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MYCOBACTERIUM AVIUM COMPLEX (MAC) ISOLATED FROM AIDS PATIENTS AND THE CRITERIA REQUIRED FOR ITS IMPLICATION IN DISEASE

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SUMMARY

Before the AIDS pandemia, the *Mycobacterium avium* complex (MAC) was responsible in most cases for the pneumopathies that attack patients with basic chronic pulmonary diseases such as emphysema and chronic bronchitis[3,6]. In 1981, with the advent of the acquired immunodeficiency syndrome (AIDS), MAC started to represent one of the most frequent bacterial diseases among AIDS patients, with the disseminated form of the disease being the major clinical manifestation of the infection[8].
Between January 1989 and February 1991, the Section of Mycobacteria of the Adolfo Lutz Institute, São Paulo, isolated MAC from 103 patients by culturing different sterile and no-sterile processed specimens collected from 2304 patients seen at the AIDS Reference and Training Center and/or Emilio Ribas Infectology Institute. Disseminated disease was diagnosed in 29 of those patients on the basis of MAC isolation from blood and/or bone marrow aspirate. The other 74 patients were divided into categories highly (5), moderately (26) and little suggestive of disease (43) according to the criteria of DAVIDSON (1989)[10]. The various criteria for MAC isolation from sterile and non-sterile specimens are discussed.

KEYWORDS: *Mycobacterium avium* complex (MAC); Acquired immunodeficiency syndrome (AIDS); Diagnostic criteria.

INTRODUCTION

Approximately 60 species are recognized today in the genus *Mycobacterium*. Among them, particularly important in terms of human pathology are those included in the *Mycobacterium tuberculosis* (*M. tuberculosis, M. bovis, M. africanum*) complex and *M. leprae*. However, at least 22 other species known by the generic term Mycobacteria other than tuberculosis (MOTT) may cause disease in human beings in the presence of certain host conditions[2].

Prior to the AIDS pandemia, MAC was mostly responsible for pneumopathies mainly occurring in adult males with some chronic basic lung disease such as pulmonary emphysema, bronchiectasia, pneumoconiosis or

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cicatricial lesions due to tuberculosis[8]. Until 1980, few cases of disseminated disease had been reported in the literature[18], and most of them affected patients with malignant hematologic disease such as hairy cell leukemia[31] and immunosuppressed by drugs or with other basic pathologies[36].

Over the last 14 years, with the advent of the acquired immunodeficiency syndrome (AIDS), several MOTT have been reported to be responsible for opportunistic infections in these patients, with those belonging to the *Mycobacterium avium-intracellulare* complex being the most frequently reported. Disseminated MAC disease started to represent the major form of clinical manifestation of disease due to these mycobacteria[8,19].

Severe immunodeficiency characterized by a CD4 lymphocyte number of less than 100/mm^3 has been considered the major risk factor for disseminated MAC disease, whose clinical manifestations are quite nonspecific: fever, night sweating, adynamia and weight loss[19], requiring a differential diagnosis from other opportunistic infection.

Although the differential diagnosis assumes the isolation and identification of MAC from sterile specimens such as blood, bone marrow, lymphonode, and liver or spleen tissue, some investigators tend to place a higher value on the isolation of these microorganisms from non-sterile sites both for a predictive indication of dissemination and for the diagnosis of infection[7,10,33]. However, in practice there are many difficulties in differentiating the three types of relationship between MAC and the human host: colonization, infection and disease[37], especially when these mycobacteria are isolated from non-sterile specimens.

The objectives of the present report are to present the results obtained by us with mycobacteriologic examination of the different specimens collected from HIV-infected patients or patients with AIDS, determine the frequency of MAC isolation and, finally, analyze and discuss the criteria available in the literature for the determination of the diagnostic value of the isolation of these mycobacteria from different sterile and non-sterile specimens.

MATERIALS AND METHODS

PATIENTS AND SOURCE OF SPECIMENS

From January 1989 to February 1991 a total of 6537 clinical specimens were processed in the Section of Mycobacteria, Department of Bacteriology, of Adolfo Lutz Institute, The specimens were obtained from patients with suspected or diagnosed HIV/AIDS seen at the Emilio Ribas Infectology Institute and/or AIDS Reference and Training Center - São Paulo (CRTA-SP).

A total of 5484 specimens collected from 2304 patients were selected from the above total on the basis of notification of the confirmed presence of AIDS. This selection was made using no. III Epidemiologic Notification Cards/AIDS of Sexually Transmissible Diseases/AIDS of the Epidemiological Surveillance Center of Department of Health of São Paulo State.

The specimens were collected according to the suspected involvement of various sites, except cerebrospinal fluid (CSF), which was collected from several patients for the control of tuberculous meningoencephalitis, and brain toxoplasmosis and cryptococcosis.

Search for mycobacteria in the different clinical specimens:

Culture: specimens of sputum, bronchial wash, gastric wash, feces, urine, biopsies, and pleural and peritoneal secretions and fluids were decontaminated by the method of Petroff and later inoculated (0.2 ml) into Lowenstein-Jensen medium[27].

Blood specimens (5 ml) were directly inoculated into biphasic culture medium consisting of Middlebrook 7H9 liquid medium (Difco) containing the anticoagulant sodium polyanethol sulfonate (0.25 g/ml), polymyxin B (200 U/ml), amphotericin B (10 mg/ml), carbenicillin (50 mg/ml) and trimetoprim (20 mg/ml), and of Lowenstein-Jensen solid medium. Bone marrow aspirates and CSF samples were directly inoculated into Lowenstein-Jensen medium (approximately 0.2 ml).

After inoculation, the culture media were incubated at 37°C and inspected weekly until the occurrence of mycobacteria colonies or for a period of up to 60 days when they were considered negative because of the absence of growth. Biopsies and skin secretions were also incubated at 28°C and 37°C.

Identification of mycobacteria: non-pigmented mycobacteria colonies with a rough aspect, growing in Lowenstein-Jensen medium only at 37°C for a period of more than seven days (slow growth), niacin producing, nitrate reducing, sensitive to paranitrobenzoic acid and resistant to 2-thiophenocarboxylic acid were identified as *M. tuberculosis*[9,32] Strains characterized as Mycobacteria other than *M. tuberculosis* (MOTT) were identified by the methods of KENT & KUBICA (1985)[22] and DAVID et al. (1989)[9].

Clinical and laboratory data:

The clinical signs and symptoms that permitted the diagnosis of disseminated disease were obtained from CRTA medical records and from the following regional hospitals: Sul, Mauá, Franco da Rocha, Brigadeiro, Ferraz de Vasconcelos, Mandaqui, Ipiranga, and Heliópolis, and from the Municipal Itaquera Hospital and the Santa Marcelina Health House.

Date of AIDS diagnoses and date of deaths for cases of disseminated MAC disease were obtained from the epidemiologic notification cards.

Data about hemoglobin, total leucocyte and lymphocyte and serum glutamic oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT) and alkaline phosphatase (AP) determinations performed on the occasion of the specimen collection that led to the diagnosis of disseminated MAC disease were also obtained from these medical records.

Definition of disseminated MAC disease and diagnostic categories:

All patients with MAC strains identified from blood, bone marrow aspirate, lymphonode and/or ganglionar secretions were considered to be cases of disseminated MAC disease[20].

Patients with MAC strains isolated from other sites were distributed into the following diagnostic categories, defined on the basis of isolation sites:

HIGHLY SUGGESTIVE OF DISEASE: when MAC strains were isolated from two or more CSF specimens;

MODERATELY SUGGESTIVE OF DISEASE: when the strains were isolated from the same non-sterile specimen two or more times, from two non-sterile specimens regardless of the number of specimens positive for each type, and from a single CSF specimen;

LITTLE SUGGESTIVE OF DISEASE: when the strains were isolated from a single non-sterile specimen.

RESULTS

MAC was isolated from the different specimens of 103 (4.5%) of the 2304 patients whose specimens were cultured (Table 1). Proportionally, there was a higher frequency of isolation of these microorganisms from blood (14.8%) and bone marrow aspirate (25.7%) samples. The table also shows the isolation of MAC from only one patient among those whose lymphonode and/or ganglionar secretions were cultured.

Table 2 shows the classification of these 103 patients into four diagnostic categories according to previously established criteria. We emphasize that MAC was isolated from non-sterile specimens collected from 60 (58.2%) of these patients.

The clinical signs and symptoms of 18 of the 29 cases of disseminated MAC disease are presented in Table 3. This analysis could not be extended to all patients because of lack of access to their data. It is interesting to mention that all of these 15 patients had fever.

TABLE 1

Frequency of MAC isolation from different specimens obtained from 2304 patients with processed cultures.

Specimen	Number of processed cultures	No. of patients with MAC	(%)
Sputum	899	36	4.0
Bronchial lavage	566	30	5.3
Gastric lavage	56	1	1.8
Feces	20	1	5.0
Urine	81	2	2.5
Cerebrospinal fluid	1372	16	1.2
Blood	122	18	14.8
Bone marrow aspirate	74	19	25.7
Ganglionary secretion	30	1	3.3
Others	150	0	0.0

TABLE 2

Distribution of patients from whom the *Mycobacterium avium* complex was isolated according to the diagnostic categories established and isolation sites.

Diagnostic category Site of isolation	No. of patients (%) (n=103)
Diagnosis of disseminated disease	**29(27.9)**
Blood	7(6.6)
Blood and sputum	1(1.0)
Blood and bronchial lavage	1(1.0)
Blood and ganglionary secretion	1(1.0)
Bone marrow	8(7.6)
Bone marrow and sputum	1(1.0)
Bone marrow and gastric lavage	1(1.0)
Bone marrow and cerebrospinal fluid	1(1.0)
Blood and bone marrow	6(5.7)
Blood, bone marrow feces	1(1.0)
Blood, bone marrow and cerebrospinal fluid	1(1.0)
Highly suggestive of disease	**5(4.8)**
Cerebrospinal fluid (3 samples)	4(3.8)
Cerebrospinal fluid (2 samples)	1(1.0)
Moderately suggestive of disease	**26(25.0)**
Sputum (2 samples)	8(7.6)
Sputum (2 samples) and bronchial lavage	2(1.9)
Sputum (3 samples) and bronchial lavage	2(1.9)
Sputum (4 samples)	1(1.0)
Sputum and Bronchial lavage	1(1.0)
Bronchial lavage (2 samples)	2(1.9)
Urine (2 samples)	1(1.0)
Cerebrospinal fluid	9(8.7)
Little suggestive of disease	**43(41.3)**
Sputum	20(19.2)
Bronchial lavage	22(21.1)
Urine	1(1.0)

The chemical and cytologic analysis of the blood of these 15 patients is presented in Table 4. Eleven of them had hemoglobin levels lower than 10 g/dl and a mean number of total lymphocytes of 787.9/mm^3.

DISCUSSION

Although the pathogenic potential of MAC and a marked degree of immunodeficiency represent preponderant factors for the development of the disease, the interpretation of MAC isolation from different specimens requires caution, especially because this is an environmental microorganism with the ability to contaminate specimens or to colonize the mucosal surfaces of human beings in a transitory manner without any harm to the host[6,11,14,15,26,28].

Thus, what are the criteria used to attribute a pathogenic role to this complex in a given patient?

In 1967, YAMAMOTO et al.[39], in Japan, proposed: **a) major criteria:** 1) isolation of MOTT four times or more with a number of colonies of more than 100 associated with the presence of clinical symptoms that might be attributed to these bacilli, and 2) presence of a lesion containing MOTT and histopathological alterations possibly due to these bacilli; **b) minor criteria:** 1) MOTT isolation more than four times or a number of colonies exceeding 100, 2) MOTT elimination during the course of the disease, 3) MOTT isolation from an organ or tissue whose histopathological characteristics are not known, and 4) intradermal reaction halo to tuberculin obtained from MOTT larger than that in response to the tuberculin protein derivative obtained from *M. tuberculosis*, or an increase in the halo in response to tuberculin obtained from MOTT coinciding with the course of the disease. The diagnosis of the disease is established when at least one of the **major criteria** or al least three of the four **minor criteria** are satisfied.

In 1970, EDWARDS[13], in Australia, adopted as a criterion for MOTT disease the presence of tissue lesions indistinguishable from active tuberculosis and the culture of the bacillus at least once from the same tissue in the absence of *M. tuberculosis*.

In 1981, the AMERICAN THORACIC SOCIETY[2] adopted as a criterion for MOTT-induced pulmonary disease the presence of an infiltrate on the chest X-ray of a patient whose pathology could not be determined by careful clinical and laboratory investigation, but in whose specimens the same mycobacterium species could be detected in the absence of other pathogens.

In 1982, AHN et al.[1], in the U.S., in a reanalysis of the above criteria, proposed the following ones for the diagnosis of pulmonary disease caused by *M. kansasii* or *M. intracellulare*: a) positive sputum bacilloscopies for two weeks or more after the beginning of chemotherapy in the presence of cavitation not explained by any other disease; b) absence of reduction in the number of colonies within one month or absence of negativity of sputum within 2 to 4 months after bronchial hygiene in the presence of infiltration without cavitation that could not be explained by any other associated disease, or c) isolation of mycobacteria from tissue biopsies in association with histopathological alterations compatible with the disease.

In 1986, TSUKAMURA[33], in Japan, proposed diagnosis of the disease based on the isolation of the same mycobacterium species from two or more cultures of sputum samples collected over a period of seven days or several isolations at two to three month intervals in the presence of a cavitary or caseo-infiltrative pulmonary lesion. He also suggested the diagnostic importance of a cavity with a sclerotic wall or cavities inside a sclerotic lesion associated with three or more cultures of monthly sputum samples collected over a period of six months with isolation of more than 100 monthly of the same mycobacterium species.

In 1989, DAVIDSON[10] in the U.S. proposed that the larger the number of positive culture and the number of colonies present in them, and the longer the period of observation of these findings, the greater the chance of the isolated mycobacterium being the agent responsible for the disease.

In 1990 the AMERICAN THORACIC SOCIETY[3], in a reevaluation of the criteria established in 1981, proposed that the diagnosis of MOTT -induced lung disease should be based on the presence of AFB in two or more sputum samples (or a sputum sample and bronchial lavage) and/or abundant MOTT growth associated with the exclusion of another morbid entities that might justify the pulmonary disease, such as fungal disease, malignant disease or tuberculosis.

However, it is important to point out that most of the above criteria still need validation.

In 1985, KIEHN et al.[24] and in 1986 HAWKINS et al.[17], in New York (U.S.A.), validated the isolation of MAC from blood, bone marrow, lymphonode or liver for the diagnosis of disseminated disease in HIV-infected patients by documenting this clinical form of the disease at autopsy in all AIDS patients studied and by isolating MAC from these specimens.

In view of the above information, in our series we defined as cases of disseminated disease the 29 patients from whose blood and/or bone marrow aspirate MAC was isolated in association or not with MAC isolation from non-sterile specimens.

Analysis of clinical signs and symptoms was performed in only 15 of the 29 cases. The presence of fever in all of the 15 patients agreed with data reported for most series in the literature[19,25]. However, analysis of the other clinical signs and symptoms among the present patients with this disease (table 3) was hampered by the fact that these clinical parameters were obtained from medical records and not by prospective patient evaluation. With respect to the laboratory data, a mean hemoglobin concentration of 9.0 g/dl and a mean total lymphocyte number of 787.9/mm^3 were the most frequent alterations. These data are similar to those reported by others[4,34].

Mean GOT, GPT and AP values were within normal levels although high serum AP levels of these patients are commonly reported[19,23].

To establish a sure diagnosis for the remaining 74 patients it would be obligatory to isolate MAC from blood or bone marrow. However, since this was a retrospective study, these specimens unfortunately were not collected and cultured for mycobacteria. Consequently we opted for the stratification of these patients into three diagnostic categories (Table 2) mainly on the basis of the postulates of DAVIDSON[10].

In the second group, categorized as highly suggestive of the disease, we included the patients with MAC isolation from one or more CSF specimens (sterile specimens). In this respect, little has been reported on the pathogenicity of MAC for the central nervous system and the possible relationship between MAC isolation from the CSF only and the existence of disseminated disease[16,21,30,35,36]. Considering that CSF is a sterile specimen, MAC isolation from it should signify disseminated disease in view of the fact that MAC access to the central nervous system would be preceded by hematogenic dissemination. On the other hand, anatomopathological substrates relating MAC isolation from CSF to diseases involving the central nervous system are still quite scarce[36]. Among the few reports citing the pathogenicity of MAC for the central nervous system is that of CHAPMAN (1960)[5], in Texas, who reported granulomatous meningitis detected at autopsy in a 13 year old boy with disseminated MAC disease and MAC isolation from the CSF before death.

Recently, JACOB et al.[21], in New York (U.S.A.), reported MAC isolation from the CSF of 15 patients and *M. fortuitum* isolation from another, all of them AIDS patients. Autopsy was performed in three of these 16 cases, revealing extensive involvement of the liver, gastrointestinal tract, bone marrow, lymphonode and central nervous system, with discrete inflammatory activity and poorly formed granulomas without giant Langhans cells. Although the material obtained at autopsy was not cultured, AFB were visualized by direct inspection at most sites. Thus, if, on the one hand this study presented the first substantial evidence in the AIDS pandemia that MAC isolation from the CSF may represent a marker of its hematogenic dissemination, on the other hand we cannot base ourselves on this finding as a reference of the pathogenicity of MAC for the central nervous system since there are no reports of the detection of AFB in the central nervous system or of the isolation of mycobacteria from brain tissue cultures.

TABLE 3

Clinical signs and symptoms of 15 patients with disseminated disease caused by *Mycobacterium avium* complex.

Signs and symptoms	No. of cases (%)
Fever	15(100.0)
Night sweating	4(36.7)
Generalized malaise	1(6.7)
Adynamia	1(6.7)
Anorexia	1(6.7)
Weight loss	1(6.7)
Generalized pain	1(6.7)
Respiratory	
Cough	4(26.7)
Dyspnea	2(13.3)
Retrosternal pain	1(6.7)
Gastrointestinal	
Diarrhea	2(13.3)
Epigastric pain	1(6.7)
Lymphadenopathy	
Localized	1(6.7)
Generalized	1(6.7)
Hepatosplenomegaly	1(6.7)

TABLE 4

Chemical and cytologic analysis of blood from 15 patients with disseminated disease caused by *Mycobacterium avium* complex.

Normal values[a]	Values obtained	No. of cases (%) (n=15)
Hemoglobin	<10	11(78.6)
(>=12g/dl)	10-12	2(14.3)
	> 12	1 (7.1)
	ND[b]	1
	X=9.05	
Total white cells	<4,000	5(35.7)
(5,000-10,000/mm^3)	4,000-10,000	9(64.3)
	ND	1
	X=4514.3	
Total Lymphocytes	<200	2(14.3)
(1,000-4,200/mm^3)	200-600	4(28.6)
	600-1,000	4(28.6)
	>1,000	4(28.6)
	ND	1
	X=787.9	
Glutamic oxaloacetic	<40	9(81.8)
transaminase	>40	2(18.2)
(14-31UI/I)	ND	4
	X=37.7	
Glutamic pyruvic	=<50	12(85.7)
transaminase	>50	2(14.3)
(8-46UI/I)	ND	1
	X=30.3	
Alkaline phosphatase	<220	7(63.6)
(80-220UI/I)	>220	4(36.4)
	ND	4
	X=180.7	

[a]According to Kjeldberg (1993)
[b]ND = not determined

In relation to the third diagnostic category, classified as moderately suggestive of the disease, according to the criteria proposed by DAVIDSON[10], the reincidence of MAC isolation from non-sterile specimens with a moderate number of colonies represents the major bacteriologic basis for the assignment of patients to this category. We also decided to assign to this category those patients with a single MAC isolation from the CSF by considering the probability of disease among them to be lower compared to the category highly suggestive of disease.

We assigned to the last category the 43 patients with the least probability of disseminated disease as indicated by a single isolation of MAC from a non-sterile specimen.

In summary, the present results indicate the need for further studies to determine the incidence and predictive

value of MAC-induced disease in each category, especially by culturing blood and/or bone marrow aspirate and by submitting to autopsy subjects suspected to have disseminated MAC disease.

RESUMO

Complexo *Mycobacterium avium* (MAC) isolado de pacientes com AIDS e os critérios exigidos para sua implicação em doença.

Anterior a pandemia de AIDS, o Complexo *Mycobacterium avium* (MAC) era responsável pela maioria das vezes, por pneumopatias acometendo pacientes com doença pulmonar crônica de base como enfisema e bronquite crônica[36]. Em 1981, com o advento da síndrome de imunodeficiência adquirida (SIDA), o MAC passou a representar uma das doenças bacterianas mais frequentes em pacientes com esta síndrome, sendo a doença disseminada a principal forma de manifestação clínica da infecção[8].

Entre Janeiro de 1989 e Fevereiro de 1991, no Setor de Micobactérias do Instituto Adolfo Lutz em São Paulo, o MAC foi isolado de 103 pacientes a partir do cultivo de diferentes espécimes estéreis e não estéreis processados, coletados de 2.304 pacientes atendidos no Centro de Referência e Treinamento AIDS e/ou Instituto de Infectologia Emilio Ribas. A doença disseminada foi diagnosticada em 29 destes, com base no isolamento do MAC a partir do sangue e/ou aspirado de medula óssea. Os outros 74 pacientes foram agrupados nas categorias altamente (5), moderadamente (26) e pouco sugestiva de doença (43) de acordo com os postulados de DAVIDSON (1989)[10]. Os diferentes critérios para valorizar o seu isolamento de espécimes estéreis e não estéreis são discutidos.

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