High Rates of Disseminated Infection due to Non-tuberculous Mycobacteria among AIDS Patients in Finland

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Objective: to determine the rate of disseminated infection due to non-tuberculous mycobacteria (NTM) among Finnish AIDS patients, and to analyse the epidemiology of these infections.

Methods: in a prospective cohort study HIV-infected patients with CD4 counts < 200 × 10⁶/l were interviewed, and had mycobacterial blood cultures performed at baseline and at 6 months, then subsequently for clinical indications; autopsies were performed on patients who died. The cohort was followed at least for 24 months or to death. Water samples were collected from the homes of patients and from the environment and cultured for organisms of the Mycobacterium avium complex (MAC). Environmental and clinical isolates were compared using pulsed field gel electrophoresis (PFGE).

Results: NTM infection occurred in 22 (43%) of 51, 19 isolates were Mycobacterium avium, two M. genavense and one M. intracellulare. Multivariate analysis identified urban residence (P = 0.04) and eating raw fish (P = 0.04) as independent risk factors. Molecular analysis revealed two clusters of related isolates (three M. avium, two M. genavense) among urban residents.

Conclusion: AIDS patients in Finland have high rates of disseminated infection due to NTM. Clusters of identical organisms and association with urban residence suggests that these are newly acquired infections in advanced AIDS.

Introduction

Disseminated infections due to non-tuberculous mycobacteria (NTM) have been reported to occur at a rate of approximately 20% per year among patients with advanced AIDS in developed countries. It has been suggested that all patients with HIV infection might ultimately develop disseminated infection with NTM if other HIV-related events could be prevented. In a recent international study we detected high rates of disseminated infection with Mycobacterium avium complex (MAC) in HIV-infected individuals in Finland and in the United States.

Additional follow-up of the patients from Finland disclosed a high cumulative incidence of disseminated NTM infection. In this report we present an analysis of rates and risk factors for disseminated mycobacterial infection among AIDS patients in Finland, and results of environmental cultures to detect the source of these infections.

Methods

Study protocol

Patients were recruited and studied as previously described. In brief, HIV-positive patients with CD4 lymphocyte counts < 200 × 10⁶/l treated in Helsinki and Tampere, Finland, were entered in the study beginning September 1991. The study was approved by ethical committees at all clinical sites. CD4 counts were performed on the day of enrollment or within 3 months prior to the enrollment. Subsequent CD4 counts were performed approximately every 6 months for clinical indications. Examinations, risk factor interviews and mycobacterial blood cultures were performed at baseline and at 6 months.

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Risk factor questions referred to the 12 months prior to enrollment. After the end of the formal study in May 1994, surviving patients were followed through November 1995. During this interval mycobacterial blood cultures were obtained for clinical indications, and autopsies were performed whenever possible on patients at Aurora Hospital. Autopsies included mycobacterial stains and cultures of mesenteric lymph nodes. None of the patients were on chemoprophylaxis for MAC during the study period. HIV antiviral therapy was at the discretion of the treating physician.

Urban vs. non-urban residence was determined by address of the permanent residence at baseline. Urban residence was defined as residence in a city with over 100,000 inhabitants and a defined central downtown area. Other sites of residence (suburban, rural) were defined as 'non-urban'.

**Laboratory studies**

The following tests were performed at baseline and at 6 months: complete blood count, blood CD4 lymphocyte count, and mycobacterial blood culture. Subsequent CD4 lymphocyte counts were performed for clinical indications at approximately 6-month intervals. Blood cultures were performed using BACTEC 13A broth medium (Becton-Dickinson Diagnostic Instrument Systems, Sparks, MD, U.S.A.). In 18 cases blood cultures were performed using both BACTEC 13A and the Isolator blood culture system (Wampole Laboratories, Cranbury, NJ, U.S.A.); results for the growth of mycobacteria were completely concordant.

Thirteen isolates from blood cultures were identified at Dartmouth–Hitchcock Medical Center as previously described. Eight isolates from blood cultures were identified at the mycobacterial reference laboratory of the Finnish National Public Health Institute at Turku. Disseminated NTM infection was defined as a blood or sterile site culture positive for a NTM.

**Molecular typing of M. avium complex**

Molecular strain typing for MAC was performed using pulsed field gel electrophoresis (PFGE) to resolve Asel digests of whole cell DNA as previously described. Restriction digests profiles were examined visually; similar patterns were compared directly in a single gel and analysed according to criteria of Tenover et al. Isolates whose profiles were indistinguishable or closely related (differed by a single genetic event, typically one to three bands) were considered to represent the same strain; multiple isolates from a single patient with monoclonal infection fit these criteria. Isolates whose profiles differed by multiple genetic events were considered to represent different strains; different strains typically had completely different profiles (> 50% bands different).

**Identification of M. genavense**

Patient samples were cultured in Bactec 13A medium. Mycobacterial DNA was isolated from the cultures by mechanical lysis with glass beads. A 1037 bp segment of the gene coding for the mycobacterial 16S rRNA was amplified with primers 264 and 285 (biotinylated). Biotinylated PCR products were rendered single-stranded by using Dynabeads M-280 Streptavidin (Dynal AS, Oslo, Norway).

Mycobacterial isolates were identified by PCR-based nucleic acid sequence analysis of the 16S rRNA fragments as described previously and sequencing reactions were performed manually. Sequence alignments in the region corresponding to positions 129 to 266 (hypervariable region A) of Escherichia coli 16S rRNA was used for identification.

**Risk factor analysis**

The rate of disseminated NTM infection was analysed for 6-month intervals to 24 months of follow-up. Rates of infection were analysed separately for three categories of baseline CD4 counts (< 50, 50 to 99, and 100 to 199 cells/mm³). Because subsequent CD4 counts were performed at intervals > 6 months, exact CD4 counts were not always available at the time of diagnosis of disseminated NTM infection. The date of diagnosis of disseminated NTM infection was analysed by quarter of the year (first quarter = January to March), excluding patients who had disseminated NTM infection at baseline.

**Environmental samples**

Water samples from natural and treated sources were collected in volumes of 50–500 ml as previously described. Water samples were processed by centrifugation or filtration as previously described and cultured on modified 7H10 agar (pH 6.5 and cyclohexamide 500 μg/ml). Water culture methods were developed for detection of MAC but not for M. genavense.

Samples of natural water from the Helsinki area included two samples from a lake, one sample from a well, and three samples from the Vantaa River (the source of water for the municipal treatment plant in Helsinki until 1982). One sample was collected at the end of a pipeline.
One sample was collected from the Helsinki municipal water treatment plant at the beginning of the water distribution system. Potable water samples were collected on four occasions during the study (3–9 months apart) at Aurora Hospital in Helsinki where > 75% of the patients were followed and treated. Water samples from Aurora Hospital included 15 samples from the inpatient ward where the patients were hospitalized, three samples from the outpatient clinic and two samples from the dental office. Potable water samples were collected from the homes of three patients in Helsinki and one in Tampere after they were diagnosed with disseminated MAC infection. Samples were also collected in downtown Helsinki on two occasions from two indoor swimming pools, a residential apartment, a business apartment, and a public cafeteria. Commercially bottled water was cultured on two occasions.

Salmonid fish originating both from salt and fresh water sources were obtained at two fish markets and at a supermarket in Helsinki on two occasions. Fish samples (peritoneum and surrounding muscles) were cultured both in Finland and in the United States. In Finland, a piece of each fish sample was ground with a mortar and a part decontaminated with benzalkonium chloride and trisodium phosphate. The samples were cultivated before decontamination in Bectec 12 B medium (Becton-Dickinson Diagnostic Instrument Systems, Sparks, MD, U.S.A.) and on modified Löwenstein-Jensen (L-J) slants (Mycobacterium 1 and 2 modified Löwenstein-Jensen medium, Orion Diagnostica, Espoo, Finland) and after decontamination only on modified L-J slants. In the U.S.A., the fish samples were cut into small pieces and ground into 2 ml sterile phosphate buffered saline using a

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**Table I.** Environmental water samples obtained for the detection of *Mycobacterium avium* complex (MAC).

<table>
<thead>
<tr>
<th>Source</th>
<th>Number of samples (potable hot water)</th>
<th>Number of samples positive for mycobacteria (potable hot water)</th>
<th>Number of samples positive for MAC (potable hot water)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Natural water</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helsinki area</td>
<td>8</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Tampere area</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><strong>Treated water</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital</td>
<td>20 (14)</td>
<td>2 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Patient homes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helsinki</td>
<td>12 (6)</td>
<td>6 (5)</td>
<td>0</td>
</tr>
<tr>
<td>Tampere</td>
<td>6 (2)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Bottled water</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Indoor pools</td>
<td>8 (4)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Other sites</td>
<td>7 (6)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>63 (32)</td>
<td>9 (6)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>
sterile mortar and pestle. Aliquots (100 µl) were plated directly onto 7H10 agar and L-J slants; the remainder was diluted to 10 ml in sterile phosphate buffered saline, centrifuged at 30 × g for 10 min and the supernatant filtered onto Millipore filters which were then decontaminated by immersing in 5% oxalic acid for 15 min, neutralized by immersion in phosphate buffered saline and then cultured on 7H10 agar as above.

**Statistical analysis**

Multivariate analysis of risks for disseminated NTM infection was performed in a model that included bronchoscopy, swimming in an indoor pool, eating raw fish, treatment with granulocyte colony stimulating factor, CD4 lymphocyte count at baseline or at 6 months, and residence at baseline.

**Results**

**Microbiology**

Seven (14%) of 51 patients had disseminated *M. avium* infection diagnosed at baseline. An additional 15 (29%) had disseminated NTM infection detected during prospective follow-up. Overall, 22 (43%) of 51 patients had disseminated NTM infection: 19 *Mycobacterium avium*, two *M. genavense* and one *M. intracellulare*. None had disseminated infection with *Mycobacterium tuberculosis*. CD4 lymphocyte count measured within 3 months of the diagnosis of disseminated NTM infection was available for 13 patients, whose median (range) CD4 count was 38 (5–159) cells × 10⁶/l.

Environmental water samples yielded three *M. avium* strains (Table I). None of the fish samples yielded MAC, and approximately 50% of fish cultures were contaminated.

**Autopsy results**

During the follow-up 38 patients died including 27 at Aurora Hospital, eight patients at Tampere and three patients at the Department of Dermatology and Venereal Diseases. Mycobacterial stains (Auramine and Kinyoun) and cultures (both on modified L-J slants and in Bactec 12B medium) were performed in the Finnish laboratory on mesenteric lymph nodes obtained on 21 patients at the time of autopsy (20 of 27 deaths from Aurora Hospital and one of eight deaths from Tampere University Hospital). Among 10 patients without a pre-mortem diagnosis of NTM infection, both stain and culture were negative. Among remaining 11 patients with a pre-mortem diagnosis of NTM infection, four were both stain and culture positive, three were stain negative and culture positive, one was culture negative and stain positive, and two were stain negative and culture negative (both with an earlier *M. avium* infection); one was stain negative with a contaminated culture (earlier *M. avium* infection).

Patients with disseminated NTM infection were treated with standard multiple drug therapy. Three patients with disseminated MAC infection died within the first month after mycobacterial blood culture was obtained. The mean (range) survival for the remaining 17 patients with disseminated NTM infection was 8 (1–30) months. Among the 11 patients for whom autopsy data was available, the mean (range) interval between the positive blood culture and either a positive culture or stain at autopsy was 7 (0–12) months. For two patients with a positive blood culture and both a negative culture and stain at autopsy the intervals were 8 and 21 months, respectively.

**Rate of infection with NTM**

The mean duration (range) of follow-up was 15.4 (0–46) months. The mean duration (range) of follow-up for patients with urban residence was 15.4 (0–46) months and for patients with non-urban residence 15.2 (0–30) months. One patient was lost to follow-up 19 months after enrolment. The median (range) CD4 count of patients at baseline was 90 (1–199) cells × 10⁶/l. The risk for disseminated NTM infection by baseline CD4 lymphocyte count and duration of follow-up are shown in Table II. Survival free of disseminated NTM infection is illustrated in Figure 2.

**Risk factor analysis**

Disseminated NTM infection was diagnosed most frequently during the quarter from July to September (eight cases), compared with the quarter from January to March.

**Table II.** Rates (percentage) of disseminated NTM infection by baseline blood CD4 lymphocyte count and duration of follow-up.

<table>
<thead>
<tr>
<th>Duration of follow-up</th>
<th>Baseline CD4 count cells/mm²</th>
<th>All patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>199–100</td>
<td>99–50</td>
</tr>
<tr>
<td>0 months</td>
<td>1/21 (5)</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td>6 months</td>
<td>0/20 (0)</td>
<td>2/10 (20)</td>
</tr>
<tr>
<td>12 months</td>
<td>2/20 (10)</td>
<td>2/8 (25)</td>
</tr>
<tr>
<td>18 months</td>
<td>1/14 (7)</td>
<td>1/6 (17)</td>
</tr>
<tr>
<td>≥24 months</td>
<td>3/12 (25)</td>
<td>0/5 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>7/23 (33)</td>
<td>5/10 (50)</td>
</tr>
</tbody>
</table>

|                      | 22/51 (43%)                 |              |
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Figure 2. Survival without non-tuberculous mycobacterial infections for patients who did not have non-tuberculous mycobacterial infections at baseline (n = 44).

(four cases), from April to June (three cases), and from October to December (two cases). However, the difference among quarters was not statistically significant (Fisher’s exact test P = 0.14). Forty-two (82%) of 51 patients had been immunized with BCG at birth. At baseline 41 patients had urban and 10 patients non-urban residence. The mean (±SD) CD4 cell count was 86 (±59) cells x 10^9/l for patients with an urban residence and 96 (±68) cells x 10^9/l for patients with a non-urban residence. (P = 0.66 by t-test); Twenty-one (51%) of 41 patients with urban residence and one (10%) of 10 patients with non-urban residence developed a disseminated NTM infection (Fischer’s exact test, P = 0.03). Multivariate analysis demonstrated that both urban residence and consumption of raw or partially cooked fish or shellfish were independently associated with risk of disseminated NTM infection (Table III).

Table III. Multivariate risk factors for disseminated mycobacterial infection

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Odds ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eat raw, partially cooked fish/shellfish</td>
<td>14.2 (1.06–190.96)</td>
<td>0.045</td>
</tr>
<tr>
<td>Reside in urban area</td>
<td>17.45 (1.06–145.69)</td>
<td>0.044</td>
</tr>
</tbody>
</table>

The blood cultures positive for M. genavense were obtained within a 2-week period in 1995 from two patients in the cohort. A third Helsinki patient with AIDS, not in the study, also had a positive blood culture for M. genavense during the same 2-week period. All three subjects lived within 1 km radius of each other. Samples from all three patients were processed during the same time in the Bactec system but were not positioned consecutively and intervening samples from other non-AIDS patients were not positive for M. genavense.

Discussion

The cumulative rate of disseminated NTM infection in this carefully followed cohort is higher than previously reported. Although some of our cases were due to NTM other than MAC, cumulative rates of MAC are also higher than reported previously. This finding suggests that AIDS patients in Finland either have reduced immunity to NTM, increased exposure to NTM in general, or to virulent NTM in particular, or a combination of these factors. The present study suggests that both exposure and immunity may be relevant.

The principal risk factor for disseminated NTM infection in Finland was residence in an urban environment. Molecular epidemiology demonstrated two clusters of infection in Helsinki (three M. avium, three M. genavense). Since MAC and presumably other NTM, appear to be acquired late in AIDS as a new infection, this suggests that urban residence is associated with an increased risk of exposure to NTM. We were unable to identify a specific risk factor within urban areas in this study, although we

Molecular epidemiology

Among 13 patients with disseminated M. avium infection, PFGE resolved 12 distinct strains. One patient had polyclonal infection with two distinct strains. Three patients diagnosed within 10 weeks were infected with the same strain of M. avium: all three lived in the inner urban area of Helsinki within a 2.5 km radius. None had been treated as in patients prior to the diagnosis of disseminated M. avium infection. The cultures of these three patients were never simultaneously in the Bactec incubator; moreover, and the tips entering the culture bottles were changed daily. PFGE patterns for the remaining 10 patients were diverse, as observed previously among AIDS patients in the U.S.A. None of the MAC isolates from treated water sources including the isolate from Aurora Hospital, matched a clinical isolate (Table I). One M. avium isolate from a natural source matched a clinical isolate. This natural isolate was cultured from a soil sample obtained in a migratory bird colony on Hailuoto Island in the Baltic Sea about 500 km north of Helsinki (Fig. 1). The patient had lived within 200 km of Hailuoto for 7 years in childhood, but had never visited the island. The patient had no history of cervical adenitis in childhood and was the only patient in the cohort with a positive skin test reaction to M. avium sensitin.

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The principal risk factor for disseminated NTM infection in Finland was residence in an urban environment. Molecular biological data also demonstrated two clusters of infection in Helsinki (three M. avium, three M. genavense). Since MAC and presumably other NTM, appear to be acquired late in AIDS as a new infection, this suggests that urban residence is associated with an increased risk of exposure to NTM. We were unable to identify a specific risk factor within urban areas in this study, although we
have previously shown that exposure to recirculating hot water systems, common in urban areas, may be a risk factor for acquisition of MAC in AIDS.7 We isolated MAC from natural water sources supplying Helsinki, and from hot water in Helsinki and documented colonization of potable water in a hospital water system. Although we were unable to match specific strains of MAC isolated from water with clinical strains, water may be colonized with different strains over time and we may not have sampled at the correct time. The identification of a common source for environmental mycobacteria may be difficult in Finland, where, in contrast to the United States, recirculating hot water systems are used not only in large institutions but also in single family residences. We were not able to isolate M. avium from commercial fish samples or from indoor pools. two sources associated with an increased risk of disseminated MAC in our epidemiological studies.4

Urban residence may also be associated with reduced immunity to mycobacterial infection. We have shown previously that lifelong occupational exposure to soil and water is associated with a reduced risk of disseminated MAC in AIDS, possibly because such exposure before HIV infection confers some degree of natural immunity to environmental mycobacteria.4 Lifelong urban residence may result in less childhood exposure and natural immunity than rural residence. In our international study the rate of disseminated MAC infection in the U.S.A. was lower in rural than in urban study sites.4 MAC colonization rates among soil and water sources in rural Finland are very high11,12 and may be the source of infection with and immunity to NTM in some residents. Since > 80% of subjects in the study had received BCG, it was not possible to determine whether childhood BCG was associated with protection against disseminated MAC in AIDS.

The rate of isolation of MAC from potable water was lower in Finland than reported from the U.S.A.13 This may be due to temperature differences between hot water systems in Finland and in the U.S.A. In the U.S.A. only six states (12%) require maximal hospital water temperatures > 50°C, whereas Finland requires a hot water temperature of 50–65°C.14 Colonization of potable water with MAC may be intermittent, with transient changes during repair work or periods of low water use contributing to transient colonization. MAC colonization of potable hospital water in this study was preceded by a major plumbing renovation during the previous 3–6 months.

The only match between a clinical and environmental isolates by molecular strain typing had no apparent epidemiological link. The patient had a positive skin test to M. avium suggesting that the patient may have been infected with M. avium in childhood, before HIV-associated immunodeficiency impaired delayed type hypersensitivity. Reactivation of childhood M. avium infection is a possibility in this patient.

We have demonstrated that AIDS patients in Finland with levels of CD4 cytopenia comparable with other cohorts in developed countries have higher rates of disseminated infection with NTM than described previously. As a consequence of this study, prophylaxis against MAC infections is offered to our patients with CD4 counts < 50 x 10⁹/l. Our observations at autopsy support the earlier finding that most AIDS patients with disseminated MAC infection have persistent tissue infection while on therapy and underscore the need for long term treatment.15 We found the risk of disseminated NTM infection was strongly associated with urban residence. This finding, together with two clusters of geographically and temporally related urban infections, suggests an increased risk of exposure to NTM in treated water sources among urban residents with AIDS. Lifelong urban residence may also be associated with decreased childhood exposure to environmental sources of NTM, and less naturally acquired immunity to NTM before the onset of AIDS.

Acknowledgements

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References

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