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DISSEMINATED *MYCOBACTERIUM AVIUM* COMPLEX INFECTION IN SOUTH AFRICAN PATIENTS WITH AIDS

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A research report submitted to the Faculty of Medicine, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree

of

Master of Medicine

In

Internal Medicine

Johannesburg, 1999

DECLARATION

I, Clive Allan Pettipher, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Internal Medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

4th day of October, 1999.

ABSTRACT

Disseminated *Mycobacterium avium* complex (MAC) is the most common systemic bacterial infection in patients with acquired immunodeficiency syndrome (AIDS) in developed countries; however, it is reported to be uncommon in Africa. The role of a prior history of tuberculous disease, and the presence of a bacille Calmette-Guerin (BCG) scar, in preventing disseminated MAC is controversial.

The aims of the study were to determine the prevalence and clinical features of disseminated MAC in black South African patients with AIDS, and to investigate its interrelationship with *Mycobacterium tuberculosis* (MTB).

All patients diagnosed with disseminated MAC at Chris Hani Baragwanath Hospital from 1997 to 1998 were evaluated retrospectively. Further, one hundred HIV positive black South African patients with CD4 counts of less than 100 cells/mm^3 admitted to hospital were subjected to routine Bactec radiometric blood cultures and prospectively followed up.

The study showed that the prevalence of disseminated MAC in black South African patients with AIDS is 10%, a prevalence similar to that in developed countries. Furthermore, a prior history of tuberculous disease or the presence of a BCG scar were found not to be protective of disseminated MAC. The clinical and laboratory manifestations were largely unhelpful in diagnosing the condition.

Mycobacteria were cultured from Bactec blood cultures in 29 of the 100 cases. Ten patients in total were diagnosed with disseminated MAC, and 58 with MTB. Of the patients with mycobacterial disease, sputum analysis alone detected disease in 53% of cases, and Bactec blood culture in 45% of cases. The two investigations used in combination made the diagnosis in 86% of cases, making this an efficient, cost effective and simple strategy.

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1 INTRODUCTION

The study describes the epidemiology of disseminated *Mycobacterium avium complex* (MAC) in black South African patients with acquired immune deficiency syndrome (AIDS). The clinical and laboratory features of hospitalized black South African patients with disseminated MAC are evaluated in separate descriptive and comparative components of the study. Further, the interrelationship of *Mycobacterium tuberculosis* (MTB) with disseminated MAC is evaluated, especially pertaining to MTB being protective over MAC. The yield of Bactec blood cultures in diagnosing mycobacterial disease in hospitalized black African patients with advanced retroviral disease is determined prospectively, and recommendations are made for its routine use in the above setting.

This study was conducted at Chris Hani Baragwanath Hospital (C.H.B.H.) which is a large South African hospital wholly representative of the black South African community, and probably many other African populations.

The opening chapter discusses the context of, and the rationale for, the study. The literature survey reviews the epidemiology, pathogenesis, clinical features, diagnosis, prognosis, prevention and treatment of disseminated MAC, and describes the controversies in the interrelationship between MTB and MAC in patients with AIDS in developed countries. The remaining chapters constitute the research content, followed by a discussion and a conclusion.

1.1. CONTEXT AND RATIONALE OF THE STUDY

Disseminated *Mycobacterium avium* complex is the most common systemic bacterial infection in patients with AIDS in developed countries. The incidence is proportional to the duration and severity of immunosuppression caused by human immunodeficiency virus (HIV) infection and is intimately related to a declining CD4 cell count. Studies from Kenya, Uganda, Tanzania and the Ivory Coast have reported disseminated MAC to be uncommon in Africa despite its ubiquitous nature. Do other African countries concur with the above findings? There have been no studies from South Africa reporting the incidence of disseminated MAC in HIV-infected individuals. Is disseminated MAC underreported or underdiagnosed in Africa? Most of the studies from Africa did not measure CD4 counts: the diagnosis of AIDS was purely a clinical one. Since disseminated MAC is far more common at low CD4 counts, especially below 100 cells/mm^3, underestimation of the disease may have occurred.

It has been widely proposed that exposure to MTB boosts broad mycobacterial immunity, the reason commonly quoted for the relative lack of disseminated MAC in HIV positive patients in Africa. The protective effect of a prior history of tuberculous disease, or the presence of a baciile Calmette-Guerin (BCG) scar (indicating previous BCG vaccination), in preventing disseminated MAC in advanced HIV infection is contested. Given the substantial morbidity and mortality of both MTB and MAC in HIV-infected persons, the relationship between these infections is important. Since no studies from Africa, or areas endemic with tuberculosis (TB), have been conducted to address this interesting association, the answer to the question remains in the balance.

The clinical and laboratory features of disseminated MAC have been described predominantly in white HIV positive patients from developed countries, subsets derived

mostly from homosexuals and intravenous drug abusers. No studies from Africa have described the clinical and laboratory manifestations of disseminated MAC in the black African predominantly heterosexual population, who are generally non intravenous drug abusers. Is there concordance of clinical features? Are these features helpful in detecting disseminated MAC in our population, or should all patients with a CD4 count <100 cells/mm^3 be routinely screened with Bactec blood cultures?

What is the yield of Bactec blood cultures in detecting mycobacterial disease (MTB or MAC) in patients with advanced HIV, and should it be recommended as part of routine screening for black African patients with AIDS who have symptoms such as fever and / or weight loss?

Context and rationale of the study not referenced. See literature survey for references.

1.2 LITERATURE SURVEY

1.2.1 EPIDEMIOLOGY

Disseminated *Mycobacterium avium* complex disease is the most common bacterial infection among persons with advanced AIDS in developed countries(1), and it has been estimated that up to one fourth of all AIDS patients will acquire this infection during their lifetime(2). AIDS case surveillance between 1981 and 1987 indicated that 5.5 percent of patients with AIDS had disseminated MAC(1). This percentage has increased steadily such that by December 1990, the cumulative incidence was 7.6 percent(2). In the United States there are only small differences between regions in the frequency of disseminated MAC, and disease occurs in all demographic groups; its incidence has not been associated with gender or HIV risk factors. In Europe and Australia, the incidence is similar to that of the United States(2).

Organisms of this complex, which comprises two closely related species, *M. avium* and *M. intracellularae*, appear to have low virulence in the normal host. Of the species composing MAC, *M. avium* is most commonly associated with disease in AIDS, and serotypes 1, 4, and 8 are the most commonly isolated from symptomatic patients(3).

Before the AIDS pandemic, disseminated infection with MAC was extremely rare; by 1980 only 24 cases had been reported in the medical literature(4). These comprised patients on long term high doses of corticosteroids, patients with acute leukaemia, lymphoma, or organ transplants.

Several factors may have contributed to the increasing frequency of disseminated MAC infection in AIDS patients. First, the increased availability of mycobacterial blood cultures has made it easier to establish the diagnosis of this infection. Second, early in the AIDS pandemic, clinicians, skeptical of the efficacy of the treatment regimens then available may

not have pursued the diagnosis too aggressively. Third, the introduction of zidovudine therapy as well as improvements in diagnosis, prophylaxis, and treatment of other opportunistic infections has resulted in a substantial increase in survival of patients with AIDS at extreme levels of immunosuppression. Since disseminated MAC occurs late in the course of HIV infection (a mean of 7 to 15 months after the diagnosis of AIDS), a greater number of patients are therefore susceptible to MAC(2, 5).

Disseminated MAC is intimately related to a declining CD4 count. The product-limit incidence of MAC bacteremia at one year after the first CD4 count was reported to be 39% for CD4 cell counts <10/mm^3, 30% for 10-19/mm^3, 20% for 20-39/mm^3, 15% for 40-59/mm^3, 8% for 60-99/mm^3, and 3% for 100-199/mm^3(6). A history of a prior AIDS-defining illness has also been associated with an increased risk of disseminated MAC(6). Investigators have speculated that most HIV-infected patients will eventually become infected with MAC should they not succumb to other HIV-related illnesses.

MAC is ubiquitous and can be recovered from water, soil, food, and animal sources. Some studies implicate contaminated water in causing disseminated MAC, through ingestion or inhalation, possibly during showering. A large environmental study of specimens from the homes of patients with disseminated MAC, found that MAC isolates of soil from potted plants were likely to be of similar serotypes to those that cause disease(7). Another large case-controlled study identified the consumption of hard cheeses to be associated with increased risk of disseminated MAC, and taking daily showers to be protective(8). All of these studies were conducted in developed countries, particularly in the United States.

A study conducted in South Africa from 1968 to 1978 isolated 792 strains of MAC from healthy rural persons, lymph node lesions of swine, animal feed and bedding, plants, dust,

and soil. Although the occurrence of serotypes 1-3 was low in all sources studied, serovars 4-24 were frequently isolated from the environment(9). Therefore MAC is ubiquitous in South Africa, and other African countries(10, 11, 12), however, disseminated MAC infection in African patients with AIDS is reported to be uncommon.

MAC was recovered from none of 95 patients (all of whom were subjected to a 13A Bactec blood culture) with advanced AIDS in Kampala, Uganda even though MAC was prevalent in soil and water samples from the area(10, 11). A further study from Kampala showed that only one of 228 febrile HIV positive patients was bacteremic with MAC on Bactec blood culture(13). A Kenyan study in 1992 isolated MAC on blood culture in 6% of patients with advanced HIV and low CD4 counts (mean of 10/mm^3). MAC bacteremia was detected significantly less frequently in the study population than MTB bacteremia(14). A study done in Tanzania, in 1995, showed that of 282 febrile HIV-infected patients admitted, 58 had MTB bacteremia, while only one patient was bacteremic with MAC(15). In a large autopsy series in Cote d'Ivoire (Ivory Coast) 5401 patients were evaluated of whom 50% were HIV seropositive. MTB was seen in 54% of cadavers with AIDS-defining pathology. No atypical mycobacteria were isolated(16).

Several factors may account for the relative rarity of MAC infection in developing countries. Exposure to MAC in the environment or water supply may be less common than in developed countries due to differences in behavior or in the supply systems. For example, potable hot water and indoor pools, which are not widely available in many developing countries have been identified as sources of MAC infection among AIDS patients in the United States(17). Impoverished patients in developing countries may be dying from more virulent infections before they are immunosuppressed enough for MAC to develop. Patients with latent tuberculosis, for example, are likely to reactivate this

mycobacterial infection before they reach the low CD4 counts associated with susceptibility to MAC. Alternatively, prior or continued exposure to MTB or nontuberculous mycobacteria may be eliciting or boosting broad antimycobacterial immunity, which remains effective against MAC even in late HIV infection(14). Further support of this comes from skin-test studies, conducted among healthy subjects, which indicate that broad mycobacterial immunity (due to prior infection with MTB, environmental bacteria, or BCG) is greater in Trinidad and Kenya than in developed countries(18). Finally, many of the African studies were conducted on HIV positive febrile patients, the CD4 counts of whom were not determined, or greater than 100 cells/mm^3. Therefore the studies from Africa may be underestimating the prevalence of MAC due to bias from poor patient selection.

There have been no studies from South Africa reporting the incidence of disseminated MAC in HIV-infected individuals.

1.2.2 PATHOGENESIS

Whereas tuberculosis results largely from reactivation of a previously dormant focus, disseminated MAC results from primary acquisition of the pathogen.

In the pre-AIDS era, the most important risk factor for the acquisition of pulmonary MAC was the presence of underlying lung disease. Chronic obstructive pulmonary disease, chronic bronchitis, bronchiectasis, pneumoconiosis, healed or active tuberculosis, pulmonary mycosis and malignancy are chronic lung diseases associated with an increased prevalence of MAC. Patients who had undergone gastrectomy were also at higher risk to develop disease. Patients with cystic fibrosis who survive into the third and fourth decades of life, are also at risk for developing pulmonary MAC(19).

MAC first colonizes the gastrointestinal or respiratory tract, and systemic dissemination follows. Focal pneumonia caused by MAC may present with or without dissemination, but dissemination usually occurs. Such pneumonias are uncommon however, occurring in only 4% of patients with disseminated MAC(20).

The gastrointestinal tract is the more common portal of entry. Nausea, vomiting and watery diarrhea, are common in disseminated MAC infection. Abdominal pain and biliary obstruction may be explained by the frequency of extensive intra-abdominal lymphadenopathy. A prospective study of HIV+ve patients with CD4 counts of less than 50cells/mm^3, found that 60% of patients with positive sputum or stool culture for MAC developed disseminated infection within one year. However, both respiratory tract specimen and stool culture had poor sensitivities (22% and 20% respectively) for bacteremia, and culture of these sites has limited usefulness as a screening test(21). Essentially, a positive sputum or stool culture is not reflective of disseminated MAC, and culture from a sterile site is mandatory for the diagnosis.

Widespread dissemination occurs, with the blood, bone marrow, liver, spleen and lymph nodes being more commonly affected, although the organism has been recovered from eye, brain, meninges, cerebrospinal fluid, heart, lung, stomach, skin, tongue, thyroid, adrenals, breast, parathyroid, kidneys, prostate, pancreas, testis and urine(2).

Microscopically, tissues are filled with foamy histiocytes packed with Ziehl-Neelsen staining acid fast bacilli, akin to lepromatous leprosy, reflecting the inability of the host to mount an effective immune response. Granulomas when present, are often poorly formed, but a few patients will have a classic granulomatous response, implying that cell mediated immunity is the predominant form of protection against the organism(2). Phagocytosis of the invading organisms appears to be unimpaired (as demonstrated by the enormous

numbers of intracellular bacilli), but macrophage mediated killing is ineffective. Recent studies have implicated lymphokines, such as interleukin-2, tumor necrosis factor and granulocyte-macrophage colony-stimulating factor, in mediating the killing of mycobacteria(22). Further , a factor in the serum of healthy donors, and missing in HIV infection, inhibits the intracellular replication of MAC(23). Cytokines have bi-directional effects on the growth of MAC, and further studies are needed to address the expression of cytokine receptors on mononuclear phagocytes from patients with AIDS, as well as local fluxes of growth enhancing and growth inhibitory cytokines in infected tissues.

1.2.3 THE RELATIONSHIP BETWEEN MAC AND MTB

It is proposed that exposure to MTB boosts broad mycobacterial immunity, the reason commonly quoted for the relative lack of disseminated MAC in HIV seropositive patients in Africa(14). However, this is controversial and has been propagated and contested by two prominent groups of investigators from the United States, in two recent journal articles. CR Horsburgh et al, in a retrospective study reported that prior tuberculous disease provides protection against MAC in HIV-infected persons with CD4 counts < 200 cells/mm^3, possibly by stimulation of mycobacterial immunity. The protective effect of a recent history of a positive purified protein derivative (PPD) from acquiring disseminated MAC, however, failed to reach statistical significance (24).

Reasons offered for the latter included that tuberculosis disease provides more persistent immune stimulation than does tuberculosis infection. Secondly, INH prophylaxis was given routinely to patients in their clinic, thereby reducing immune stimulation by persistent tubercle bacilli; PPD-positive persons who do not receive INH (such as those in Africa) may therefore have protection that could not be demonstrated in their study. Thirdly, skin test results are known to be an inexact marker for protective immunity to mycobacterial

infection. Lastly, because a 5mm reaction was used to define a positive test, some persons identified as infected with mycobacteria may instead have only been sensitized to environmental mycobacteria. This paper was criticized for their inclusion of patients recently on tuberculosis treatment, as at least two of the commonly used tuberculosis drugs, rifampin and ethambutol, are known to be active against MAC; therefore these patients could have inadvertently been receiving MAC prophylaxis. Also, the ability of severely immuno-compromised patients to mount an adequate immune response against tuberculosis enough to protect them from acquiring MAC infection would be considerably impaired. In fact, second episodes of tuberculosis, by both reactivation and re-infection, in AIDS patients who live in endemic areas are not uncommon, despite the theoretical protection provided by the first infection. Accordingly, a significant protection derived from acquired immunity against another mycobacterial species is questionable(25).

In a prospective observational cohort study, Sterling et al concluded that a history of MTB infection or disease was not associated with protection against subsequent disseminated MAC disease in HIV-infected persons(26). Since protective immunity may be less likely to develop at lower CD4 counts, their results did not change significantly even when they included only those persons who developed tuberculosis when CD4 cells exceeded 100/mm. In fact, persons with extra-pulmonary tuberculosis were at increased risk for disseminated MAC, particularly at low CD4 levels. Other immunologic factors, including decreased production of interferon in response to mycobacterial antigens may have contributed to the latter result.

Given the substantial morbidity and mortality of both MTB and MAC in HIV-infected persons, the relationship between these infections is important.

1.2.4 CLINICAL FEATURES

Elucidation of the clinical syndrome caused by MAC in patients with AIDS was initially hampered by the perception that MAC was merely a colonizer and did not contribute to morbidity, and that no effective therapeutic regimens were available. However, evidence has demonstrated that disseminated MAC makes a significant contribution to morbidity in AIDS, and that anti-mycobacterial chemotherapy is associated with amelioration of symptoms, and with increased survival(2).

The following clinical features have been described predominately in white HIV+ve patients from developed countries, subsets derived mostly from homosexuals and intravenous drug abusers.

A prodrome can be identified 3 months before the first positive blood culture. Fever, drenching night sweats and weight loss are the hallmark of disseminated MAC in AIDS patients. Diarrhea, malaise and anorexia are frequently present. Widespread involvement of the reticuloendothelial system is common and results in hepatomegaly, splenomegaly and lymphadenopathy. Anemia may be profound necessitating frequent blood transfusions, and elevated alkaline phosphatase indicates an increased bacterial burden of MAC in liver and bone marrow. An elevated serum LDH level has also been associated with disseminated MAC, a marker previously noted to be a harbinger of *Pneumocystis carinii* pneumonia(27).

A study from Taiwan compared the clinical features of disseminated tuberculosis and disseminated MAC in patients with AIDS. In their analysis, hepatosplenomegaly, leukopenia, elevated serum alkaline phosphatase and γ-glutamyl transpeptidase favored the diagnosis of disseminated MAC over disseminated MTB(28). Although these clinical and laboratory manifestations are non specific, and may be confused with other AIDS defining illnesses, and with advanced HIV itself, the occurrence of these symptoms in the setting of

a patient with a CD4 count <100cells/mm³ provides some handle to the diagnosis of disseminated MAC, and Bactec blood cultures should then be undertaken.

In contrast to non-AIDS patients, MAC is rarely pathogenic in the lungs. Diffuse interstitial infiltrates, nodular lesions, endobronchial lesions, and adenopathy have been described(29). Distinguishing MAC from MTB is not possible radiographically in advanced HIV-infected patients; only cultural confirmation can definitively separate the two. The identification of AFB in the sputum even in the absence of radiographic findings mandates empirical therapy for MTB because it is not conclusively possible to distinguish these two mycobacteria morphologically and MTB is much more common in this setting.

No studies from Africa have described the clinical and laboratory manifestations of the black African predominantly heterosexual population, who are generally non intravenous drug abusers.

1.2.5 DIAGNOSIS

The diagnosis of disease due to MAC requires isolation of the organism and compatible clinical features. Colonization can occur in both normal and immune-compromised hosts and must be distinguished from true disease because of therapeutic implications. In contrast to the more virulent MTB, the identification of MAC in an isolated sputum culture does not constitute definite evidence of disease. MAC can colonize healthy persons and has a propensity for colonizing patients with underlying pulmonary disease. The presence of radiographic changes compatible with MAC, and not attributed to another disease process (i.e. tuberculosis or fungal disease) and two or more positive sputum or bronchial washings with moderate to heavy growth of MAC is considered sufficient evidence of MAC disease (to be differentiated from disseminated MAC)(30). Similarly a positive stool culture for

MAC does not imply disease. For a diagnosis of disseminated MAC to be made, isolation of the organism from a sterile site is required.

Blood cultures are highly sensitive for the detection of disseminated MAC in AIDS patients. A single blood culture has a high diagnostic yield (90-95% sensitive), subsequent cultures provide marginal improvement in detection and should be used in undiagnosed cases when symptoms are suggestive of MAC(31). Tissues such as bone marrow, liver, and lymph nodes may be infected before bacteremia is persistent, so biopsy and culture of these tissues may be useful in the early detection of disseminated MAC(32).

In the Bactec radiometric system, 5mls of blood from the patient is inoculated into a 13A culture media which contains a radiolabeled substrate that is metabolized in the presence of mycobacteria to carbon dioxide and detected by radiorespirometric methods. In AIDS patients, a growth signal can usually be detected within 8-14 days although specimens with low colony counts may require up to 4 weeks(19). Once sufficient growth is achieved, the diagnosis of MAC can be made in a few hours using DNA probes that hybridize specifically with the RNA of the organism. Polymerase chain reaction assays are also available to detect MAC directly in clinical specimens.

False positive cultures due to contaminated laboratory reagents have been reported.

1.2.6 PROGNOSIS

When disseminated MAC disease was first recognized in AIDS, it was unclear whether it contributed to mortality or was simply a marker of profound immunosuppression. This question and the lack of drugs with clinical efficacy led to apathy; clinicians did not believe that diagnosis and treatment of disseminated MAC were worthwhile. Horsburgh et al changed this perception after publishing their large retrospective study of 1011 AIDS

patients, demonstrating shortened survival (median of 4 months) in those patients with disseminated MAC when compared with matched controls (median < .1 months)(33). Further, Chin, et al demonstrated that MAC bacteremia was independently associated with increased risk of death and that treatment prolongs survival(34). If left untreated the course of disseminated disease is progressive clinical deterioration. The shortened survival is thought to be due to severe weight loss and immune suppression, caused by malnutrition, which hastens death from other infections. In other cases death simply results from inanition(2).

1.2.7 TREATMENT

Treatment of disseminated MAC with at least a two or three drug regimen including clarithromycin or azithromycin is recommended(35). Ethambutol is often chosen as the second agent, together with one or more of the following as third or fourth agents: clofazimine, rifabutin, rifampin, ciprofloxacin, and sometimes amikacin. Therapy is lifelong. Practitioners, or patients may elect to stop treatment if there is failure to respond clinically or microbiologically.

1.2.8 PREVENTION

Recent analyses demonstrated that rifabutin prophylaxis extends the quality of life and prolongs survival, as well as being cost effective. Rifabutin prophylaxis is recommended in patients with HIV infection with a CD4 count <100cells/mm^3 It should be continued indefinitely unless treatment for disseminated infection becomes necessary(35). Clarithromycin has been approved by the Food and Drug Administration as the second prophylactic agent for MAC.

1.3 PURPOSE OF THE STUDY

Part 1. To determine the number of cases of disseminated MAC in adults diagnosed per year over a 5 year period from 1994 to 1998 in a large black South African hospital in an attempt to assess the magnitude of the disease, and to correlate this with the prevalence found in part 2.

To determine in a retrospective study, the clinical and laboratory manifestations of black South African patients diagnosed with disseminated MAC.

Part 2. To determine in a prospective study of 100 patients who are HIV positive with a CD4 cell count of less than 100/mm^3, the prevalence of MAC in advanced retroviral disease in South Africa. The interrelationship of MTB with disseminated MAC will also be evaluated in patients from this part of the study

Part 3. To determine, in a country endemic with tuberculosis, if a prior history of tuberculous disease, or the presence of a BCG vaccination scar, protects against disseminated MAC in patients with AIDS.

Part 4. To compare the clinical and laboratory manifestations of patients with AIDS and disseminated MAC to two groups of patients, matched for age and sex and with a CD4 count of less than 100/mm^3. The latter two groups are as follows:

- Patients with AIDS and tuberculosis (organ or disseminated)
- Patients with AIDS only.

This is an attempt to separate the symptom complex of black South African patients with AIDS and disseminated MAC from matched groups (with and without MTB disease).

2 MATERIALS AND METHODS

2.1 OVERVIEW

The study has two arms: the retrospective and prospective arms.

There are four parts to the study. Parts 1 and 2 are descriptive, while parts 3 and 4 are comparative.

2.2 DETAILED DESCRIPTION

Part 1. Retrospective arm.

The number of patients diagnosed with disseminated MAC annually at the C.H.B.H. microbiology laboratory from 1994 to 1998 was recorded retrospectively.

All MAC culture positive specimens obtained from sterile sites from the beginning of 1997 to the end of 1998 were evaluated and patient files studied for clinical and laboratory details. Sterile culture sites analyzed were bone marrow, blood, pleural fluid, urine, cerebrospinal fluid, lymph node aspirates, and peritoneal fluid. Clinical and laboratory details included, where possible, the age, sex, HIV status, CD4 cell count, the presence of splenomegaly, anemia[1], leukopenia[2], thrombocytopenia[3], pancytopenia[4], the absolute lactate dehydrogenase and alkaline phosphatase values, the sterile site where MAC was isolated, and outcome.

Survival was evaluated after 12 months, from the date the specimen was received at the laboratory. Information on outcome was obtained through reports from families, from patient files, and from the Department of Home Affairs.

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[1] Anemia defined as Hb <10g/dl
[2] Leukopenia defined as WCC <4 x 10^9/L
[3] Thrombocytopenia defined as platelet count <100 x 10^9/L
[4] Pancytopenia defined as all of the above

Inclusion criteria:

- Patients who are sterile site culture positive for MAC.
- Adult black South Africans attending C.H.B.H. from 1997-1998.

Part 2. Prospective arm.

Patients identified with clinically advanced retroviral disease who were either under my care, identified by other doctors in my ward, or simply admitted to the adult general medical unit in which I was working at C.H.B.H, were approached for the study. After verbal consent, I interviewed all patients (and / or relatives), performed a physical examination and recorded their data. A Bactec blood culture and CD4 count (if not done within 1 month) was obtained from all patients. Five milliliters of blood was inoculated into a Bactec blood culture bottle either by myself or by a trained phlebotomist using sterile technique with alcohol swabbing. Blood cultures were followed up for growth using the Bactec system. All blood culture positive specimens underwent routine morphological examination and Gen-Probing to determine the mycobacterial species.

One hundred patients meeting the inclusion criteria were studied.

Inclusion criteria:

- Patients who are HIV+ve.
- Patients with a CD4 count <100 cells/mm^3.
- Adult black South Africans admitted to C.H.B.H.

Exclusion criteria:

- Patients refusing or unable to give consent.
- Patients less than 18years of age.

Part 3. Is MTB protective of MAC in patients with AIDS?

Patients were derived from the prospective and retrospective arms.

The proportion of HIV+ve patients with a prior history of tuberculous disease, infected with disseminated MAC (derived from the retrospective and prospective arms) was compared to the proportion of HIV+ve patients with a prior history of tuberculous disease but not infected with MAC (derived from the remainder of the prospective arm; the control).

Patients who were currently receiving TB therapy were counted as not having a prior history of tuberculous disease, unless they had tuberculosis prior to their current event. This is so, as to count only those persons who have developed immunity to MTB from a prior disease, in order to determine if there is 'cross-over' immunity to MAC.

From the same comparative groups, the proportion of HIV+ve patients with a visible BCG scar and infected with disseminated MAC, was compared to the proportion of HIV+ve patients with a visible BCG scar but not infected with MAC.

Exclusion criteria:

- Patients who are HIV negative.
- Patients on whom clinical data was insufficient (regarding a prior history of tuberculosis or a visible BCG scar).

Part 4. Clinical and laboratory manifestations.

Patients derived from the prospective and retrospective arms.

The clinical and laboratory manifestations of patients with AIDS and disseminated MAC (derived from the retrospective and prospective arms), were compared to the

clinical and laboratory manifestations of two groups of patients (derived from the remainder of the prospective arm).

The latter two groups of patients were divided as follows:

- Patients with AIDS (CD4 count < 100cells/mm^3) and tuberculosis (organ or disseminated)
- Patients with AIDS (CD4 count < 100cells/mm^3) and no mycobacterial disease (control).

All groups were matched for age (p = 0.33) and sex (p = 0.16) using nonparametric statistical analysis (Kruskal-Wallis analysis of variance by ranks).

The clinical and laboratory manifestations included weight loss[5], drenching night sweats, fever[6], diarrhea[7] abdominal pain, oral candidiasis, significant generalized lymphadenopathy, hepatomegaly, splenomegaly, anemia, leukopenia, thrombocytopenia, pancytopenia, CD4 count, alkaline phosphatase and lactate dehydrogenase values.

Exclusion criteria:

- Patients who are HIV-ve.
- Patients co-infected with MTB and disseminated MAC.

2.3 SETTING

Chris Hani Baragwanath Hospital is a 3300 bed public sector university hospital serving Soweto and surrounding communities. There is an average of 2500 medical admissions per

[5]Weight loss defined as > 10% loss of body mass over 6 months.
[6] Fever defined as temperature >37.5 degrees Celsius.
[7] Diarrhea defined as three or more loose or watery stools per day.

month. In March 1997, 26% of all medical admissions were HIV positive (persona communication, Dr A Karstaedt).

2.4 STUDY POPULATION

Part 1. All patients diagnosed with disseminated MAC at C.H.B.H. over two years from 1997-1998.

Part 2. Patients identified with clinically advanced retroviral disease who were either under my care, identified by other doctors in my medical ward, or simply admitted to the adult general medical unit in which I was working at C.H.B.H. in 1998.

Part 3. Patients derived from parts 1 and 2 who were HIV seropositive.

Part 4. Patients derived from parts 1 and 2 who were HIV seropositive and were not co-infected with MAC and MTB.

2.5 LABORATORY METHODS

Isolation of mycobacteria was performed using either the Bactec radiometric system (using the Bactec 13A vials) or the Bactec 9000MB system (using the Bactec MYCO/F LYTIC vials). Bactec 13A contains the Middlebrook 7H13 culture medium, which contains a radio-labeled substrate that is metabolized in the presence of mycobacteria to $^{14}CO_2$, and detected by radiometric methods(36). Bactec MYCO/F LYTIC culture medium is a Middlebrook 7H9 and brain heart infusion broth formulation with a sensor which can detect decreases in oxygen concentration resulting from microorganism metabolism and growth. The sensor is monitored by the Bactec 9000MB system for increasing fluorescence which is proportional to the decrease in oxygen.

Positive Bactec cultures were confirmed by morphological examination of Ziehl-Neelson stained smears from these vials. Gen-Probing was performed on all confirmed positive cultures to determine the mycobacterial species. Gen-Probe is a rapid DNA probe test,

which utilizes the technique of nucleic acid hybridization for the identification of mycobacteria isolated from culture. After ribosomal RNA is released from the organism, the complementary chemiluminescent labeled single stranded DNA probe combines with the target organism's ribosomal RNA to form a stable DNA:RNA hybrid. The labeled hybrid is then measured in the Gen-probe luminometer (Gen-Probe, San Diego, California, USA).

2.6 DATA ANALYSIS

Statistical analysis was performed using Statsoft, 1997, statistica for windows, statistical software. A Non parametric test (Kruskal-Wallis analysis of variance by ranks) was used for comparison of groups. Categorical variables were compared using the two tailed Fisher exact chi squared test. A P value of < 0.05 was considered significant.

2.7 ETHICAL ISSUES

Prior to undertaking the study, the protocol was submitted to, and approved by the Committee for Research on Human Subjects (medical), University of the Witwatersrand, Johannesburg (Appendix A).

Recruitment of patients in part 2 of the study was guided by the following ethical principles:

- An information sheet was provided to all patients briefly explaining the purpose of the study, and the implications for them (appendix B).
- If unable to read, the contents were verbally explained.
- Patients were assured about anonymity.
- It was voluntary for the patients to participate in the study.
- Verbal consent was sought.

3 RESULTS

Part 1. Retrospective arm.

There were a total of 58 cases of disseminated MAC diagnosed at C.H.B.H. from 1994 to 1998. The number of cases increased exponentially over the years as depicted in figure 1. All cases of disseminated MAC were included irrespective of their HIV status.

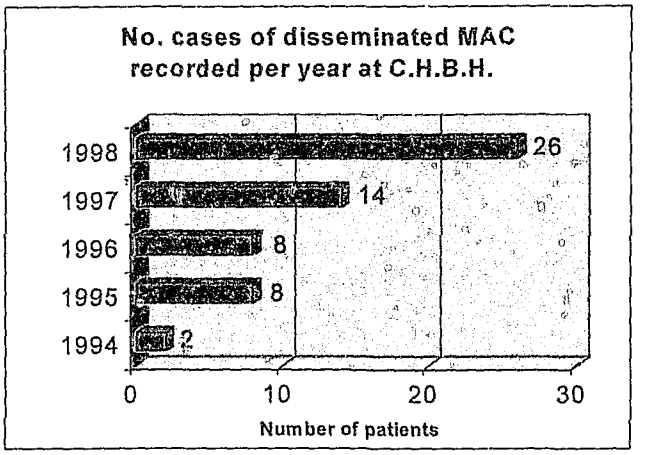


Figure 1.

The HIV status of patients with disseminated MAC diagnosed at C.H.B.H. from 1997 to 1998 was determined. Thirty three patients were HIV seropositive, 3 patients were HIV seronegative, and the HIV status of 4 patients was unknown as depicted in figure 2.

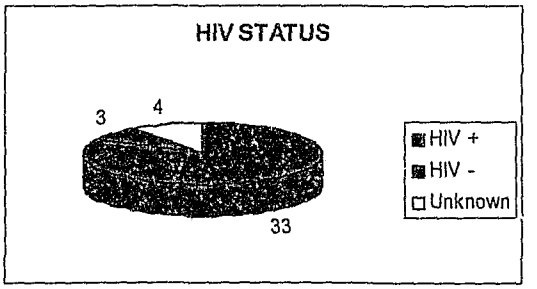


Figure 2.

Those patients with disseminated MAC who were HIV -ve had the following diagnoses:

1. Portal hypertension and hypersplenism.
2. Non Hodgkins lymphoma.
3. Bronchiectasis.

HIV testing was not conducted on four cases as there were no clinical features of retroviral disease. Their HIV status is presumed negative, and their final diagnoses were as follows:

1. Hepatocellular carcinoma and haemosiderosis.
2. Diabetic ketoacidosis.
3. Cirrhosis with liver failure.
4. Gunshot chest and abdomen with paraplegia. Admission included a protracted ICU stay

The age distribution was skewed to the left showing a preponderance of young adults with advanced HIV and MAC (median: 38years, range: 20-65years) as depicted in figure 3. Patients who were HIV seronegative tended to be older (median of HIV seropositive patients: 33years, median of HIV seronegative patients: 46years)

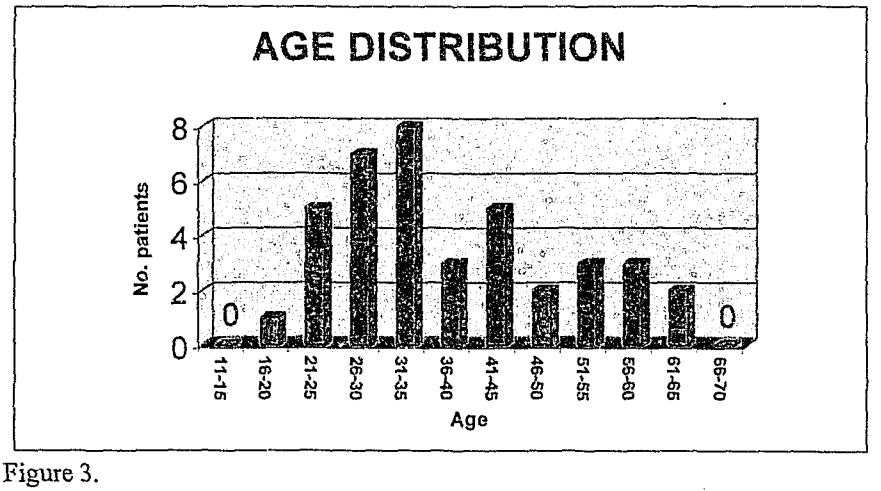


Figure 3.

There was no significant difference in the sex ratio among patients with disseminated MAC as depicted in figure 4.

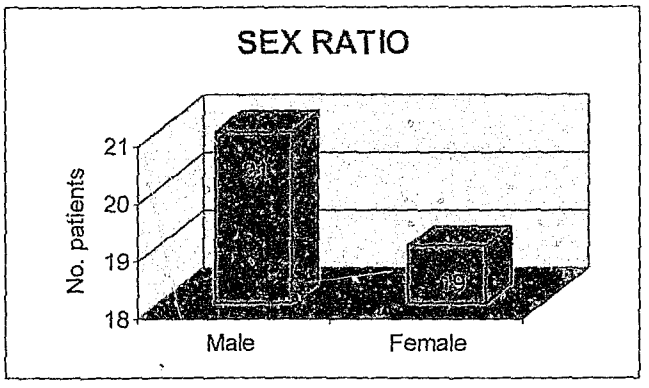


Figure 4.

CD4 counts of the HIV positive patients with disseminated MAC are illustrated in figure 5. Twenty two out of 27 patients tested (81%) had CD4 cell counts below 50/mm³ and 17 out of 27 patients (63%) had CD4 counts below 25/mm³ (mean: 43, median: 19, range: 0-457).

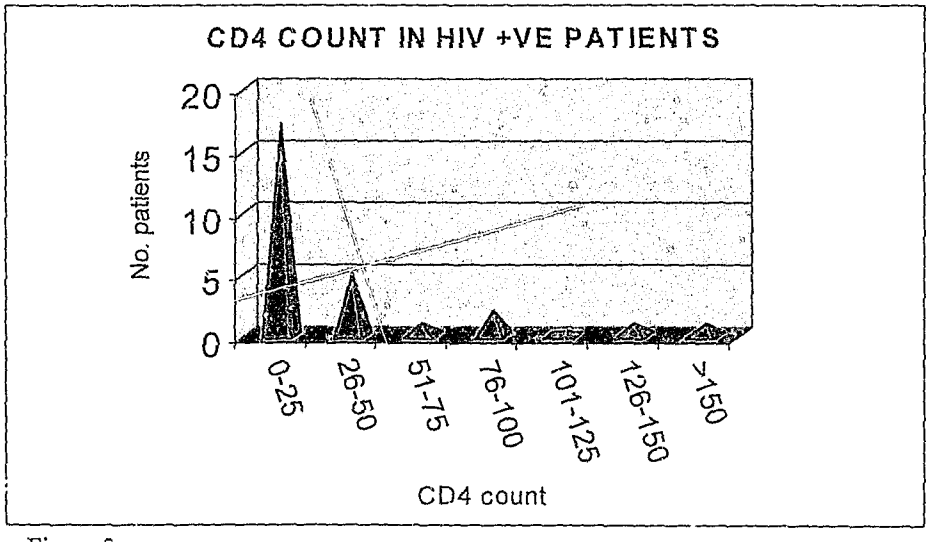


Figure 5.

Splenomegaly occurred in 7 out of 38 patients (18%) as depicted in figure 6.

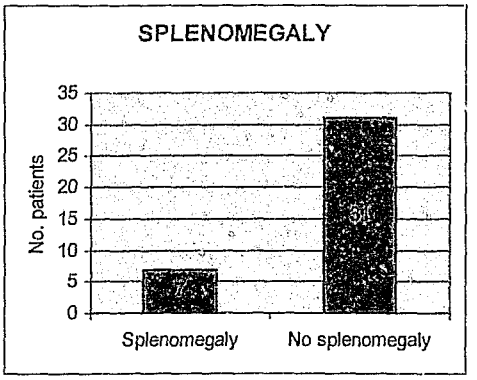


Figure 6.

Anemia[1] occurred in 70%, leukopenia[2] in 50%, thrombocytopenia[3] in 40%, and pancytopenia[4] in 20% of patients with disseminated MAC, as shown in figure 7.

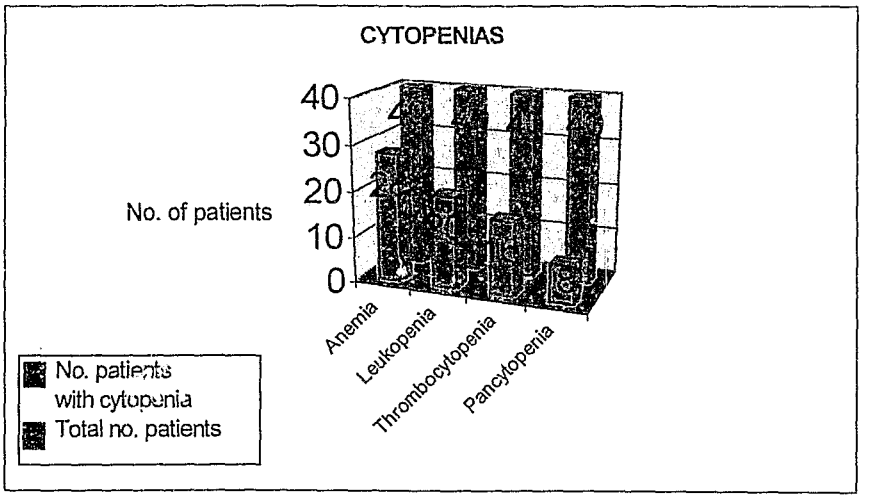


Figure 7.

[1] Anemia defined as Hb <10g/dl.
[2] Leukopenia defined as white cell count $<4x10^9/L$.
[3] Thrombocytopenia defined as platelet count $<100x10^9/L$.
[4] Pancytopenia defined as all of the above.

The range, median, and mode of lactate dehydrogenase, and alkaline phosphatase values of patients with disseminated MAC are represented in figures 8 and 9 respectively.

Range, median, and mode of lactate dehydrogenase[1]

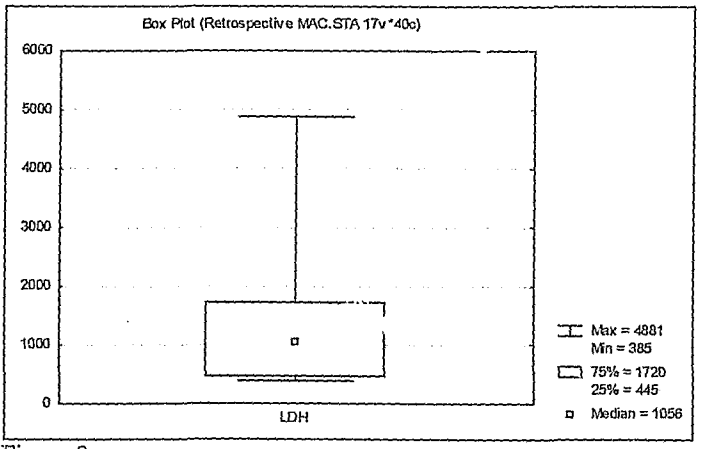


Figure 8.

Range, median, and mode of alkaline phosphatase[2]

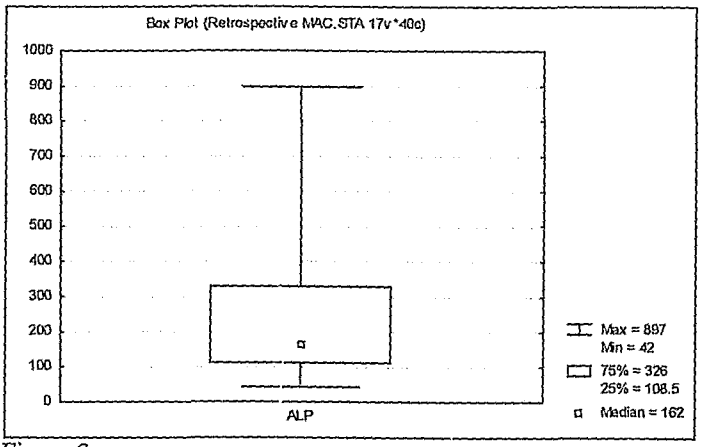


Figure 9.

[1] Normal reference range: 100-430U/L
[2] Normal reference range: 40-120U/ml

From the 40 patients, MAC was cultured from a number of sterile sites as depicted in figure 10. Fourteen cultures were from bone marrow, 17 from blood, 5 from pleural fluid, 2 from urine, 3 from cerebrospinal fluid, 2 from lymph node aspirates and 1 from peritoneal fluid. Four patients cultured MAC from two sterile sites.

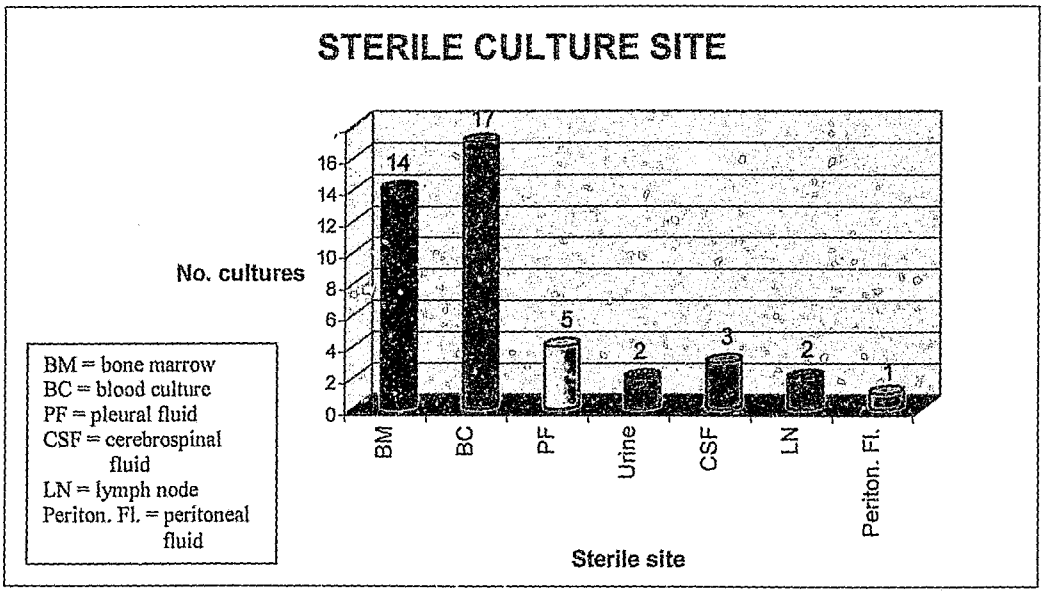


Figure 10.

Patient outcome after 12 months from the date the specimen was received at the laboratory is recorded in figure 11. Twenty-six patients had died, 2 were alive, and 12 were lost to follow up. Two patients died during their hospital admission. Of the survivors, both were on treatment for disseminated MAC.

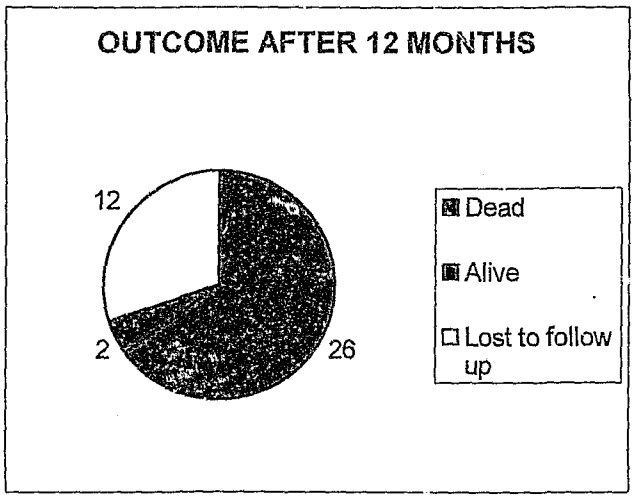


Figure 11.

Part 2. Prospective arm

Mycobacterial blood culture results.

Two hundred and six patients were entered into the study. One hundred and six patients were excluded on the basis of their HIV status or high CD4 count. Of the remaining 100 patients, 29 grew mycobacteria on their blood cultures. Twenty patients were bacteremic with MTB, and seven with MAC. Co-infection with both organisms occurred in a further two patients as depicted in figure 12.

BACTEC BLOOD CULTURES

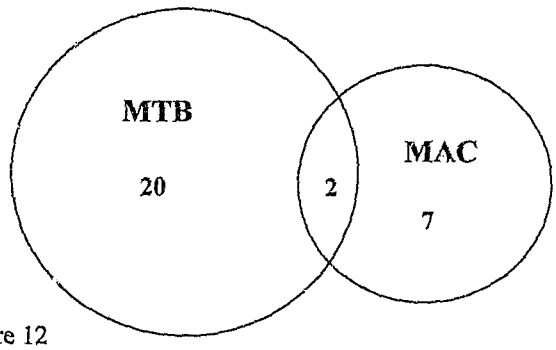
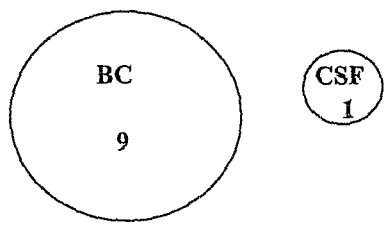


Figure 12

Sterile site cultures of MAC

Disseminated MAC was diagnosed on cerebrospinal fluid Bactec culture in one more patient from the study as depicted in figure 13. Ten patients out of 100 were finally diagnosed with disseminated MAC

TOTAL MAC CULTURE POSITIVES



KEY: BC = blood culture
CSF = cerebrospinal fluid

Figure 13

The interrelationship of MAC and MTB

MTB was diagnosed in 58 patients by a number of investigations, the relative importance of these is depicted in figure 14.

TOTAL MTB CULTURE POSITIVES

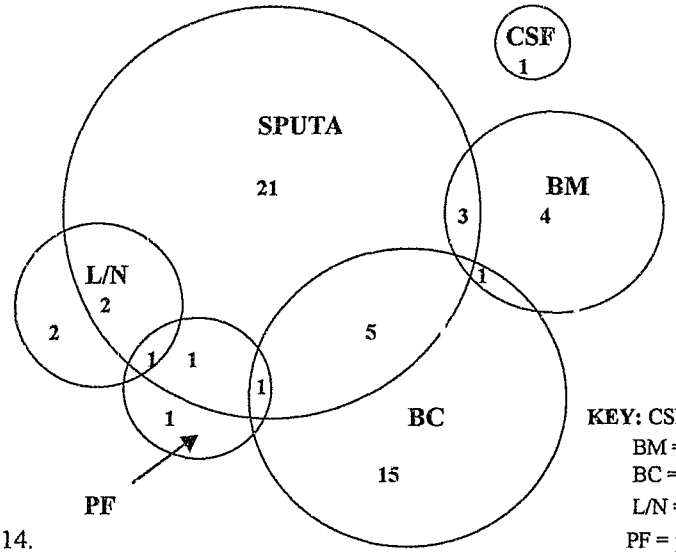


Figure 14.

The relative importance of all investigations in diagnosing mycobacterial disease in HIV seropositive patients with CD4 cell counts < 100/mm is depicted in figure 15.

COMBINED MAC & MTB CULTURE POSITIVES

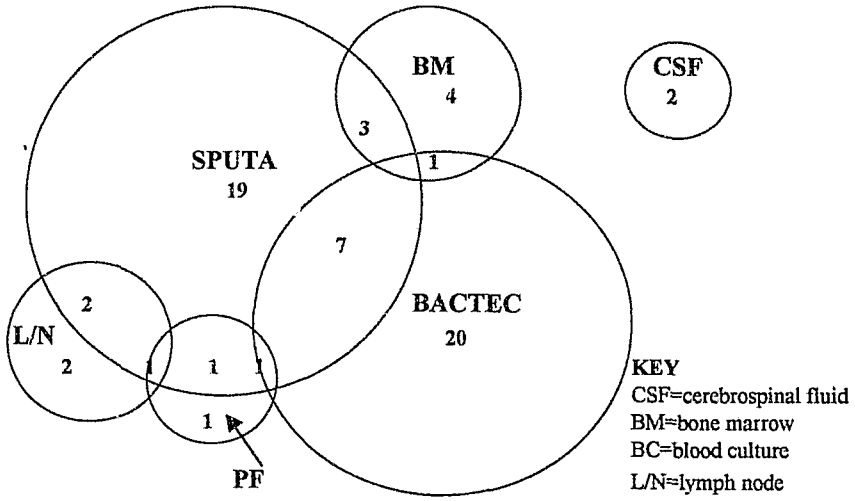


Figure 15

Part 3. Is MTB protective of MAC in patients with AIDS?

There were a total of 33 patients diagnosed with disseminated MAC and advanced retroviral disease at C.H.B.H. in 2 years from 1997 to 1998.

- Three out of 26 patients (seven patients were excluded due to lack of data) had a previous history of tuberculous disease. Two of these patients were diagnosed with tuberculosis 10years before, and one patient 9 months before, the onset of disseminated MAC. Compared to 17 out of 84 patients from the prospective arm of the study (control), this was shown **not** to be statistically significant ($p = 0.39$), implying that a prior history of tuberculous disease was **not** protective of disseminated MAC in patients with AIDS (Table 1).
- Three out of 10 patients (low numbers as data could not be extracted from the retrospective arm of the study) with disseminated MAC had a visible BCG scar compared to 32 out of 62 patients from the prospective arm of the study (control). This was shown not to be statistically significant ($p = 0.31$), implying that BCG is not protective of MAC in patients with AIDS. (Table 1)
- Co-infection with MTB and MAC occurred in 12 of the original 33 cases.
- Despite being on TB treatment, one patient (from the prospective arm) tested Bactec blood culture positive for MAC. Similarly, one patient who defaulted TB treatment 5 months before, tested Bactec blood culture positive for MAC.

Table 1. A prior history of tuberculosis or BCG vaccination and its protection against disseminated MAC.

	No. patients with MAC	Control	P value
Prior history of tuberculosis	3 (11.5%)	17 (20%)	0.39
No prior history	23 (88.5%)	67 (80%)	
BCG scar	3 (30%)	32 (52%)	0.31
No BCG scar	7 (70%)	30 (48%)	

(P value obtained using the two tailed Fisher exact, chi squared test)

Part 4. Clinical and laboratory manifestations.

The clinical and laboratory manifestations of HIV+ve patients with disseminated MAC were similar to those of patients with advanced HIV and tuberculous disease, and again similar to those of patients with simply advanced HIV. Exceptions included the presence of diarrhea, leukopenia, and the absence of lymphadenopathy, which were all associated with a positive blood culture for MAC compared to MTB (Table 2). Statistical analysis was performed using the two-tailed Fisher exact chi squared test.

Using non-parametric tests (Kruskal-Wallis analysis of variance by ranks), patients from the MAC group had statistically lower mean white cell counts ($p < 0.05$) compared to the other two groups (table 3). Similarly, patients from the MAC group had statistically lower mean CD4 counts ($p = 0.014$, Kruskal-Wallis analysis of variance by ranks).

Of interest, the CD4 counts of those patients with MAC who were co-infected with MTB were statistically higher ($p = 0.0014$) than those who were not co-infected (non- parametric Mann-Whitney U test). Mean of co-infected group: 92/mm^3. Mean of MAC group: 19/mm^3.

Table 2. Clinical and laboratory features of patients with disseminated MAC compared to two control groups, one with MTB and one without mycobacterial disease.

Clinical features	No. with MAC	No. with MTB	p value	No MAC nor MTB	P value
Weight loss					
Yes	21	54		36	
No	0	0	1	0	1
Fever					
Yes	15	41		22	
No	3	12	0.74	12	0.2
Night sweats					
Yes	7	37		21	
No	5	14	0.48	13	1
Diarrhea					
Yes	13	15		15	
No	1	19	**0.0029**	9	0.059
Abdominal pain					
Yes	6	12		7	
No	2	11	0.11	14	0.09
Oral candida					
Yes	16	42		28	
No	2	10	0.7	6	0.7
Lymphadenopathy					
Yes	0	15		8	
No	17	38	**0.014**	28	**0.014**
Hepatomegaly					
Yes	8	21		13	
No	11	33	1	23	0.77
Splenomegaly					
Yes	3	4		3	
No	16	50	0.37	33	0.4
Laboratory features					
Anemia					
Yes	16	40		23	
No	5	14	1	13	0.38
Leukopenia					
Yes	14	18		15	
No	7	36	**0.018**	21	0.25
Thrombocytopenia					
Yes	6	10		9	
No	15	44	0.36	27	0.76
Pancytopenia					
Yes	3	5		6	
No	18	49	0.67	30	1

Table 3. Cytopenias and other laboratory values in patients with disseminated MAC compared to two control groups, one with MTB and one without mycobacterial disease.

	MAC	MTB	HIV only	P value
Hb(n)	21	54	36	
Hb(mean)	7.9	8.6	9.2	0.38
WCC(n)	21	54	36	
WCC(mean)	3.9	6.0	5.7	**<0.05**
Plat(n)	21	54	36	
Plat (mean)	158	184	201	0.33
LDH(n)	5	10	6	
LDH(mean)	1451	1180	1309	0.25
ALP(n)	11	26	10	
ALP(mean)	238	150	177	0.85

4 DISCUSSION

Disseminated MAC is reported to be the most common systemic bacterial infection in patients with AIDS in developed countries. Conversely, studies from Africa have shown disseminated MAC infection in AIDS patients to be uncommon, despite its ubiquitous nature on the continent. This study showed that the total number of patients with disseminated MAC diagnosed per year at C.H.B.H. has increased exponentially from 1994 to 1998, and that the prevalence of disseminated MAC in black South African patients with advanced retroviral disease (CD4 counts < 100/mm^3) is 10%, a prevalence similar to that of first world countries(1,2).

The actual number of cases of disseminated MAC recorded per year at C.H.B.H. is a gross underestimation of the extent of the disease seen at our hospital. Ten of the 26 patients diagnosed with disseminated MAC in 1998 were from the prospective arm. These patients were sporadically derived from one medical ward out of five over a twelve month period. They represented only a small fraction of patients with advanced retroviral disease seen in one year at C.H.B.H., and yet contributed almost 40% to the number of cases of disseminated MAC diagnosed in that year. The reason for underdiagnosis of the infection is probably a combination of ignorance among doctors of the disease, apathy in performing Bactec blood cultures, and therapeutic nihilism; doctors are pessimistic in managing a disease in patients with advanced HIV for which treatment is prolonged, expensive, and only marginally prolongs life in a country that has many other pressing primary health care priorities.

A prior history of tuberculous disease and the presence of a BCG scar was shown in this study not to be protective of disseminated MAC. South Africa is a developing country, is endemic with tuberculosis, and yet disseminated MAC occurs in our population with a

prevalence similar to developed countries where tuberculosis is not endemic. These important findings refute claims that exposure to tuberculosis boosts broad mycobacterial immunity, and protects against MAC disease.

Reasons why other African countries record low prevalence rates of disseminated MAC need to be fully elucidated. Studies conducted in Africa on the prevalence of disseminated MAC were mostly done on patients without CD4 counts, or on patients with a wide range of CD4 counts. This may have led to an underestimation of the prevalence of MAC in AIDS patients in Africa. Another possible explanation is that exposure to MAC in the environment or water supply may be less common in certain African populations due to differences in behavior or differences in the water supply systems compared to patients from developed countries. Alternatively, impoverished patients in developing countries may be dying from more virulent infections before they are immunosuppressed enough for MAC to develop. Patients with latent tuberculosis, for example, are likely to reactivate this mycobacterial infection before they reach the low CD4 counts associated with susceptibility to MAC.

The retrospective study revealed that up to 17.5% of patients with disseminated MAC were HIV negative, a figure disproportionately high due to the relatively low numbers in the HIV positive group. Few cases of disseminated MAC have been reported prior to the HIV pandemic (only 24 cases in total up to 1980). Therefore, 7 cases over two years is a significant finding, and is a representation of the volume of samples processed by our laboratory at C.H.B.H. All of the HIV negative patients had an underlying illness that rendered them in some way severely immunocompromised.

The age distribution of patients with disseminated MAC was skewed to the left showing a preponderance of young adults with advanced HIV. Patients who were HIV negative tended to be older.

There was an equal sex ratio reflecting the epidemiology of HIV in Africa being prevalent in a heterosexual population. Most studies from developed countries concerning disseminated MAC were conducted predominantly in the homosexual white male and intravenous drug abuser subsets.

Eighty one percent of patients tested in the retrospective study had CD4 counts below 50/mm^3, and 63% of patients had CD4 counts below 25/mm^3. This conforms to reports of disseminated MAC being intimately related to a declining CD4 count, signifying severe immunosuppression. Of interest, CD4 counts of those patients with MAC who were co-infected with MTB were statistically higher ($p = 0.0014$) than those who were not co-infected (non- parametric Mann-Whitney U test which compensates for extremes of ranges). Since overwhelming tuberculosis is known to cause immunosuppression in its own right, HIV positive patients with tuberculosis may be susceptible to MAC at higher CD4 counts. Thus, it is probably not the absolute CD4 count that is singularly important for the acquisition of disseminated MAC, but one's overall level of immunity. One patient with disseminated MAC had an unusually high CD4 count of 457cells/mm^3. Since this patient was also diagnosed with tuberculous meningitis, this may account for the above finding. Unfortunately this patient was part of the retrospective study, and her result cannot be confirmed.

The retrospective study shows the positive correlation of splenomegaly, anemia, leukopenia, thrombocytopenia, and elevated alkaline phosphatase and lactate dehydrogenase levels in patients with disseminated MAC. However, compared to

hospitalized HIV positive patients (with and without MTB) matched for age, sex, and with CD4 counts of less than 100cells/mm^3, these associations were not specific for disseminated MAC, and may simply reflect features of advanced immunosuppression. One exception was leukopenia whereby using nonparametric tests (Kruskal-Wallis analysis of variance by ranks), patients from the MAC group had statistically lower mean white cell counts ($p < 0.05$) compared to the other two groups (table 3). However, since the CD4 counts from the MAC group were statistically lower on entry of patients to the study, leukopenia may simply reflect sample bias, i.e. it is not disseminated MAC that causes leukopenia, but rather MAC disease occurs in patients who are leukopenic. The presence of diarrhea, and the **absence** of lymphadenopathy, were the only other clinical features shown in the study to be statistically associated with a positive blood culture for MAC using the two tailed Fisher exact chi squared test (Table 2). A recent study from Taiwan also showed the absence of lymphadenopathy, in patients with disseminated MAC, to be statistically significant compared to those with disseminated MTB who had similar levels of immunosuppression(28). A possible explanation for this is that the architecture of lymph nodes breaks down in patients with advanced HIV infection and patients subsequently loose their lymphadenopathy(37). Absence of lymphadenopathy in patients with disseminated MAC may therefore simply be a manifestation of AIDS. Apart from the presence of diarrhea and the absence of lymphadenopathy, the clinical and laboratory manifestations were largely unhelpful in making a diagnosis of disseminated MAC in hospitalized black African patients with AIDS. Bactec blood culture is the only reliable investigation, and is thus recommended in the appropriate clinical setting of HIV positive patients with CD4 counts less than 100cells/mm^3.

Contrasting this, in white HIV positive patients from developed western countries, subsets derived mostly from homosexuals and intravenous drug abusers, fever, drenching night sweats and weight loss were found to be the hallmark of disseminated MAC. Diarrhea, malaise and anorexia have been reported to be frequently present. Widespread involvement of the reticuloendothelial system resulting in hepatomegaly, splenomegaly and lymphadenopathy are clinical signs that occur with statistical significance. Profound anemia necessitating frequent blood transfusions, and elevated serum alkaline phosphatase and lactate dehydrogenase levels are further indicators of disseminated MAC in this population subset(21, 26). Reasons why these clinical and laboratory features were not as prevalent in the population studied may reflect ethnic differences, although it probably relates to the two control groups used in this study. Patient controls from other studies were recruited from out patient centers (presumably non-acutely ill), whereas not only were our two control groups recruited in hospital (predominantly acutely ill), but also the one control group by definition had MTB which may mimic many of the clinical and laboratory features of disseminated MAC. Many of the patients from the remaining control group were acutely ill from a variety of other illnesses including acute community acquired pneumonia, *Pneumocystis carinii* pneumonia, meningitis, septicemia, gastroenteritis, pelvic inflammatory disease, Kaposi's sarcoma, non specific manifestations of which may overlap with disseminated MAC.

The prospective arm showed that a single Bactec blood culture, taken from hospitalized black South African AIDS patients with CD4 counts of < 100cells/mm^3, detected mycobacterial disease in 29% of cases. This represents a period prevalence of 29% extending over the study period. Bactec blood culture is therefore an extremely efficient,

cost effective and simple investigation in the above clinical context. MTB was diagnosed in 20%, MAC in 7% and co-infection with both organisms in a further 2% of cases, using this investigation alone. These figures are consistent with data from African studies that show MTB to be the most common cause of septicemia in advanced retroviral disease, although they reported much lower prevalence rates of disseminated MAC(5, 6).

Of these 100 HIV positive patients with CD4 cell counts less than 100/mm^3 a further single case of disseminated MAC was diagnosed on CSF Bactec culture, and a further 36 cases of MTB were diagnosed by various other methods as depicted in figure 14. In total, 64 patients had mycobacterial disease: 10 patients with disseminated MAC, and 58 patients with MTB. Co-infection with MTB and MAC occurred in 4 of these patients.

Sputa analysis alone made the diagnosis in 53% of cases, and Bactec blood culture analysis made the diagnosis in 45% of cases. The two investigations used in combination made the diagnosis in 86% of cases, making this an extremely efficient, cost effective, and simple strategy for diagnosing mycobacterial disease that can easily be performed in an out patient setting. In a resource poor setting, Bactec blood culture could be performed after a negative sputum result in an attempt to save costs. In AIDS patients, a growth signal on Bactec culture can usually be detected within 8-14 days, although specimens with low colony counts may require up to 4 weeks for a positive result(19). Thus if a more rapid diagnosis is required, other investigations including bone marrow aspirate and trephine and liver biopsy are alternatives to the above strategy.

Mycobacterial disease is therefore exceedingly common in black South African patients with AIDS admitted to hospital, the infection occurring in 64% of cases. It often presents atypically and commonly with systemic involvement. With the combination of sputa and

Bactec blood culture analysis alone, the condition can be diagnosed in 86% of cases, making the use of these two investigations in the appropriate clinical setting, mandatory. Prophylaxis against mycobacterial disease in black South African AIDS patients with CD4 counts of less than 100cells/mm^3 needs to be considered. Isoniazid and rifabutin given to patients in the above setting is a reasonable option, but further research concerning this is still needed.

4.1 LIMITATIONS

Part 1. Retrospective studies may result in inaccuracies when extracting data from patients' clinical files. Data was not recorded in the study if not specified clearly in these files. Where data was considered absolutely essential, (especially concerning the prior history of MTB in patients with disseminated MAC infection) patients were traced and information was verified verbally.

Part 2. Patient recruitment was not consecutive in part 2, allowing for the possibility of bias.

Part 3. Lack of data, especially pertaining to the number of patients with BCG scars may have resulted in the lack of statistical significance. Inaccuracies in obtaining a prior history of tuberculosis may have occurred due to lack of data recorded in patients' files.

Part 4. groups were relatively small, especially the MAC group (n = 21). Larger numbers may have resulted in other clinical parameters reaching statistical significance, although from a clinical standpoint of reaching a firm diagnosis of disseminated MAC, this would probably still be unhelpful. Clinical data was not always available from patients' files.

Lastly, laboratory contamination of specimens is always possible. Steps as outlined in laboratory methods were taken in order to eliminate this problem and to ensure correct results. Culture results were always correlated with the patients' clinical condition as a further control to detect any irregularities.

5 CONCLUSION

This study showed that disseminated MAC in black South African patients with AIDS (CD4 counts < 100/mm³) is not uncommon, in conflict with reports from other African countries. The total number of patients with disseminated MAC diagnosed per year at C.H.B.H. increased exponentially from 1994 to 1998, and the prevalence is 10%, a prevalence similar to developed countries not endemic with tuberculosis. Furthermore, a prior history of tuberculous disease, and the presence of a BCG scar was found not to be protective of disseminated MAC.

In contrast to white HIV positive patients from developed countries, subsets derived mostly from homosexuals and intravenous drug abusers, the clinical and laboratory manifestations of disseminated MAC were diagnostically unhelpful in hospitalized black South African patients with AIDS. Bactec blood culture was the only reliable investigation in consistently diagnosing the disease and is recommended in the routine evaluation of these patients.

Mycobacteria were cultured from a single Bactec blood culture in 29 of the 100 HIV positive black South African patients who were admitted to hospital with CD4 counts of less than 100 cells/mm³. Ten patients were finally diagnosed with disseminated MAC (9 on blood culture alone), and 58 with MTB. Co-infection occurred in 4 of the cases. Of the patients diagnosed with mycobacterial disease, sputum analysis alone detected disease in 53% of cases, and Bactec blood culture in 45% of cases. The two investigations used in combination made the diagnosis in 86% of cases, making this an extremely efficient, cost effective, and simple strategy in the appropriate clinical context with applications in an out-patient setting.

6 REFERENCES

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APPENDIX A

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Pettifor

CLEARANCE CERTIFICATE PROTOCOL NUMBER M980823

PROJECT Disseminmed Mycobacte.ium Avium Complex Infection In South African Patients With AIDS

INVESTIGATORS Dr CA Pettifor

DEPARTMENT Dept of Medicine, Baragwanath Hospital

DATE CONSIDERED 980823

DECISION OF THE COMMITTEE *

Approved unconditionally

DATE 980910 CHAIRMAN .....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Dr AS Karstadt
Dept of Dept of Medicine, Baragwanath Hospital

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DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee.

DATE 13/10/98 SIGNATURE .....

PROTOCOL NO.: M 980823

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX B

PATIENT INFORMATION SHEET

Your doctor has informed me that you may be suffering from tuberculosis.
One of the ways we can detect this infection, is by putting 5mls (one teaspoon) of your blood into a special culture bottle, and then sending it off to the laboratory for growth of the bacteria. If we indeed find that you do have tuberculosis, we are able to treat you with medicines that can cure you of this sickness, providing you take your treatment.

I am doing a study on patients who, like yourself, may be suffering from tuberculosis and would like to ask you a few questions about the symptoms you may be experiencing. I would also like to examine you briefly for signs of tuberculosis. This should take about five minutes, and you will not experience any discomfort.
We will need to take 5mls of your blood for the tuberculosis test if your doctors have not done so already.

Participation is voluntary
You are completely free to choose whether or not to take part in the study. If you decide not to participate, you will not be penalized in any way whatsoever, and will continue to receive the same medical attention you previously enjoyed.

Confidentiality
Your details will be kept confidential. Any information given to the health authorities or published will not show your name or the names of other patients.

Benefits
We will follow up your results carefully, and inform you immediately if your blood cultures become positive for tuberculosis.

If you have any questions, I will gladly answer them to the best of my knowledge.

DR C.A. PETTIPHER.
PHYSICIAN AT CHRIS HANI BARAGWANATH HOSPITAL.

Author Pettipher C A
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