Incidence and molecular typing of *Mycobacterium kansasii* in a defined geographical area in Catalonia, Spain

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SUMMARY

A retrospective population-based study was conducted between January 1990 and December 1998 to investigate the incidence of *Mycobacterium kansasii* disease and the heterogeneity of the isolates in a well-defined geographical area in Catalonia, Spain. A total of 136 patients were identified. Overall incidence and incidence in AIDS patients was 1.5 (95% CI 1.2–1.8) and 1089.6 (95% CI 689–1330) cases/100,000 persons per year respectively, which is comparable to that reported from most of other geographical areas. Surprisingly, although 7 subtypes of *M. kansasii* have been consistently reported, in the present study 91 of the 93 isolates (97.8%) tested for genotype were subtype I, regardless of HIV status of the patients. In conclusion, the high rate of infection observed in the AIDS population contributes significantly to the burden of the *M. kansasii* disease in our area. *M. kansasii* disease in our geographical area was almost exclusively caused by subtype I regardless of HIV status.

INTRODUCTION

*Mycobacterium kansasii* is one of the most frequent non-tuberculous mycobacterial pathogens isolated from clinical specimens. It has been recovered in almost every part of the world; however, its incidence varies significantly from area to area [1, 2]. Annual rates reported range from 0.5 cases/100,000 in non-endemic areas to figures as high as 34.3 cases/100,000 in certain parts of Central Europe [3–5]. The advent of the acquired immunodeficiency syndrome (AIDS) epidemic has substantially changed the scenario of *M. kansasii* infections: among human immunodeficiency virus (HIV)-infected subjects, incidence has been estimated to be far higher than in the general population, and disseminated disease is frequently found [2, 6–8].

The heterogeneity of the *M. kansasii* group has recently been demonstrated [9–12]. To date, seven subtypes (I–VII) have been identified by PCR–restriction fragment length polymorphism (PCR–RFLP) of the *hsp65* gene [11–14]. This heterogeneity may have pathogenic, clinical and epidemiological implications, as the available data have suggested [11, 12, 14]. Although a variety of subtypes have been identified in different geographical areas in Europe [12, 14], subtype I seems to be the most frequent pathogenic subtype isolated. However, little is known concerning many epidemiological features such as its natural reservoir, its transmission routes and geographical differences.

The aims of our study were to investigate the incidence of *M. kansasii* disease and to assess its...
heterogeneity in a well-defined geographical area in Catalonia, Spain.

**METHODS**

**Setting**

The health region ‘Costa de Ponent’ in Catalonia, Spain, has seven hospitals serving 104 municipalities and a population of 1,153,851. The referral centre for the area is the Hospital Universitari de Bellvitge (HUB), a 1000-bed teaching hospital which admits only adult patients. Its Microbiology Department is the referral laboratory for the identification of mycobacteria.

**Design**

A retrospective, population-based study took place between January 1990 and December 1998. Two studies were performed (Fig. 1).

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**Fig. 1.** Study profile. On the right, the two substudies performed: (a) mean annual incidence in the health region; and (b) clinical and genotypic assessment according to HIV status.
Estimation of the incidence

All cases of *M. kansasii* disease, identified by reviewing the log of positive cultures of the HUB and a survey of the hospitals of the area, were included. Mean annual incidence was estimated using the average of the 1991 and 1996 censuses (1 134 474) [15]. Because the estimated number of HIV infections was not available for this area, we were unable to calculate the incidence in the whole HIV population. So incidence in these patients refers exclusively to those with a diagnosis of AIDS. For incidence in AIDS population, the estimates of the mean number of living AIDS patients per year in the health region, provided by the Centre for Epidemiologic Study of AIDS in Catalonia (CEESCAT), were used.

Molecular typing

This substudy was performed in 93 patients diagnosed and treated at the referral centre (HUB). These 93 patients came from every municipality with at least one case of *M. kansasii* disease. One isolate per patient was processed for genotyping.

Definitions

HIV-1 infection was made by antibody enzyme immunoassay (EIA) and confirmed by Western blot. Diagnosis of AIDS was established using the 1993 revision of the Centers for Disease Control and Prevention’s surveillance case definition [16]. Cases of *M. kansasii* disease were considered definite if they met the American Thoracic Society’s criteria for diagnosis of disease caused by non-tuberculous mycobacteria [17]. Probable cases were defined as at least one positive culture sputum, respiratory symptoms, resolution with specific therapy and no other concomitant infection. Disseminated disease was defined as isolation of *M. kansasii* from sites other than or in addition to lungs, skin, or cervical or hilar lymph nodes [17].

Microbiological studies

The clinical specimens from non-sterile body sites were processed following the conventional digestion-decontamination procedure [18]. Smears were stained by auramine–rhodamine fluorochrome. Positive slides were confirmed by the Ziehl–Neelsen stain [19]. Samples (0·5 ml) of specimens were inoculated into BACTEC 12B vials and onto Löwenstein–Jensen medium slants. Blood samples were inoculated into BACTEC 13A vials. All isolates were identified by conventional phenotypic methods (growth rate, pigmentation, and biochemical tests). In addition, DNA AccuProbe assay was used at the time of isolation [19]. Molecular typing was performed by PCR–RFLP analysis of the *hsp65* gene [12, 13, 20].

Statistical analysis

Qualitative comparisons were done using the $\chi^2$ test or Fisher’s exact test when appropriate. Differences between quantitative variables of the three
groups (HIV-1-positive, HIV-1-negative and HIV-1-unknown) were evaluated by Kruskal–Wallis test. A $P$ value of less than 0.05 was considered statistically significant. The statistical analysis was performed with SPSS software (version 6.6.2, SPSS, Chicago, IL, USA).

**RESULTS**

A total of 147 patients with isolates of *M. kansasii* were identified during the study period (Fig. 1). Of these 136 (92.5%) had evidence of disease: 49 patients (36%) were HIV-1-positive, 34 patients (25%) were HIV-1-negative and 53 patients (39%) had no serological HIV test performed.

**Incidence of *M. kansasii* disease**

The overall incidence in the ‘Costa de Ponent’ health region was 1.5 cases/100,000 persons per year (95% CI 1.2–1.8) and 1089.6 cases/100,000 AIDS patients per year (95% CI 688.6–1330.2). The

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Fig. 3. Map of the health region ‘Costa de Ponent’, Catalonia, Spain, showing its 104 municipalities and the seven hospitals surveyed. The box in the top right-hand corner shows the location of the health region in Catalonia. HUB, Hospital Universitari de Bellvitge.
distribution of incidence throughout the study period is shown in Figure 2: rates of *M. kansasii* disease ranged from 1.2 in the 1994–1995 period to 1.8 in the 1992–1993 period in the general population, and from 802 in the 1998 period to 1686 in the 1992–1993 period in the AIDS population. Twenty-one of the 104 municipalities accounted for the total number of cases of *M. kansasii* disease in the health region studied. The two municipalities with the highest rates of the disease (5.1 and 2 cases/100,000 persons per year respectively) had a mean higher population density than the rest of the health region (1323 and 20,569 inhabitants/km² respectively vs. 204, *P*<0.05) (Fig. 3).

**Molecular typing**

Genotyping was performed in the 93 patients diagnosed and treated at the referral centre (HUB). These patients came from every municipality with at least one case of *M. kansasii* disease. The genotype was type I in 91 (97.8%) of the 93 patients studied; two patients, both HIV-infected, had type VI isolates. PCR–RFLP (*hsp65*) patterns of the two subtypes of *M. kansasii* isolated are shown in Table 1.

**Clinical characteristics**

As for clinical, radiographic and microbiological findings, 89 (95.7%) of 93 had a definite diagnosis. The probable cases corresponded to 4 HIV-positive patients with respiratory symptoms, minor abnormalities on chest radiograph (slight bilateral reticular interstitial pattern), positive sputum cultures, response to specific therapy and no other microorganisms isolated. Of the 39 HIV-positive patients, 23 (58.9%) were intravenous drug users. The median CD4 count was 24 cells/mm³ (range, 1–220) and all but one had a previous diagnosis of AIDS. HIV-infected patients were younger than non-infected subjects (median age, 32 vs. 54 years, *P*<0.001), had more disseminated disease (43.6 vs. 4.2%, *P*<0.001), and less frequently cavitation on chest radiograph (22.9 vs. 62.5%, *P*<0.001). The main clinical, radiographic and microbiological findings of the 93 patients evaluated are shown in Table 2.

**DISCUSSION**

The incidence of *Mycobacterium kansasii* disease found in our area is comparable to the figures reported in United States [1–3] and far lower than that observed in an endemic area in Central Europe [4, 5]. However, there was a substantial disparity in distribution, with clustering in urban and densely populated areas. Indeed, a small number of municipalities accounted for the majority of the cases, and others did not present a single diagnosis during the study period. The municipalities where incidence was highest are industrial areas with high population densities, a finding that corroborates previous reports suggesting an association of these factors with clustering of *M. kansasii* infection [2, 4, 21]. Incidence among AIDS patients was more than 700-fold higher than in the general population. However, a substantial decline of *M. kansasii* was observed among HIV-1-infected patients in the last period of the study, a trend that has been confirmed in another study [22].

Interestingly, and somewhat unexpectedly, all but two patients were infected with type I. Although type I is the subtype that is most frequently isolated from clinical specimens, the other subtypes, particularly II, have also been found in humans. In fact, types I and II account for more than 90% of *M. kansasii* infections in humans [11, 12, 14]. Extrapolating from other reports in several European countries such as Italy, France, Germany and Switzerland, we would have expected isolates of type II to account for at least 30% of infections in our series [11–14]. However, our results seem to confirm the absence of type II in our area, as Alcaide et al. [12] reported a few years ago. There are three possible reasons for the absence of *M. kansasii* subtypes other than type I in our study. First, there may have been differences in the microbiological methods used in the laboratory; nevertheless, the conventional and molecular techniques used in the present study readily detect and identify the different subtypes of *M. kansasii*. Secondly, there may have been differences in the susceptibility to infection of the population exposed in a certain geographical area. There is substantial evidence of an association between *M. kansasii* other than type I and HIV-1-positive status [14, 23]. Since, a high

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Table 1. PCR–RFLP (*hsp65*) patterns of *M. kansasii* isolated after digestion with BstEII and HaeIII

<table>
<thead>
<tr>
<th><em>M. kansasii</em> subtype</th>
<th>Bands (bp) by BstEII</th>
<th>Bands (bp) by HaeIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>240, 210</td>
<td>140, 105, 80</td>
</tr>
<tr>
<td>VI</td>
<td>240, 135, 85</td>
<td>140, 105, 70</td>
</tr>
</tbody>
</table>
proportion of patients (42%) in our study were infected with HIV-1, some infections with other subtypes should have been found, as has recently been reported [14]. Thirdly, the disparity in the geographical distribution of subtypes observed may reflect the existence of different ecosystems for these microorganisms [14]. This explanation seems to be the most plausible hypothesis. Therefore, the M. kansasii disease in our geographical area must be due to the existence of only one type (subtype I) in the environment.

As for the clinical picture, our findings are similar to those reported elsewhere in the literature. Chronic, progressive and cavitary lung disease, closely resembling tuberculosis, is the most frequent clinical presentation of M. kansasii disease in non-HIV subjects [2, 6, 24–27], and extrapulmonary disease is uncommon in the absence of a concurrent immunosuppression [26]. In HIV-1-infected patients, M. kansasii disease appears in the context of advanced immunosuppression and frequently involves extrapulmonary sites [28–32].

Although genotyping was not performed in the total isolates recovered, we do not think that this is a significant limitation in the present study. The results obtained from the molecular typing of the 93 isolates available are representative of the whole geographical area investigated. In fact, the genotype was assessed in at least one patient from each municipality with cases of M. kansasii.

In summary, the incidence of M. kansasii disease in our geographical area is comparable to that observed in most regions reported. The distribution of the cases was heterogeneous, with clustering in urban and densely populated areas. The rate of infection among AIDS patients was far higher than the incidence observed in the general population. Interestingly, M. kansasii disease in our geographical area was almost exclusively caused by subtype I, regardless of HIV status.

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