Clinical and Epidemiological Study of Disease Caused by *Mycobacterium kansasii* in the Metropolitan Area of Bilbao, Spain

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OBJECTIVE: Epidemiological description of individuals from whom *Mycobacterium kansasii* isolates were obtained in respiratory samples, and analysis of the isolates using molecular biological techniques.

MATERIAL AND METHODS: A descriptive retrospective/prospective study was carried out from January 1994 to April 2002 in Basurto Hospital and Santa Marina Hospital and from January 2000 to April 2002 in Cruces Hospital, Galdakao Hospital, and San Eloy Hospital. Diagnosis of the disease was performed according to American Thoracic Society criteria; other definitions were also applied to allow inclusion of all cases. Disease caused by *M kansasii* in patients who were not infected with the human immunodeficiency virus (HIV) was compared with disease caused by *Mycobacterium tuberculosis* in a control group. Polymerase chain reaction was applied with analysis of restriction fragment length polymorphisms to differentiate between species of mycobacteria and classify them into genotypes. Amplified fragment length polymorphisms were used to recognize clones within each genotype.

RESULTS: The patient charts of 334 patients in which an isolate of *M kansasii* had been recorded were reviewed. We considered 220 patients to be suffering from disease caused by *M kansasii* (American Thoracic Society criteria along with probable disease according to established definitions). The disease was more frequent in male patients (n=185; 84.1%) and in individuals who were not HIV positive (n=184; 83.6%). The highest incidence of disease in the Bizkaia region was found in Margen Izquierda-Encartaciones, where the rate was 8.05 per 100 000 inhabitants. In the Bilbao area, the highest rate was found in the districts lying on the outskirts. The underlying diseases were tuberculosis (20.5%), chronic obstructive pulmonary disease (25.9%), pulmonary neoplasia (7.7%), silicosis (0.9%), chronic liver disease (11.4%), and duodenal ulcer (8.6%). The most frequent constitutional symptoms were fever (39.1%), loss of appetite (23.2%), and weight loss (33.3%). Among the respiratory symptoms, the most outstanding were cough (70.9%) and expectoration (62.3%). The most frequent radiographic patterns were cavitation and pulmonary infiltration. The most common treatment regimen was rifampicin, isoniazid, and ethambutol (43.4%), and the average duration was 12 months in patients who were HIV negative. Analysis of antibiotic sensitivity, performed on 56 strains, revealed that 100% were resistant to isoniazid, while none displayed rifampicin resistance. Thirty-four cases of disease caused by *M kansasii* were compared with 68 cases of tuberculosis, all of them without HIV infection. The comparison revealed a predominance of smokers, respiratory symptoms, and cavitation in patients with disease caused by *M kansasii*. The majority of the isolates (98.5%) corresponded to genotype I. A total of 8 clones were obtained; the clones designated 1 and 3 were more common in HIV-positive and HIV-negative individuals respectively.

CONCLUSIONS: In recent years, there has been an increase in the number of patients with disease caused by *M kansasii* in the province of Bizkaia. The disease is more frequent in male patients, individuals who are HIV negative, and in urban areas. In addition, more respiratory symptoms and a higher incidence of cavitation were found in patients with disease caused by *M kansasii* than in those with tuberculosis. Genotype I is the most common isolate, and clones 1 and 3 affect 80% of patients suffering from the disease.

Key words: *Epidemiology. Human immunodeficiency virus.* M kansasii.

Estudio clinicoepidemiológico de la enfermedad por *Mycobacterium kansasii* en el área urbana de Bilbao

OBJETIVO: Descripción epidemiológica de los individuos con aislamiento de *Mycobacterium kansasii* en muestras respiratorias y análisis de estos aislamientos mediante técnicas de biología molecular.

MATERIAL Y MÉTODOS: Se realizó un estudio retrospectivo, prospectivo y descriptivo de enero de 1994 a abril de 2002 en los hospitales de Basurto y Santa Marina, y de enero de 2000 a abril de 2002 en los hospitales de Cruces, Galdakao y San Eloy. Se aplicaron los criterios de la American Thoracic Society para el diagnóstico de enfermedad y se utilizaron otras definiciones para abarcar todos los casos. Se comparó la enfermedad por *M. kansasii* en pacientes sin infección por el virus de la inmunodeficiencia humana (VIH) con un grupo control con enfermedad por *Mycobacterium tuberculosis*. Se aplicó la reacción en cadena de la polimerasa con análisis de RFLP *(restriction fragment-length polymorphisms)* para diferenciar las especies de micobacterias y subtipificación en genotipos, y la AFLP *(amplified fragment-length polymorphisms)* para reconocer clones dentro de cada genotipo.

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RESULTADOS: Se revisaron 334 historias clínicas de pacientes en los que existía un registro de aislamiento microbiológico de *M. kansasii*. Consideramos que 220 eran enfermos (criterio de la American Thoracic Society más enfermedad probable de definiciones creadas). La enfermedad era más frecuente en varones (n = 185; 84,1%) y en personas sin infección por el VIH (n = 184; 83,6%). La tasa de incidencia de enfermedad más alta en la comarca de Bizkaia fue en Margen Izquierda-Encartaciones, con un 8,05 por cada 100.000 habitantes, y en el Área de Bilbao, en los distritos de la periferia. Las enfermedades de base fueron: tuberculosis (20,5%), enfermedad pulmonar obstructiva crónica (25,9%), neoplasia de pulmón (7,7%), silicosis (0,9%), hepatopatía crónica (11,4%) y gastrectomía (8,6%). Los síntomas constitucionales más frecuentes fueron: fiebre (39,1%), anorexia (23,2%) y disminución de peso (33,3%). Entre los síntomas respiratorios destacaron la tos (70,9%) y la expectoración (62,3%). Los patrones radiológicos más frecuentes fueron cavitación e infiltrados pulmonares. La pauta de tratamiento más habitual fue rifampicina, isoniacida y etambutol (43,4%), y el tiempo medio de duración fue de 12 meses en las personas sin infección por el VIH. En el estudio de sensibilidad realizado en 56 cepas, el 100% fue resistente a isoniacida y ninguna mostró resistencia a rifampicina. Se compararon 34 casos de enfermedad por *M. kansasii* con 68 casos de tuberculosis, todos sin infección por el VIH, y se obtuvieron los siguientes resultados: predominio de fumadores, de síntomas respiratorios y de cavitación en los pacientes con enfermedad por *M. kansasii*. El 98,5% de los aislamientos pertenecieron al genotipo I. Se obtuvieron un total de 8 clones; el clon denominado 1 fue más frecuente en personas con infección por el VIH y el denominado 3 en los que no la presentaban.

CONCLUSIONES: Se ha registrado un aumento del número de pacientes con enfermedad por *M. kansasii* en la provincia de Bizkaia en los últimos años. Dicha enfermedad es más frecuente en varones, personas sin infección por el VIH y zonas urbanas. Asimismo, se han encontrado más síntomas respiratorios como manifestaciones clínicas y mayor presencia de cavitación como hallazgo radiológico en la enfermedad por *M. kansasii* al compararla con la tuberculosis. El genotipo I es el aislado con más frecuencia, y los clones 1 y 3 afectan al 80% de los individuos enfermos.

Palabras clave: *Epidemiología. Virus de la inmunodeficiencia humana.* M. kansasii.

Introduction

Environmental mycobacteria currently represent between 10% and 30% of all mycobacteria isolated in the majority of microbiology laboratories. They are found in soils, water, animal products, and various foodstuffs. From these sources they can contaminate clinical samples, transiently colonize body surfaces, and under certain circumstances, cause disease. Eighty percent of the isolates are clinically significant. Both the natural reservoir, which could be water,[1,2] and the routes of human infection (possibly inhalation of aerosols[3,4]) are unknown. Disease is found mainly in metropolitan areas and is associated with mining and regions of heavy industry. Contagious transmission has not been demonstrated. Differences in presentation exist according to serological status for the human immunodeficiency virus (HIV). Thus, in patients who are not infected with the virus, the clinical and radiologic characteristics are similar to those of tuberculosis. In individuals infected with HIV, the disease appears in advanced stages of immunodeficiency. A wide geographic variability has been demonstrated, with the incidence ranging between 0.5 and 1 case per 100 000 person years, and up to 16.6 cases per 100 000 in some areas of Eastern Europe.[5] In Spain, the disease has been encountered mainly in La Rioja, Andalusia, Asturias, Catalonia, and the Basque Country.

According to the Microbiological Information System of the Autonomous Community of the Basque Country (SIMCAPV),[6] the number of isolates of *Mycobacterium kansasii* reported by hospitals in Bizkaia is much higher than that of neighboring provinces, where it is almost never isolated. This situation, observed in recent years, led us to undertake a review of our cases in an effort to describe the epidemiological characteristics of our patients, determine whether they fulfill American Thoracic Society (ATS) criteria, assess progression following diagnosis and treatment, differentiate between populations with and without HIV infection, make comparisons with tubercular disease, and relate clinical data to epidemiological typing.

Materials and Methods

A descriptive retrospective/prospective study of *M kansasii* isolates was carried out from January 1994 to April 2002 in Basurto Hospital and Santa Marina Hospital, and from January 2000 to April 2002 in Cruces Hospital, Galdakao Hospital, and San Eloy Hospital. A clinical protocol was designed for data collection. The ATS criteria[7] were applied along with other adapted criteria created by us in order to group all of the cases, as well as the 1993 criteria of the Center for Disease Control (CDC) for the diagnosis of HIV or AIDS.[8] The variables collected in the protocol (patient details, medical history, clinical manifestations, radiographic findings, treatment initiated, and disease progression) were analyzed in the Epi-Info 2000 database (CDC, Atlanta, GA, USA) for both the HIV-positive and the HIV-negative population. The clinical features of the two groups were compared using a χ^2 test (with the Yates correction where necessary) and the Fisher exact test. Statistical significance was established at $P<.05$.

A group of 34 HIV-negative patients who presented with *M kansasii* disease was compared with a control group of 68 HIV-negative patients with disease caused by *Mycobacterium tuberculosis*. Patients in the control group were diagnosed in the same period and had a similar sex and age distribution.

We created a series of definitions to embrace all of the cases that did not meet the ATS criteria, as has been done by other authors[9]:

– Definitive disease: when all of the ATS criteria were met.

– Probable disease: all ATS criteria met except the microbiological criteria (but with more than one isolate and/or presence of bacilli).

– Possible disease: symptoms and radiographic findings consistent with either *M kansasii* disease or another disease, with a single microbiological isolate.

– Probable definitive disease: symptoms and microbiological criteria met but with normal findings in chest radiography (not those defined by the ATS).

– Lung colonization: a single microbiological isolate.

– Gastrointestinal colonization: isolates only from feces.

Using the polymerase chain reaction with analysis of restriction fragment-length polymorphisms (RFLP),[10,11] we amplified the hsp65 gene with the primers Tb11 and Tb12. A 439 bp fragment was obtained and digested with *Bst*II and *Hae*III. Amplified fragment-length polymorphisms (AFLP)[12,13] were assessed by selective amplification of fragments of *Apa*I-digested DNA using 3 different primers (A, B, and C). The combination of patterns generated with the 3 primers defined a clone.

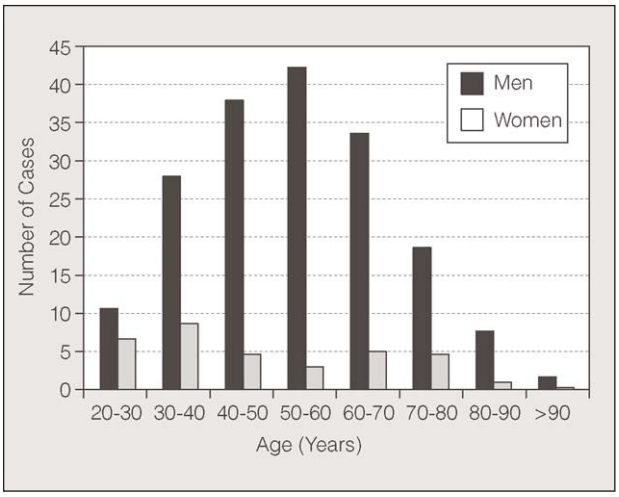

Figure 1. Age distribution of *M kansasii* disease according to patient sex.

Results

The medical histories of 334 cases in which an isolate of *M kansasii* had been recorded were reviewed. Of these, we found that 199 patients (56.6%) met the ATS criteria for the definition of *M kansasii* disease. Using our own definitions, 21 patients (6.3%) fell into the category of probable disease. We considered both this group and the group of patients that met the ATS criteria to present authentic disease, and we therefore refer to 220 patients with *M kansasii* disease in the statistical analysis. Table 1 shows the percentages corresponding to each of the definitions.

Epidemiological Characteristics of M kansasii *Disease*

Of the 220 patients with *M kansasii* disease, 185 (84.1%) were men and 35 (15.9%) were women (ratio of men to women, 5:1). The distribution according to serological status for HIV was 184 (83.6%) HIV-negative patients and 36 (16.4%) HIV-positive patients, a ratio that is similar to that for men to women. The mean age was 53.01 years (range, 19-94 years) for men and 48.2 years (range, 26-83) for women. A peak incidence was observed for men between 50 and 60 years old. In women, although the age distribution was more homogeneous, there was a clear increase between 30 and 40 years. Figure 1 shows the age distribution of the disease according to patient sex. The distribution of the disease by district and serological status for HIV in the 126 patients resident in Bilbao is shown in Table 2. The highest incidence was found in districts 2 (Uribarri), with an incidence of 5.4 per 100 000 person years, 5 (Casco Viejo), with an incidence of 6.94 per 100 000, and 7 (Rekalde), with an incidence of 4.78 per 100 000. The lowest incidence was found in districts 6 (Abando), with an incidence of 1.89 per 100 000, and 1 (Deusto), with an incidence of 2.67 per 100 000.

In Figure 2, which shows the distribution of patients in the metropolitan area of Bilbao, it can be seen that the cases are grouped in the periphery or form a cordon around the center of the city, which remains clear.

The distribution of the cases of *M kansasii* disease between 2000 and April 2002 for the health districts of Bizkaia was as follows: 46 (38.3%) lived in the Bilbao area, 48 (40%) in Margen Izquierda-Encartaciones, 13 (10.8%) in Uribe, 12 (10%) in Interior, and 1 (0.8%)

TABLE 1
Classification of Patients With *Mycobacterium kansasii* Isolates

	Number of Cases (%)
Colonization	58 (7.4)
Possible	19 (5.7)
Probable-definitive	37 (11.1)
Probable	21 (6.3)
Definitive	199 (59.6)

TABLE 2
Distribution of 126 Patients in Bilbao With *Mycobacterium kansasii* Disease According to District and HIV Status*

HIV	District 1: Deusto	Distrito 2: Uribarri	District 3: Otxarkoaga	District 4: Begoña	District 5: Casco Viejo	District 6: Abando	District 7: Rekalde	District 8: Basurto	Total
Positive	2	3	3	3	8	1	6	0	26
Negative	11	10	11	12	22	8	14	10	98
Total	13	13	14	15	30	9	20	10	124
Incidence (per 100 000 person years)	2.67	5.4	5.15	3.47	6.94	1.89	4.78	3.31	

*HIV indicates human immunodeficiency virus.

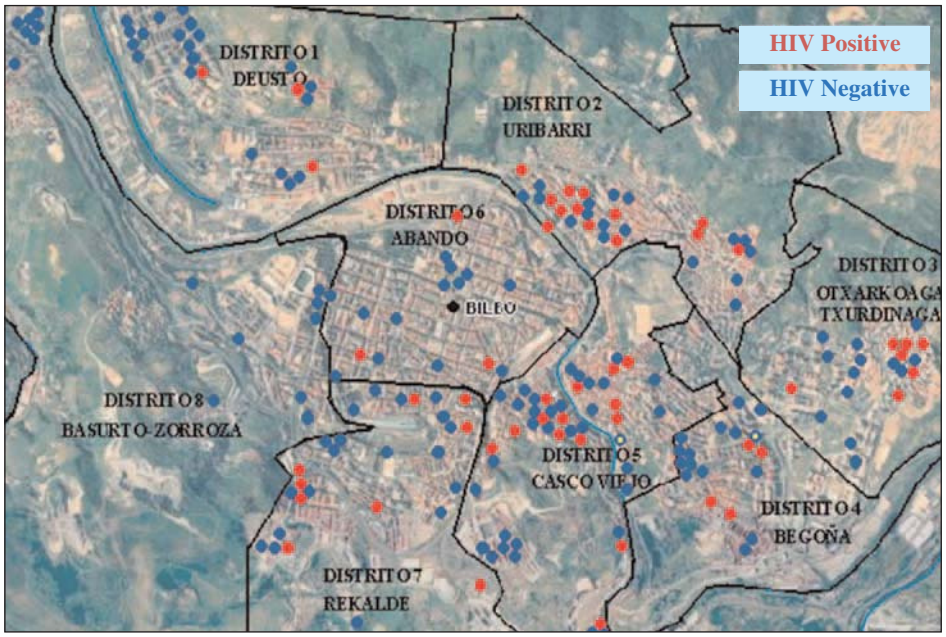

Figure 2. Map of Bilbao, Spain.

lived outside the province of Bizkaia (Table 3). The rates per 100 000 individuals were highest in Margen Izquierda-Encartaciones (8.05 per 100 000), followed by Bilbao (6.49 per 100 000). Uribe and Interior showed the lowest rates (3.24 and 2.05 per 100 000, respectively).

A single case was found in which another family member was affected: a man was diagnosed with the disease months after it was diagnosed in his mother.

We did not find statistically significant differences in the underlying diseases, shown in Table 4, according to serological status for HIV, except in the case of individuals with chronic obstructive pulmonary disease, which showed a higher prevalence in HIV-negative patients, and in the greater number of HIV-positive patients with chronic liver disease. In terms of toxic habits, 163 (74.1%) patients were active smokers or had smoked in the past. The clinical presentation of the patients with *M kansasii* disease was as follows: The most common constitutional symptoms were fever (86 patients; 39.1%) and weight loss (73 patients; 33.3%). Notable among the respiratory symptoms were presentation of the disease with cough in 156 cases (70.9%) and with expectoration in 137 (62.3%). Symptoms of increased dyspnea (53 patients; 24.1%), chest pain (45 patients; 20.5%), and hemoptysis (40 patients; 18.2%) were less frequent. Statistically significant differences in the presentation of the disease between HIV-positive and HIV-negative patients were observed for fever, weight loss, and loss of appetite, as general symptoms, and cough and expectoration, as respiratory symptoms.

The most commonly seen radiographic patterns were pulmonary infiltrates and cavitation. Both findings were seen in 106 patients. Although the distribution of affected pulmonary areas was variable, both infiltrates and cavitation were most commonly associated with the right upper lobe. Likewise, unilateral involvement was more frequent than bilateral. There were no significant differences in the radiographic manifestations of HIV positive or negative patients.

Of the 220 patients considered to present *M kansasii* disease, 201 (34 HIV positive and 167 HIV negative)

TABLE 3
Distribution of *M kansasii* Disease According to Health District

Health District	Number of Cases	Rate (per 100 000)
Bilbao	46	6.49
Margen Izquierda-Encartaciones	48	8.05
Uribe	13	3.24
Interior	12	2.05

TABLE 4
Underlying Disease of 220 Patients with *Mycobacterium kansasii* Disease*

Underlying Disease	HIV Status		Total (%)
	Positive	Negative	
Tuberculosis	9	36	45 (20.5)
COPD	0	57	57 (25.9)
Silicosis	0	2	2 (0.9)
Lung cancer	0	17	17 (7.7)
Other neoplastic disease	0	15	15 (6.8)
Chronic liver disease	10	15	25 (11.4)
Duodenal ulcer	1	18	19 (8.6)
HIV positive (without other disease)	16	0	16 (7.3)
None or not reported	0	0	24 (10.9)
Total	36	160	220

*COPD indicates chronic obstructive pulmonary disease.
HIV, human immunodeficiency virus.

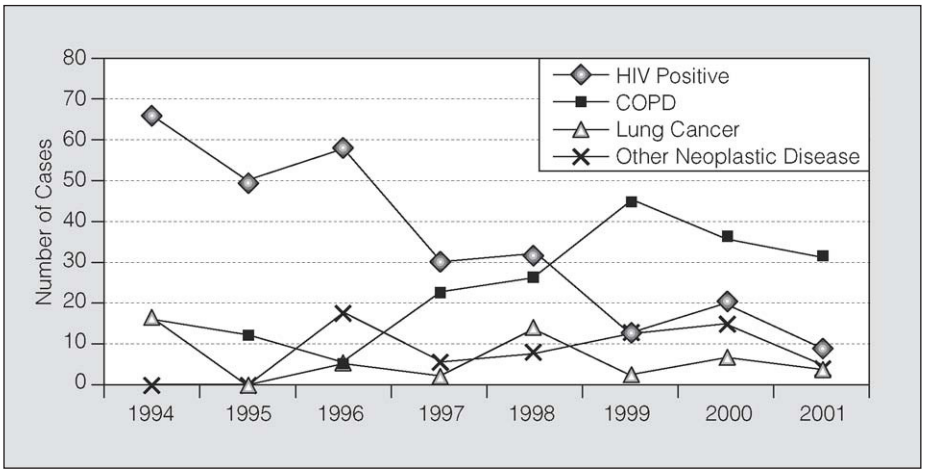

Figure 3. Progression of patients with *M kansasii* isolates. HIV indicates human immunodeficiency virus; COPD, chronic obstructive pulmonary disease.

received antimycobacterial treatment. Of the 19 in whom treatment was not initiated (2 HIV negative and 17 HIV positive), 8 died prior to diagnosis of the disease. The most frequently established initial treatment regimen (Table 5) was rifampicin, isoniazid, and ethambutol, prescribed in 85 cases (38.6% of the patients with *M kansasii* disease). Of those whose treatments were subsequently changed, in 73 (33.2%) cases the change was to specific treatment for *M kansasii* when this species was isolated. The most common regimens prescribed subsequently are shown in Table 6.

The mean duration of treatment was 13 months (range, 2-18) in the group of patients who were infected with HIV and 12 months (range, 1-24) in the HIV-negative group. In Galdakao hospital, a 9-month treatment was established with only 2 drugs (rifampicin and ethambutol). The clinical progression was good for 101 of the 114 treated patients in whom data could be collected (6 HIV positive and 95 HIV negative); 24 died (13 HIV positive and 11 HIV negative). The radiographic progression was favorable in 62 of the 121 treated patients in whom data could be collected (8 HIV positive and 54 HIV negative). The disease was reactivated in 7 patients (1 HIV positive and 6 HIV negative). *M kansasii* was isolated from nonpulmonary sites in 7 cases (6 HIV positive and 1 HIV negative).

The characteristics of the patients from whom *M kansasii* isolates were obtained in respiratory samples at the Basurto and Santa Marina hospitals changed between 1994 and 2002. Initially most patients were HIV positive, but this number was reduced over time and the group of patients with chronic obstructive pulmonary disease assumed greater significance. The percentage of patients with neoplasms remained unchanged (Figure 3).

Epidemiological Characteristics of the Group With Probable-Definitive Disease

As described in the materials and methods section, the group of patients with probable-definitive disease presented normal chest radiographs (they did not meet the radiographic criteria of the ATS for the definition of *M kansasii* disease).

The group contained 37 patients (30 men and 7 women; 13 were HIV positive and 24 HIV negative), all of whom showed a radiographic pattern that was considered normal according to ATS criteria. Treatment was received by 32 patients. Of the 5 patients in whom treatment was not implemented, all of them HIV negative, 2 had died prior to diagnosis of the disease. Of the patients who were treated, 7 died (5 HIV positive and 2 HIV negative). Reactivation of the disease was observed in 1 patient (HIV negative). The disease spread, as shown by isolates of *M kansasii* obtained in nonpulmonary samples, in 5 patients, all of whom were HIV positive.

Epidemiological Characteristics of the Group With Possible Disease

This group contained 19 patients (7 HIV positive and 12 HIV negative). Of these patients, 9 received treatment (4 HIV positive and 5 HIV negative).

TABLE 5
Most Frequently Established Treatments (Initial Regimen)*

Initial Regimen	Frequency (%)
r + h + e	85 (38.6)
r + h + pz	75 (34)
r + h + pz + e	20 (9)
r + e	7 (3.2)

*Total number of patients with *M kansasii* disease, 220. r indicates rifampicin; h, isoniazid; pz, pyrazinamide; and e, ethambutol.

TABLE 6
Most Frequent Subsequently Established Treatment Regimens*

Treatment	Frequency (%)
r + h + e	73 (33.2)
r + h	2 (0.9)
r + e	2 (0.9)
h + e + of	2 (0.9)

*Total number of patients with M kansasii disease, 220. r indicates rifampicin; h, isoniazid; e, ethambutol; and of, ofloxacin.

Colonization

M kansasii isolates were considered to be colonizations in 52 patients (15 HIV positive and 37 HIV negative). Seven of these patients received treatment (5 HIV positive and 2 HIV negative). Status as an HIV carrier at the time of treatment initiation was statistically significant in this group.

Sensitivity Testing of M kansasii

Analysis of 56 strains was performed in a reference center; all displayed resistance to isoniazid (1 µg/mL), streptomycin, pyrazinamide, and paraaminosalicylic acid. Resistance to kanamycin was observed in 47 strains (97.7%), 3 (5.6%) were resistant to cycloserine, 1 (1.8%) was resistant to ethionamide, and 7 (12.5%) to ethambutol. None of the strains were resistant to rifampicin (1 µg/mL).

Differences Between M kansasii *Disease and Disease Caused by M tuberculosis*

We compared 34 patients suffering from *M kansasii* disease—22 men and 12 women, with a mean age of 54.3 (range, 19-71) and 52.5 years (range, 26-75), respectively—with 68 patients suffering from disease caused by *M tuberculosis*—44 men and 24 women, with a mean age of 54.8 (range, 20-71) and 53.16 (range, 18-85), respectively. All of the patients included in the comparison were HIV negative. No significant differences in medical history were found between the two groups. A higher proportion of smokers was found in patients with *M kansasii* disease.

The radiological pattern at the time of diagnosis revealed a higher proportion of patients presenting cavitation in the group with *M kansasii* disease than in patients in whom *M tuberculosis* was isolated. Pleural effusion was a more frequent manifestation of *M tuberculosis* disease.

M kansasii disease presented with more respiratory symptoms than did disease caused by *M tuberculosis*. Cough, expectoration, and hemoptysis were encountered more frequently in patients with *M kansasii* disease than in those suffering from disease caused by *M tuberculosis*, an effect that was statistically significant. No significant differences in the presentation of general symptoms were seen between the 2 diseases.

Molecular Biological Analysis of M kansasii *Isolates*

Typing was performed in 135 of the 334 patients with *M kansasii* isolates.

Polymerase chain reaction-RFLP technique. One hundred and thirty-three of the isolates (98.5%) were demonstrated to belong to genotype I and 2 isolates (1.5%) to genotype II. No other genotypes were obtained in the clinical samples. The distribution according to the modified ATS classification applied in the clinical study was as follows: isolates from the cases that presented disease (definitive and probable) all belonged to genotype I, while cases with genotype II were considered to be either colonization or disease presenting with normal radiography (probable-definitive).

AFLP technique. A total of 8 clones (1-8) of genotype I were obtained in the group of 135 isolates studied. Considering the isolates irrespective of the ATS classification, clones 1 and 3 were the most common, showing hardly any difference between the HIV-positive and HIV-negative populations. When considered in terms of the modified ATS classification, clone 3 was the most common isolate in the definitive disease group. Seven clones were found within the group designated "disease." Clones 1 and 3 affected 80% of patients within this group, with a higher proportion of HIV-negative patients corresponding to clone 3 (51.2%) and a higher proportion of HIV-positive patients corresponding to clone 1 (58.8%). The distribution of clones according to age, sex, districts of the Bilbao area, Bizkaia regions, and hospitals in Bizkaia was heterogeneous. Microbiological analyses performed in isolates from patients in the same family unit (3 isolates from the mother and 1 from the son) revealed that all belonged to genotype I. According to AFLP analysis, the first isolate from the mother belonged to clone 1, while the subsequent isolates from the mother and the isolate from the son belonged to clone 3.

Discussion

This study is one of the few studies of *M kansasii* undertaken in Spain to employ molecular biological analysis alongside assessment of clinical implications. This allowed us to determine that 98.5% of the strains belonged to genotype I, the most prevalent isolate of *M kansasii* of human origin worldwide.[14-16] This is in contrast to a study undertaken in Switzerland[17] in which genotypes other than genotype I were isolated and genotype II was associated with HIV carriers. Alcaide et al[14] found only genotype I in their strains. We identified 8 clones within genotype I. Two appeared in 80% of individuals with the disease; one of these was more common in HIV-negative patients and the other in carriers of HIV. The distribution of the remaining clones was heterogeneous. It is important to define the genotype of *M kansasii* isolates for epidemiological studies of pathogenicity, modes of transmission, and modes of disease acquisition.

According to the Microbiology Newsletter of the Basque Autonomous Community, *M kansasii* isolates are confined to the province of Bizkaia and are not found in neighboring provinces. Likewise, the rates encountered differ according to the area. The health district of Margen Izquierda-Encartaciones has the

highest rate of disease and Interior the lowest. All districts of Bilbao were affected, with a higher rate in peripheral areas, which are the most heavily populated and have fewer economic resources. In turn, the cases were grouped according to neighborhoods, with the center of the city being least affected. However, we have been unable to distinguish between the possibility that there is less disease in this area or that patients are treated privately. The results of a study undertaken in San Francisco in which patients were localized on a map of the city can be extrapolated to those found here.[18] The figures in Margen Izquierda-Encartaciones were intermediate, with a rate of 8.05 cases per 100 000 inhabitants, when compared with data obtained in the former Czech Republic (17.6 per 100 000) and the USA (2.4 per 100 000).[5]

We observe a predominance of the disease in men, a finding that is in agreement with other studies, with the exception of that of Lillo et al,[9] where the proportions are much closer. In our group, we find a high percentage of HIV-negative patients, while the majority of studies make reference to HIV carriers.[19,20] We have identified 2 types of patient: those that present an underlying disease and others designated "healthy." The latter group represented 13%, and of those 74% were smokers, a figure that is similar to the series of Evans et al.[21] Among the "healthy" patients, the percentage of patients who were smokers was lower than in the study of Bloch et al[18] (40.3%).

M kansasii infection is acquired from environmental sources and does not appear to be transmitted between individuals. Nevertheless, some cases of intrafamilial infection have been reported[22] and in this study we found a case involving a mother and son. The mother was infected with 2 different strains, both of genotype I. The first strain belonged to clone 1 and the second to clone 3. The son was also infected with clone 3, one of the most common. This leads us to question whether it is a case of person to person transmission or infection from a common source. The observation of 2 clones appearing at separate points in time in the case of the mother leads to a new question regarding *M kansasii* disease: should we talk about reactivation, reinfection, or coinfection with 2 clones and selection of 1 clone by treatment? More in-depth molecular understanding of microorganisms facilitates the discovery of clinical situations that might otherwise remain hidden.

Disease caused by *M kansasii* is similar to pulmonary tuberculosis in terms of clinical and radiographic presentation.[23,24] The most common radiographic findings in this study were cavitation, predominantly in the right upper lobe, and pulmonary infiltrates; both findings were independent of HIV status. We found a substantial group of patients who displayed normal chest radiography (37 patients: 13 HIV carriers and 24 who were HIV negative).

The exact incidence of infection by *M kansasii* would have to be defined in various populations along with an evaluation of the clinical significance of a single isolate. The usefulness of the ATS definition of *M kansasii* disease has been questioned in relation to countries with a high incidence of tuberculous disease. The ATS criteria for defining a case are strict.[25,26] Based on these criteria, 59.6% of the patients included in our study would have the disease. If we add those patients classified as having probable disease, the percentage would rise to 69.5%, and if we add those with normal chest radiographs, we would reach 70.8%. In vitro sensitivity tests show a lower activity than against *M tuberculosis*. Our strains showed uniform sensitivity to rifampicin, ethambutol, amikacin, ofloxacin, and azithromycin. Patients responded well to the established treatment. We observed reactivation in 3.18% of patients, the majority of whom were HIV negative (6:1 ratio). The relapse rates published in the literature are highly variable, ranging from the 13 cases described by Martínez et al[27] corresponding to an index of zero, to the higher rate of 15.3% reported by Garrós et al.[28] The percentages reported by the latter group generally vary between 2.5% and 10%.

In this study, we observed dissemination of the disease in HIV carriers.

There is some disagreement over the use of isoniazid, due to the high resistance shown in vitro.[29-31] In this series, only Galdakao hospital used 2 drugs for treatment. The results of a recent prospective study by the British Thoracic Society comparing HIV-negative and HIV-positive patients treated for 9 months with rifampicin and ethambutol concluded that isoniazid is not necessary.[32] In our series, all of the HIV-positive patients from whom positive isolates were obtained received treatment.

In recent years, an increase has been observed in the number of cases of *M kansasii* disease. More diagnoses are made as a result of a better understanding of the disease and the improvement of laboratory techniques. The reservoir remains to be identified due to the fact that there was an increase and subsequent decline in this mycobacterium in certain areas of the province of Bizkaia.

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