Non-tuberculous mycobacteria disease as a cause of hospitalization in HIV-infected subjects

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Summary

Objectives: The present study characterized and determined the prevalence of mycobacterial diseases (tuberculosis (TB) and non-tuberculous mycobacteria (NTM)) as a cause of hospitalization among HIV-infected subjects consecutively admitted to a large metropolitan hospital during 2001/2002.
Methods: Hospital discharge diagnoses were established for 521 HIV-positive patients.
Results: Respiratory disease accounted for 49% of the admissions. Community acquired pneumonia (CAP) was the main cause of respiratory disease (52%) followed by *Pneumocystis carinii* (PCP, 24%), non-tuberculous mycobacteria (NTM, 11%) and *Mycobacterium tuberculosis* (TB, 9%). *Mycobacterium tuberculosis* disease was established using bacteriological, clinical and radiographic criteria. NTM disease was defined following the American Thoracic Society criteria. NTM was disseminated in the majority of cases (19 *Mycobacterium avium* complex (MAC), one *Mycobacterium kansasii*). Nine patients had respiratory disease (seven MAC, one *Mycobacterium fortuitum*, one *Mycobacterium kansasii*) and one had gastrointestinal disease caused by MAC. Mortality was 10% for NTM disseminated cases; none of the TB patients died over the course of the study. The length of hospitalization for NTM patients was longer (15 ± 13 days) than for other respiratory cases (10 ± 10, $p = 0.04$).

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Conclusions: NTM disease along with its related mortality is a significant pathology as a cause of hospitalization among HIV-infected individuals.

Introduction

Non-tuberculous mycobacteria (NTM) are ubiquitous organisms, different from *Mycobacterium tuberculosis* (TB), and represented by more than 65 different species.[1] The most frequent forms of NTM include *Mycobacterium avium* complex (MAC, 61%), *Mycobacterium fortuitum* (19%) and *Mycobacterium kansasii* (10%), with smaller percentages of *Mycobacterium gordonae* and *Mycobacterium chelonae*.[1,2] The routine application of the liquid medium-based Bactec 460 TB system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD) and the DNA hybridization-based assay has increased the sensitivity and specificity of NTM diagnosis and significantly reduced the time required for isolation and identification.

In a substantial percentage of cases, patients with NTM have underlying conditions (genetic or structural in nature) or severe immunosuppression (congenital or acquired)[3—6] that increase their vulnerability to NTM. Some, but not all, NTM patients have a history of underlying chronic lung disease.[7]

In recent years, NTM disease has become particularly relevant because of the HIV pandemic.[8—10] The atypical mycobacterium *Mycobacterium avium* complex (MAC) has emerged as one of the frequent opportunistic infections isolated in AIDS patients. The frequency of diseases due to NTM has been rising worldwide as well as in the USA, and NTM may be a frequent and considerable cause of morbidity/mortality in immunocompromised subjects.[11] Prior to the introduction of highly active antiretroviral therapy (HAART), it was estimated that up to 43% of AIDS patients would acquire NTM during their lifetime.[8,12] Delayed diagnosis may, thus, result in increased mortality rates.[10]

As the prevalence of HIV in Florida, particularly in Miami, is one of the highest nationwide, it is expected that TB and NTM disease will become relatively more significant.[13] Because this is not yet a reportable illness in the USA, the task of estimating incidence and prevalence has been difficult.[9,14] The clinical significance of NTM infections in HIV seropositive patients has not been clearly defined, and the importance of NTM diseases as a cause of hospitalization remains uncertain. The present study was designed to characterize, determine the prevalence and better appreciate the role of mycobacterial diseases, particularly NTM, as a cause of hospitalization among HIV-infected subjects admitted to a large metropolitan hospital, during the HAART era (2001/2002). The present study will provide the data necessary to revise strategies in clinical, microbiological, and public health approaches to mycobacterial disease and will provide updated information on the HAART era.

Materials and methods

Study subjects

Men and women 18 years of age or older with confirmed HIV infection, and consecutively admitted to Jackson Memorial, a central referral hospital, were eligible for enrollment. Jackson Memorial Hospital, a 479-bed tertiary care teaching institution, is affiliated to the University of Miami. The hospital has the largest tuberculosis clinic in Dade County and is the major AIDS treatment center in the region. All mycobacterial isolates from this area are referred to the Mycobacteriology Laboratory at Jacksonville for final identification.

Following admission, stable patients were contacted and fully informed about the study. Written informed consent was obtained from all participants. The Human Studies Committee of the University of Miami School of Medicine Institutional Review Board approved the project.

A physician with experience in the care of HIV-infected individuals completed all the enrollment procedures. Following an interview, a physical examination was conducted. Blood was drawn to determine CD4 cell count as part of the admission laboratory diagnostic procedures. Immunological tests were ordered, if necessary, as part of the study procedures.

Medical information

Research data were derived from two main sources: medical records and study questionnaires. A physician recorded complete past and family medical history of opportunistic infections, allergies and chronic illnesses. History of medication included past and current antiretrovirals, anti-TB prescriptions and chemoprophylaxis. Our definition of HAART was a combination of three drugs that

included a single protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI), or any regimen containing more than one PI or more than 4 antiretrovirals. Participants were classified as taking HAART if: 1) they reported to both the study interviewer and medical team at admission that they were taking HAART; 2) viral load was not greater than 50 000 copies/ml and a reduction from a previous viral load test was evident; and 3) pharmacy records indicated that participants obtained their prescribed medication.

We defined 'effective HAART' as those antiretroviral combination regimens that permitted undetectable viral loads to be achieved; otherwise, therapeutic regimens were classified as 'non-effective HAART'.

The medical study team, comprised of three HIV clinician/researchers, compared and validated the data. All available clinical data, antiretroviral medications, antimicrobial treatments, laboratory results, radiology studies (i.e., X-rays, scans) and responses to treatments were reviewed to confirm the diagnosis. Medical chart review permitted the most precise cause of disease (i.e., infectious pathogen) to be defined.

Hospitalization

Computerized diagnostic code data included number of previous admissions to the hospital, date of present admission, length of stay, and presumptive admission diagnosis. Discharge data coding included the main discharge diagnosis and a list of diagnostic exams and procedures that had been performed.

Diagnostic criteria for TB disease

Two out of three criteria were used to confirm TB disease: (1) acid-fast bacilli (AFB) culture, positive for TB from any tissue or fluid; (2) positive smear; and (3) clinical (signs/symptoms), abnormal chest X-ray and CT scan compatible with TB disease.[15]

Diagnostic criteria for NTM disease

Confirmation of NTM disease was based on American Thoracic Society (ATS) criteria[16]: (1) repeated isolation of atypical mycobacteria from three or more respiratory samples (i.e., culture + AFB); and (2) an abnormal chest radiograph, or the presence of one or more symptoms indicative of pulmonary disease which responded to therapy. NTM pulmonary disease was established with isolation/culture in respiratory samples (i.e., sputum, bronchial wash) and negative blood culture. Extrapulmonary disease was diag-

nosed when mycobacteria were isolated from only one location other than lung, pleura, blood, or bone marrow. Disseminated NTM disease was established when multiple organs were involved, when mycobacteria were isolated from blood or bone marrow, or when infection manifested as miliary disease.

Chest radiograph reports were abstracted. The pattern and location of parenchymal infiltrates (interstitial, alveolar, reticulonodular, or mixed) and/or cavities were noted. The size and location of pleural effusions or fibrosis were also summarized.

Mycobacteria strains

Clinical specimens were sent for routine mycobacterial cultivation to the Mycobacteriology Laboratory of the Florida State Department of Health. Samples were processed using Bactec 12B and Bactec 13A liquid medium or solid media, such as Lowenstein—Jensen slants or Middlebrook 7H10 or 7H11 slants or plates. Bactec cultures were considered to be positive if actively growing (test culture with growth index >300). Isolates were characterized using standard microbiological criteria.

PCR restriction digestion with endonucleases BstE II and Hae III

Mycobacteria from liquid or solid media were suspended in 250 μl of 10 mM Tris-EDTA and centrifuged. The clinical specimens were centrifuged at 14 000 rpm for 5 min. The pellets were washed twice and DNA was isolated using standard procedures. Amplifications were carried out in thin-wall reaction tubes using an automated thermal cycler and according to the manufacturer's instructions (Perkin—Elmer, GeneAmp PCR System 9600). Positive and negative controls were included in all the experiments with clinical specimens.

Amplicons were digested with endonucleases BstE II and Hae III. Aliquots of amplified samples (15 μl) were loaded on 3% NuSieve agarose gels in Tris-acetate-EDTA (TAE) buffer and subjected to electrophoresis in mini gel boxes for 60 min at 160 V. The gels were stained with ethidium bromide at 0.5 μg/ml, observed under ultraviolet light for specific DNA bands and photographed.

HIV disease status

The diagnosis of AIDS was made using the Centers for Disease Control surveillance criteria.[17] CD4 cell counts and viral load measurements were

abstracted from medical records or obtained at admission for the study.

Flow cytometry was used to quantify the percentage and absolute numbers of T lymphocyte subpopulations. A four-color direct immunofluorescence procedure (Becton Dickinson, San Jose, CA) was used to determine the percentages and absolute counts of the helper inducer T cells (CD3+/CD4+).

A reverse transcriptase polymerase chain reaction (Roche) was used to quantify HIV viral load. The current version of this assay has a reportable range of >200 to 750 000 RNA copies per ml of plasma.

Statistical analysis

The data were analyzed using SPSS version 11 and SAS 8. Following descriptive statistical analyses, mean variables were compared using Student's *t*-test and one-way ANOVA procedures. The relationships between main variables were examined with Pearson's correlation coefficient. In the final model, regression logistic analyses were used to predict the risk factors associated with the development of mycobacterial disease.

Results

Study population

Five hundred and twenty-one HIV-positive patients (300 men, 221 women) were admitted to Jackson Memorial Hospital between September 2001 and December 2002 and enrolled in the study. The main characteristics of the study subjects are presented in Table 1. Participants ranged in age from 20 to 72 years (42 ± 9 years). More than half (63%) of the HIV-infected patients admitted to regularly using at least one illicit drug at some point during his or her life. One hundred and fifty-five participants (30%) admitted to using some form of illegal substance within 30 days of their interviews. The most common drugs used were crack/cocaine (44%) and marijuana (44%). Other illicit drugs (amphetamines, heroin) were regularly used by less than 3% of the HIV study participants. A tendency for crack/cocaine users to be hospitalized with NTM (OR = 2, 95% CI 1—4.2, *p* = 0.08) was observed. No other relationship was observed between drug abuse and mycobacterial disease.

Despite the prevalent use of drugs, only 7% reported drug abuse as the source of HIV infection whereas most study participants reported unprotected sex as a route of transmission. Most participants (89%) reported unprotected heterosexual contact, 3% reported homosexual transmission, and only 1% reported bisexual contact. Men who had sex with men were four times more likely to have NTM as a cause of hospital admission (OR = 4.8, 95% CI 1—20, *p* = 0.05). On the contrary, all TB cases occurred among heterosexuals.

More than half of the participants were African—American (61%), 18% were Hispanic, 17% Haitian, and the remaining 4% were Non-Hispanic white. As

Table 1 Principal characteristics of study group.

Variable	Study subjects (*n* = 521)	NTM[a] (*n* = 30)	Tuberculosis (*n* = 24)
Gender			
Male	300 (58%)	21 (70%)	15 (63%)
Female	221 (42%)	9 (30%)	9 (37%)
Age (years)	42 ± 9	39 ± 9	43 ± 8
Ethnicity			
African—American	315 (61%)	20 (67%)	6 (25%)
Haitian	90 (17%)	4 (13%)	11 (46%)
Hispanic	92 (18%)	6 (20%)	6 (25%)
Caucasian	20 (4%)	0	1 (4%)
CD4 cell count (cells/mm^3)	131 ± 177	79.2 ± 167.53	132.3 ± 132.45
Viral load (copies/ml)	262126 ± 512291	386811 ± 277978	293225 ± 202538
AIDS	416 (80%)	30 (100%)	24 (100%)
HIV	105 (20%)	0	0
HAART[b]			
Yes	203 (39%)	12 (40%)	9 (38%)
No	318 (61%)	18 (60%)	15 (62%)

[a] NTM, Non-tuberculous mycobacteria.
[b] HAART, highly active antiretroviral therapy.

shown in Table 1, no significant differences regarding gender, age or income were observed between HIV-infected subjects diagnosed with NTM disease ($n = 30$) and those diagnosed with TB ($n = 24$). Most of the NTM patients were African—American (67%) and no case was observed among Non-Hispanic Whites. On the contrary, a significantly lower proportion of African—Americans were hospitalized with tuberculosis. TB patients were predominantly Hispanic Blacks (Haitian, 46%). A significantly higher proportion of Hispanic Blacks (12%) were diagnosed with tuberculosis than African—Americans (2%; $X^2 = 16$, $p < 0.0001$). The proportion of White Hispanics (6.5%) with TB was higher than the percentage of African—Americans that developed TB (2%; $X^2 = 3.8$, $p = 0.05$). Almost half of the NTM patients (40%) and 38% of the TB patients reported being on HAART at admission.

Discharge diagnosis

Respiratory diseases accounted for 49% of the total admissions. Community acquired pneumonia (CAP) was the main cause of respiratory disease (52%), followed by *Pneumocystis carinii* (PCP, 24%), NTM (11%) and TB (9%).

Mycobacterial isolation in HIV-infected subjects

Non-tuberculous mycobacteria were isolated in the sputum, smear and/or cultures of 63 HIV-infected patients (12% of the total study population). In almost half of these cases (48%), the isolated NTM was the cause of the disease (as per ATS criteria). In two patients, both NTM and TB were isolated (TB with MAC and *M. gordonae*, respectively), but the final diagnosis was TB. Three other presumptive NTM cases were excluded from the analyses because NTM was not isolated. Excluding the above five cases, the prevalence of NTM disease was 11% in patients with a lower respiratory infection ($n = 265$). The majority of the patients with NTM disease (67%) had disseminated disease (19 MAC and 1 *M. kansasii*). The remaining 10 cases involved respiratory disease (seven MAC, one *M. fortuitum*, one *M. kansasii*) or a gastrointestinal illness (MAC). TB was diagnosed in 24 HIV-infected patients. The prevalence of tuberculosis in the current study was 9%.

The most frequently isolated NTM was MAC (71%, $n = 45$) with 32% in blood and 68% in sputum, followed by *M. fortuitum* in sputum ($n = 12$) and *M. kansasii* ($n = 6$) with 83% from sputum and one specimen in blood. Single isolations in sputum occurred for *M. chelonae, M. gordonae,* and *M. simiae*.

HIV disease status

On average, the study population had been HIV positive for 7 ± 6 years. According to CDC criteria, 80% of the patients had AIDS status and the remaining 20% were HIV symptomatic. The mean CD4 cell count of the total group was $131 \pm 177/mm^3$ with one third (33%) of the group having cell counts more than $200/mm^3$. The mean viral load was $262\,126 \pm 512\,291$ copies/ml; 5.6% ($n = 17$) had undetectable viral load levels. Only 39% of the study population was using HAART and the rest were either receiving one medication or none (61%).

NTM/TB cases and HIV

A greater proportion of NTM patients (93%) had CD4 cell counts $<200/mm^3$ as compared to TB patients (79%, $p = 0.003$). It needs to be noted that although most patients with NTM (73%) had less than 50 CD4 cells, 17% had between 51 and 200 cells and 10% had cell counts of more than 200. Univariate analyses demonstrated a significantly higher risk of being hospitalized with NTM disease among patients with CD4 cell counts below 200 (OR = 6.6, 95% CI 1.5—24.6, $p = 0.003$) when compared to patients hospitalized with TB disease. Additional analyses (CATMOD) indicated that after controlling for HAART, those with CD4 cell counts below $200/mm^3$ were three times more likely to be hospitalized for NTM than for any other non-respiratory cause ($p = 0.04$). CD4 cell counts were significantly lower in participants who developed disseminated NTM disease (30.9 ± 47) as compared to those with a localized disease (80.5 ± 76.4, $p = 0.05$). No significant differences in mean viral load were observed among patients hospitalized with localized, disseminated NTM disease or admitted with TB. Additional analyses demonstrated that viral loads of TB patients tended to be significantly higher than the viral loads of patients hospitalized with non-infectious diseases ($391\,035 \pm 77\,875$ versus $196\,017 \pm 21\,850$, $p = 0.07$). Of interest, however, is the fact that patients with NTM had significantly higher viral loads than those with non-infectious diseases ($195\,145 \pm 21\,725$; $p = 0.03$).

At admission, less than half of the study group was using HAART (39%). Forty percent of the NTM group and 38% of the TB patients were administered HAART. CD4 cell counts were significantly lower in the non-HAART group (HAART: 169.8 ± 211 versus non-HAART: 106.48 ± 149, $p = 0.0001$). Of those subjects receiving HAART, 6% developed NTM. Nevertheless, none fulfilled our criteria of 'effective HAART' (undetectable viral loads) even though

viral burden was reduced under the current regimens.

At admission, 25% of the NTM cases reported taking azithromycin, 40% of the cases were prescribed but were not adherent, and the remaining population was not taking any prophylaxis at the time of admission.

Clinical and radiographic features

Clinical findings and symptoms of all patients were non-specific and included cough, fever, chills, fatigue, and weight loss. Most patients with TB and NTM disease had abnormal chest X-rays (TB = 75%, NTM = 73%). There were some differences in the radiographic features of NTM disease compared to TB. One third (33%) of our participants had a bilateral diffuse infiltrate. In contrast to previous studies describing lingular involvement, the disease was seen predominantly in the apical lobes (24%), 14% in the lower lobes with an additional 16% having involvement of the pleura and effusions. Only 8% had involvement of the middle lobe or the lingula, and the remaining percentage (5%) had lymphadenopathy.

In chest X-rays the most frequent pathological lesions of NTM cases included interstitial infiltrates in 33% of the cases[7] contrasting with none among TB cases. Pleural effusions (20%, $n = 6$) and lymphadenopathies[18] were other common abnormalities. In contrast to previous reports, bronchiectasis was not the main radiological feature. Chest radiographs were frequently normal despite active pulmonary infection during the first 1–2 weeks of the disease, and subsequent studies demonstrated one of the above abnormalities.

Mycobacteria and other risk factors

Pre-existing disease
Medical history of a chronic disease (i.e., chronic obstructive pulmonary disease (COPD), renal disease) was noted in 21% ($n = 110$) of the study population; only 13% ($n = 4$) of the patients diagnosed with NTM had been diagnosed with a chronic respiratory disease. Pre-existing malignant disease was not associated with either NTM ($p = 0.69$) or with TB ($p = 0.83$). Past respiratory disease (COPD, TB or other lower respiratory infections), another potential risk factor, was not related to increased susceptibility for NTM ($p = 0.24$) or TB ($p = 0.17$). Most of the study participants who developed a mycobacterial disease were malnourished (85% albumin <3.5 g/dl); mean albumin levels were similar in patients with NTM (2.8 ± 0.5) and TB (2.6 ± 0.7). Significant differences, however, were evident when patients with disseminated NTM (2.8 ± 0.6) were compared to those with localized NTM disease (2.1 ± 0.27, $p = 0.03$).

Tobacco use
The overall rate of current smoking was 59.8% for men and 40.2% for women. A large percentage of the non-smoking men (54%), and 46% of the non-smoking women reported never using tobacco. Though smoking patterns were similar in NTM and TB patients, participants who developed a localized respiratory disease were more likely to be heavy smokers (17 ± 14 mean cigarettes/day) relative to those who developed a disseminated disease (7 ± 4).

Morbidity/mortality

Hospital duration and classification score for AIDS hospitalization[19] were evaluated. The length of hospital stay varied from 1 to 78 days with a mean across the study group of 9.7 ± 10 days. TB patients were hospitalized for an average of 7.7 ± 2.6 days, which was significantly shorter than the duration of patients with non-respiratory infections (9 ± 10 days, $p = 0.02$). Furthermore, diagnosis and time to initiate treatment was significantly lower in TB patients (4.4 ± 1.1 days as compared to 27 ± 6 days, $p = 0.001$) in the NTM patients.

Of interest, there was a significant positive correlation between number of days to initiate therapy and CD4 cell counts ($p = 0.04$). Univariate analyses indicated that if CD4 cell counts were below $100/mm^3$, physicians were more likely to diagnose NTM in the first week of admission (95% CI 1.03–6.64, $p = 0.04$). Moreover, if CD4 counts were dichotomized above and below 200 the mean time to initiate treatment was 22 ± 5 days as compared to 50 ± 17 ($p = 0.05$). Furthermore, three patients with more than 150 CD4 cells/mm^3 required a re-admission to the hospital before NTM disease diagnosis was achieved and two more were diagnosed and treated in the outpatient clinic. In consequence, the length of hospital stay was longest in the NTM patients (19.5 ± 4 days, $p = 0.02$). Univariate analyses further confirmed that patients with NTM disease were four times more likely than any other group to stay longer than the average length (10 days) for the total cohort ($n = 521$, $p = 0.001$).

Table 2 describes the morbidity/mortality characteristics of the patients with mycobacterial disease. Evaluation of mortality indicated that 10% of the patients with disseminated NTM disease died during the hospitalization period (#4 in the classification of AIDS hospitalization).[19] None of the patients with TB died during hospitalization.

Table 2 Morbidity/mortality characteristics of patients with mycobacterial disease.

Variable	NTM disease (n = 30)	TB disease (n = 24)
Patients with localized pulmonary mycobacteria	9 [a]	19
Disseminated disease	20 (67%)	5 (21%)
Prevalence	11%	9%
Mortality	3 (10%)	None

[a] An additional participant had a GI NTM disease.

Multivariate analyses

Multivariate analyses in this group of hospitalized patients indicated that high viral loads (OR = 7, p = 0.02) and CD4 cell counts less than 200/mm^3 (OR = 2, p = 0.05) predict NTM disease. When data were analyzed among those respiratory-compromised, cigarette smoking also remains significant (OR = 1.8, p = 0.05).

Our findings regarding disseminated NTM disease, however, differ and reveal that CD4 cell counts below 50, instead of 200, are the main variable associated with dissemination (OR = 13, p = 0.001) as well as detectable viral loads (OR = 4, p = 0.42). HAART, race/ethnicity, self-reported route of HIV transmission were no longer significant in the models.

Discussion

To our knowledge, this is the first prospective study to evaluate both tuberculous and non-tuberculous mycobacteria as causes of hospitalization in HIV-infected subjects during the HAART era. Results of this investigation indicate that, after community acquired pneumonia (CAP) and *Pneumocystis carinii* pneumonia (PCP), NTM is the third most frequent cause of hospitalization due to a lower respiratory disease. Furthermore, the prevalence of NTM disease in hospitalized subjects is actually higher (11%) than the prevalence of TB disease (9%) in an area that has one of the highest TB rates among cities in the USA. In agreement with these findings, Benator and Gordin[20] have reported that mycobacterial disease is now the second most frequent cause of illness in AIDS patients receiving PCP prophylaxis. In the present study, half of those with isolated NTM met the ATS criteria for NTM disease. Diagnosis was confirmed in 30 patients resulting in a 6% prevalence of NTM disease consistent with previous studies reporting prevalence rates of between 3 and 13%.[4,8,10,12,21—23]

The confirmed isolation of NTM in 11% of the HIV-infected subjects is in agreement with previous international reports.[8,10,12,21—23] Data suggest that the frequency of NTM infection and disease is increasing worldwide and geographically varies from 5 to 37% in Latin America to 25 to 50% in European studies.[8,18,23] Although strongly associated with the HIV epidemic, global increases in NTM cases have been observed in both HIV-infected and non-infected populations.[8,10,12,21,24] Nonetheless, O'Brien et al. in a report from Australia found that the incidence had increased most notably in non-HIV-infected individuals (4.4/100 000 population versus 1.7/100 000 in HIV-infected individuals).[25]

In agreement with earlier studies[1,7,12,14,21,22,24,26—29] the organisms most commonly isolated as the cause of NTM disease in the present report, were *M. avium* and *M. intracellulare* (collectively known as the *Mycobacterium avium* complex (MAC)), followed by *M. fortuitum* and *M. kansasii*. Most of the NTM cases occurred among African—Americans, in agreement with Bloch et al.[27] and Pitchenik et al.[29] However, data regarding race are conflicting, with most studies reporting higher rates of NTM among whites.[1,7,12,22,26—29]

Although no significant differences between route of transmission and specific mycobacteria were observed, a higher prevalence of NTM was observed among homosexuals. This is in accord with previous publications noting higher prevalences of *Mycobacterium avium-intracellulare* among homosexuals.[30—32] In addition, exposure to *M. avium* glycopeptidolipid antigens also seems to be more prevalent in HIV-seropositive homosexuals than among control populations. Demographic features and route of transmission, as a potential risk factor, will require further consideration.

Our findings reveal a higher mortality in NTM patients (10%) than in TB-infected patients, none of whom died over the course of the study. Additionally, NTM patients, particularly those with CD4 counts of less than 200, were diagnosed and treated later in the course of hospitalization and suspected only when other diagnoses had been eliminated and the patient continued to be symptomatic. These data underscore the importance of continuing to include NTM in the differential diagnosis of HIV-infected subjects, even if they have CD4 cell counts of more than 100.

Physicians treating HIV-infected subjects need to consider NTM as a potential and frequent cause of

disease. In support of this proposition, O'Brien et al.[13] have suggested that with a decrease in the incidence of TB, NTM disease will rise. The prevalence of NTM disease along with its related mortality show the necessity for considering NTM as an opportunistic infection and evaluating it as a reportable disease. This applies to patients with disseminated disease, particularly those with a less-indolent course, for whom more days of hospitalization are required before final diagnosis. Careful screening will be necessary to ensure specific treatment, prevent inappropriate chemoprophylaxis, and re-hospitalizations to reduce the risk of resistance or dissemination of NTM disease.

Of importance and in contrast to previous reports, most of the present NTM cases (67%) were disseminated; in O'Brien's study,[13] for example, as many as 80% were pulmonary cases. Although our study participants had respiratory symptoms, NTM was mainly isolated in blood (67%), with or without isolation from respiratory specimens, and only 33% were from respiratory samples (bronchial wash, sputum, smear). As expected, lower CD4 cell counts were observed in those with disseminated disease.

In agreement with previous studies, higher smoking rates were observed in those with NTM disease.[25] Our studies extended these published reports by specifying that the association is relevant only to pulmonary disease. It should be noted that *Mycobacterium avium-intracellulare* could be recovered from tobacco, cigarette paper, and cigarette filters from several cigarette brands, thus providing a potential explanation for the increased risk of pulmonary disease.[4,14]

Malnutrition (lower mean albumin levels) and lower viral loads associated with NTM pulmonary disease were, however, unanticipated and require further evaluation.

The introduction of HAART has dramatically changed the clinical prognosis for HIV-infected patients in decreasing mortality, morbidity, and the need for hospitalization.[26] Nevertheless, almost half (40%) of the patients admitted with NTM disease were receiving HAART at the time of hospital admission, although undetectable viral loads had not been achieved. These data, in agreement with those published by Nuñez and colleagues,[33] emphasize that even with administration of HAART, subjects with low CD4 cell counts and detectable viral loads may still be at increased risk of developing opportunistic infections (i.e., PCP, NTM). It should be noted that, in contrast to Nuñez's report, we did not observe a temporal relationship between the development of NTM and the receipt of antiretroviral therapy.

The high prevalence of both infection and disease suggests that the frequency of NTM as a cause of disease is increasing, and careful surveillance must be implemented. Enhanced awareness and additional culturing for both pulmonary and disseminated disease may also be related to the increased reports of NTM disease.[16,20] The lack of systematic reporting of NTM disease in the USA, as well as in other countries, limits the ability to derive accurate estimates of infection and disease.[27] Better methods for early detection and therapy of NTM disease need to be developed and prevention strategies implemented. Without appropriate screening tools, reporting systems, and early diagnosis, the problem may continue to grow and become a far more important issue than current rates suggest.

Acknowledgments

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