficiency syndrome (AIDS). A review. **Brain**, 111: 245-266, 1988.
13. HORSBURGH Jr., C. R. - *Mycobacterium avium* complex infection in the acquired immunodeficiency syndrome. **New Engl. J. Med.**, 324: 1332-1338, 1991.
14. HUGH, R. & GILARDI, G. L. - *Pseudomonas*. In: LENNETTE, E. H.; BALOWS, S.; HAUSLER Jr., W. J. & TRUANT, J. P., ed. *Manual of clinical microbiology*. Washington, American Society for Microbiology, 1980. p. 288-317.
15. INDERLIED, C. B.; KEMPER, C. A. & BERMUDEZ, L. E. M. - The *Mycobacterium avium* complex. **Clin. Microbiol. Rev.**, 6: 266-310, 1993.
16. JACOB, C. N.; HENEIN, S. S.; HEURICH, A. E. & KAMHOLZ, S. - Nontuberculous mycobacterial infection of the central nervous system in patients with AIDS. **Sth. med. J. (Bgham., Ala.)**, 86: 638-640, 1993.
17. KLATT, E. C.; JENSEN, D. F. & MEYER, P. R. - Pathology of *Mycobacterium avium-intracellulare* infection in acquired immunodeficiency syndrome. **Hum. Path.**, 18: 709-714, 1987.
18. KLOOS, W. E. & SCHLEIFER, K. H. - Simplified scheme for routine identification of human *Staphylococcus* species. **J. clin. Microbiol.**, 1: 82-88, 1975.
19. LANDGRAF, I. M. & VIEIRA, M. F. P. - Biotypes and serotypes of *Haemophilus influenzae* from patients with meningitis in the City of São Paulo, Brazil. **J. clin. Microbiol.**, 31: 743-745, 1993.
20. LEVY, R. M.; BREDESEN, D. E. & ROSENBLUM, M. L. - Neurological manifestations of the acquired immunodeficiency syndrome (AIDS): experience at UCSF and review of the literature. **J. Neurosurg.**, 62: 475-495, 1985.
21. MARTIN, T.; CHEKE, D. & NATYSHAK, I. - Broth culture: the modern "guinea-pig" for isolation of mycobacteria. **Tubercle (Edinb.)**, 70: 53-56, 1989.
22. MORLA, N.; GUIBOURDENCHE, M. & RIOU, J. Y. - *Neisseria spp* and AIDS. **J. clin. Microbiol.**, 30: 2290-2294, 1992.
23. OGAWA, S. K.; SMITH, M. A.; BRENNESSSEL, D. J. & LOWY, F. D. - Tuberculous meningitis in an urban medical center. **Medicine**, 66: 317-326, 1987.
24. RAVIGLIONE, M. C.; TIERNO, P. M.; OTTUSO, P. & DAVIDSON, M. - Group G streptococcal meningitis and sepsis in a patient with AIDS. A method to biotype group G *Streptococcus*. **Diagn. Microbiol. infect. Dis.**, 13: 261-264, 1990.
25. SHAFER, R. W.; KIM, D. S.; WEISS, J. P. & QUALE, J. M. - Extrapulmonary tuberculosis in patients with human immunodeficiency virus infection. **Medicine**, 70: 384-397, 1991.
26. SILVA, E. A. M.; MELLES, C. E. A.; SATO, D. N. et al. - Bacteriological and epidemiological aspects of the diagnosis of *Mycobacterium tuberculosis* as the etiological agent of meningitis. **Rev. Hosp. S. Paulo Esc. paul. Med.**, 1: 74-76, 1989.
27. SLUTSKER, L.; CASTRO, K. G.; WARD, J. W. & DOOLEY Jr., S. W. - Epidemiology of extrapulmonary tuberculosis among persons with AIDS in the United States. **Clin. infect. Dis.**, 16: 513-518, 1993.
28. SMALL, P. M.; SCHECTER, G. F.; GOODMAN, P. C. et al. - Treatment of tuberculosis in patients with advanced human immunodeficiency virus infection. **New Engl. J. Med.**, 324: 289-294, 1991.
29. VESTAL, A. L. - *Procedures for the isolation and identification of mycobacteria*. Washington, U.S. Department of Health, Education, and Welfare, 1976. (HEW publication CDC n° 76-8230).
30. WALLACE, J. M. & HANNAH, J. B. - *Mycobacterium avium* complex infection in patients with the acquired immunodeficiency syndrome. A clinicopathologic study. **Chest**, 93: 926-932, 1988.
31. WITT, D. J.; CRAVEN, D. E. & McCABE, W. R. - Bacterial infections in adult patients with the acquired immune deficiency syndrome (AIDS) and AIDS-related complex. **Amer. J. Med.**, 82: 900-906, 1987.

Recebido para publicação em 18/04/1994.
Aceito para publicação em 17/06/1994.