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Original

Andrés González-García[1]
Lorena Carpintero[2]
Jesús Fortún[2]
Enrique Navas-Elorza[2]
Pilar Martín-Dávila[2]
Santiago Moreno[2]

Changes in tuberculosis in human immunodeficiency virus infected patients in a Spanish tertiary hospital (1995-2013)

[1]Department of Internal Medicine, University Hospital Ramón y Cajal, University of Alcalá, IRYCIS, Madrid. Spain.
[2]Department of Infectious Diseases. University Hospital Ramón y Cajal, University of Alcalá, IRYCIS, Madrid. Spain.

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ABSTRACT

Objectives. Although the incidence of human immunodeficiency virus (HIV)-associated tuberculosis (TB) has decreased, changes in other characteristics of the disease are largely unknown. To describe the trends in TB in patients infected with HIV from 1995 to 2013.

Methods. We review all cases of TB in a tertiary hospital in Madrid, Spain.

Results. Among 1,284 patients diagnosed of TB, 298 (23%) were coinfected with HIV. The prevalence of HIV infection during the period of study has decreased from 40% to 14% (p for the trend < 0.001). Clinical presentation has also changed. Although pulmonary and extrapulmonary TB has remained unchanged, miliary presentation has significantly decreased (from 36% to 22%, p = 0.005). The 4-drug regimen was the preferable scheme, with higher implementation at the end of the study period (82% from 1995-1999 to 95% in 2010-2013, p = 0.43). Factors such as treatment failure (OR: 11.7; CI 95%: 3.12-44.1) and miliary form (OR: 2.8; CI 95%; 1.09-7.3) were independently associated with TB related mortality, while the longer duration of treatment was as a protective factor (OR 0.7; CI 95%: 0.6-0.8).

Conclusions. HIV has decreased very significantly as a risk factor for the development of TB. Despite improvement in the treatment of both TB and HIV, and in overall mortality, deaths attributable to the disease in this population remain high mostly in miliary and relapsing forms.

Key-words: Tuberculosis, VIH, Acquired Immune-deficiency Syndrome, Antiretroviral Therapy, Multidrug Resistant Tuberculosis.

Correspondence:
Andrés González García.
Department of Internal Medicine. University Hospital Ramón y Cajal.
Carretera Colmenar Km 9,4 28034 Madrid. Spain.
Phone: +34913368402.
E-mail: andres_gonzalez_garcia@hotmail.com

Cambios en los pacientes coinfectados por tuberculosis y por el virus de la inmunodeficiencia humana en un hospital terciario español (1995-2013)

RESUMEN

Objetivos. Aunque la incidencia de la coinfección por el virus de la inmunodeficiencia humana (VIH) y la tuberculosis (TB) ha disminuido, los cambios ocurridos en otras características de la enfermedad son desconocidos. El objetivo de nuestro trabajo fue describir las tendencias en los pacientes con tuberculosis infectados por el VIH en un periodo de casi dos décadas (1995-2013).

Métodos. Se revisaron todos los casos de TB en un hospital terciario en Madrid, España.

Resultados. De los 1.284 pacientes diagnosticados de TB, 298 (23%) estaban coinfectados por el VIH. La prevalencia de la infección por el VIH durante el periodo del estudio disminuyó del 40% al 14% (p para estudio de tendencias < 0,001). La presentación clínica también se modificó. Aunque las formas pulmonares y extrapulmonares permanecieron invariables a lo largo del estudio, la presentación miliar disminuyó de modo significativo (del 36% al 22%, p = 0,005). El esquema de 4 fármacos fue el mayormente elegido, con un incremento de la implementación de dicho tratamiento al final del periodo de estudio (82% desde 1995-1999 frente a 95% en el periodo 2010-2013, p = 0,43). El fracaso del tratamiento (OR 11,7; IC 95%: 3,12-44,1) y las formas miliares (OR 2,8; IC 95%; 1,09-7,3) se asociaron de forma independiente con la mortalidad atribuida a TB, mientras que la mayor duración del tratamiento se comportó como un factor protector (OR 0,7; IC 95%: 0,6-0,8).

Conclusión. La infección por el VIH ha disminuido de forma significativa como factor de riesgo principal del desarrollo de TB en nuestro medio. A pesar de la mejora en el

tratamiento de la TB y de la infección por el VIH, así como una menor mortalidad total, los fallecimientos atribuidos en esta población permanecen muy elevados, sobre todo en las formas recidivantes y en la TB miliar.

Palabras clave: Tuberculosis, HIV, Síndrome de inmunodeficiencia adquirida, Tratamiento Antiretroviral, Tuberculosis Multirresistente.

INTRODUCTION

The importance of tuberculosis (TB) in Western countries has been dropping during the last years [1]. Despite the decline in its incidence, more than 9 million people were diagnosed with TB in 2015 [2], and it is estimated that 1.5 million deaths could be related to TB worldwide. Traditionally, Spain ranks one of the regions in European Union in TB and acquired immunedeficiency syndrome (AIDS) incidence [3].

Since the introduction of highly active antiretroviral therapy (HAART) the number of patients infected with HIV and TB is declining. However, the burden of this association remains too high in resource-poor settings with more than 80% of notified TB patients being HIV infected [2].

In our environment, different changes in HIV epidemic have modified the TB pattern. The present study aimed to describe the changes in demographical and clinical characteristics as well as in treatment outcomes of HIV infected patients with TB over a long period in Spain.

METHODS

Study design and setting. We conducted an observational retrospective study at the Ramón y Cajal Hospital, a 1200-bed tertiary referral center in Madrid that provides medical care to a population of 600,000 inhabitants.

Patients. We included all adult patients with a TB diagnosis from January 1995 to December 2013. TB was considered only when *Mycobacterium tuberculosis* was isolated from culture of a clinical sample or, in the absence of identifying the organism, a compatible clinical picture together with the finding of granulomas in a tissue biopsy and/or an elevated adenosine deaminase level in an organic fluid plus a positive PCR for *M. tuberculosis* in a clinical sample. Patients who were less than 16 years old were excluded from the analysis. HIV-infected patients were selected from the TB cohort. All the patients included in the study were admitted to the Respiratory Isolation Unit, where HIV testing is routinely performed to all patients with TB. In order to make comparative analysis, the years of the study were divided into four periods: 1995-1999, 2000-2004, 2005-2009 and 2010-2013.

Patients were identified by cross-matching two hospital registries: the Microbiology Department database and the internal server of the center with discharge diagnoses. Data on all patients were obtained from the medical records. Ethics consent was obtained and approved by the local ethic committee of our institution.

Definitions. TB was considered to be pulmonary if *M. tuberculosis* was isolated in culture of respiratory samples or/and if chest X-ray was suggestive of pulmonary involvement. The extrapulmonary forms were considered if the isolation was from a non-pulmonary source or histologically confirmed in patients with TB proven by culture. In extrapulmonary patients with negative cultures, TB was established if: adenosine deaminase cut-off levels were higher than 35 Ud/L in pleural and peritoneal liquid and more than 6 Ud/L in cerebrospinal fluid; a positive polymerase chain reaction (PCR) in smear other than sputum and positive acid fast bacilli (AFB) was obtained. TB was considered to be miliary if chest X-ray showed a miliary pattern and TB was confirmed by culture of pulmonary or non-pulmonary samples.

Data collection. The following data of the diagnosis of TB was registered: date of diagnosis, type of TB and socio-demographic variables that included age, gender, and country of origin. Associated risk factors and comorbidities (smoking habit, drug use, alcohol abuse, diabetes, neoplasia, chronic renal disease, liver disease, chronic obstructive pulmonary disease, malnutrition and social status) were also recorded. Regarding tuberculosis, the following variables were included: date of diagnosis, type of TB, microbiological and other diagnostic procedures (tuberculin skin test (TST), AFB smear, PCR, culture, histological examination, ADA and drug susceptibility testing), antituberculosis drugs administered, duration of treatment, associated adverse events, treatment adherence and outcome. Finally, the following information related to HIV infection was registered: risk practice for and time of the HIV infection, last count of CD4+ T-lymphocytes, and plasma viral load before the diagnosis of TB, antiretroviral treatment administered before the diagnosis of TB. As a retrospective cohort, no information could be collected on the timing of initiation of antiretroviral therapy, nor on the development of immune reconstitution inflammatory syndrome (IRIS) in patients who initiated treatment after the diagnosis of TB. The main point of the study, trends in HIV, is hard enough to prevail over less relevant complications such as side effects not affecting prevalence.

Laboratory procedures. *M. tuberculosis* culture of samples was performed according to Tacquett & Tison method and inoculated on solid media, Lowenstein Jensen and Coletsos media (Bio Medics SL, Tres Cantos, Madrid, Spain) and, since 1996, in liquid medium (Veersa TREK system, formerly ESP culture System II). The strains isolates were identified using DNA probes (Gen Probe, San Diego, California, USA) and phenotypic tests. *In vitro* susceptibility tests were undertaken according to Canetti's method on Lowenstein Jensen medium and 7H10 agar medium until 1996, and later in liquid medium with antibiotic concentrations according to the manufacturer's protocol (Versa TREK system, formerly ESP culture System II). Multidrug resistant TB (MDR-TB) was defined when the organism was resistant to at least isoniazid and rifampin. If the organism had additional resistance to a fluoroquinolone and a second-line injectable drug was considered extensively drug-resistant TB (XDR-TB).

Transcription-mediated amplification was applied for molecular diagnosis. The Amplified *M. tuberculosis* Direct Test (AMTD; Gen-Probe Inc.) is a rapid isothermal (42º C) method based on the amplification of 16S-rRNA. Reverse transcriptase is used to copy rRNA to a cDNA-RNA hybrid, and the chemiluminescent method is then applied using specific DNA probes. This procedure was directly applied on clinical samples, including sputum, bronchoalveolar lavage, tissue biopsy specimens and urine.

Outcome. Treatment for TB was considered completed if correct therapy and follow-up were confirmed and clinical features showed a favorable outcome. In patients who had abandoned therapy and who had received more than one course of therapy, only the last episode was included for analysis. Patients whose smear or culture remained positive during the monitoring in an outpatient setting were considered failure.

Statistical analysis. The epidemiology of the disease and changes occurring during the study period as well as risk factors related to mortality were analyzed. The descriptive statistical analysis included medians and interquartile ranges (IQR) for continuous variables, and frequencies and proportions for categorical variables. The chi square test and Student t test were used to compare continuous and categorical data, respectively. Odds ratio (OR), 95% confidence interval (CI), and P values were estimated. A $P < 0.05$ was considered statistically significant.

Univariate and multivariate logistic regression analyses were performed to assess factors associated with poor outcomes. Multiple logistic regression analysis was used to determine the independent risk factors associated with mortality. All variables with a $p< 0.1$ in the univariate analysis, as well as those clinically significant that could have an impact on mortality, were entered in the multivariate model. Software SPSS Statistics 19® was used for the statistical analyses.

RESULTS

Patients characteristics. During the study period 1,284 patients were diagnosed with TB. HIV infection at the time of TB diagnosis was present in 298 patients (23.2%). The diagnoses of HIV-infection and TB were made concurrently in 67 cases (22.4%). The median CD4+ T cell count and plasma HIV RNA was 100 cells per cubic millimeter and 100000 HIV RNA copies per milliliter, respectively. HAART was being administered to only 42 cases (14%). Characteristics of the patients according to the HIV infection status are shown in table 1. HIV infected patients were significantly younger (mean age: 36 vs. 47, $P < 0.001$), predominantly men (79% vs. 59%, $P < 0.001$), and with more comorbidity, regarding coinfection with hepatitis C virus infection (57% vs. 7%, $P <0.001$), alcoholism (27% vs. 19%, $P <0.001$) and drug abuse (65% vs. 3%, $P < 0.001$).

There were significant changes in the different study periods. From 1995 to 2013, the prevalence of HIV infection decreased from 40% to 14% (X^2 trend: 94.5; $P < 0.001$, figure 1) while the median age at diagnosis of patients with HIV infection and TB increased (35 to 45 years, $P < 0.001$). Regarding the clinical forms, both pulmonary (85% in 1995-1999 to 89% in 2010-2013, $P = 0.7$) and extrapulmonary (61% in the two periods, $P = 0.68$) involvement remained stable. However, miliary TB showed a significant decrease in the period of study, from 36% in 1995-1999 to 22% in 2010-2013 ($P = 0.005$) (figure 2).

Table 1 Patients's characteristics at diagnosis of tuberculosis according to HIV status.

	HIV positive (n= 298)	HIV negative (n= 986)	P value
Gender, male	236 (79%)	585 (59%)	< 0.001
Age, years	36 (32-41)	47 (29-64)	< 0.001
Foreign-born	30 (10%)	274 (28%)	< 0.001
Alcohol abuse	80 (27%)	191(19%)	< 0.001
Injection drug users	195 (65%)	30 (3%)	< 0.001
Chronic liver disease	111 (37%)	60 (6%)	< 0.001
HCV	169 (57%)	53 (7%)	< 0.001
Chronic renal disease	2 (4%)	36 (1%)	< 0.001
Diabetes Mellitus	3 (1%)	89 (9%)	< 0.001
Pulmonary TB	249 (84%)	669 (68%)	< 0.001
Extrapulmonary TB	180 (60%)	437 (44%)	< 0.001
Miliary TB	86 (29%)	30 (3%)	< 0.001
Previous TB episode	35 (12%)	97 (10%)	0.34
Marginality	45 (15%)	35 (3%)	< 0.001

Data are reported as number (%) of patients or main value (interquartile range). HIV = human immunodeficiency virus, TB = tuberculosis, HCV = Hepatitis C virus.

Diagnostic procedures. A tuberculin skin test (TST) was performed in 161 (54%) patients. In 93 patients (58%) the TST was positive. The sputum smear was positive in 154 of 272 (57%), and the culture was positive in 205 out of 272 (75%). PCR in sputum was positive in 37 out of 45 patients (82%). The remaining specimens submitted for microbiological studies and the results are summarized in table 2. Of note, the yield of sputum smears and cultures, as well as that of other microbiological and biochemical studies did not change during the study period.

Anti-tuberculosis drug resistance. Susceptibility testing was undertaken in 268 isolates (90%). Resistance ≥ 1 drug was detected in 37 isolates (14%). Primary isoniazid resistance was documented in 8 cases (3%). MDR-TB and XDR were observed in 10 (4%) and 10 (4%) isolated, respectively. Most resistant isolates were detected in the first period, although changes in the rates of resistance during the study period were not significant.

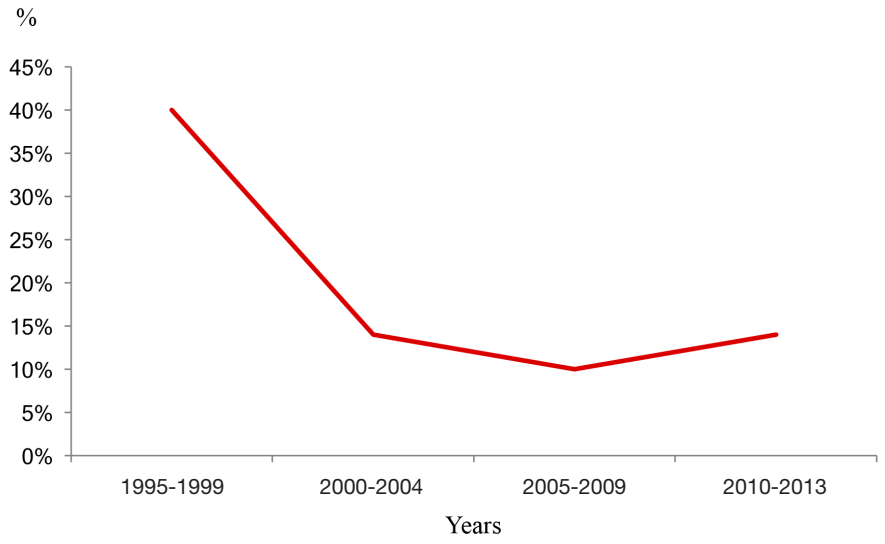

Figure 1 | **Changes in human immunodeficiency virus prevalence among patients with tuberculosis from 1995 to 2013.**

Data are expressed as number of HIV-infected patients with tuberculosis/total number of patients with tuberculosis.

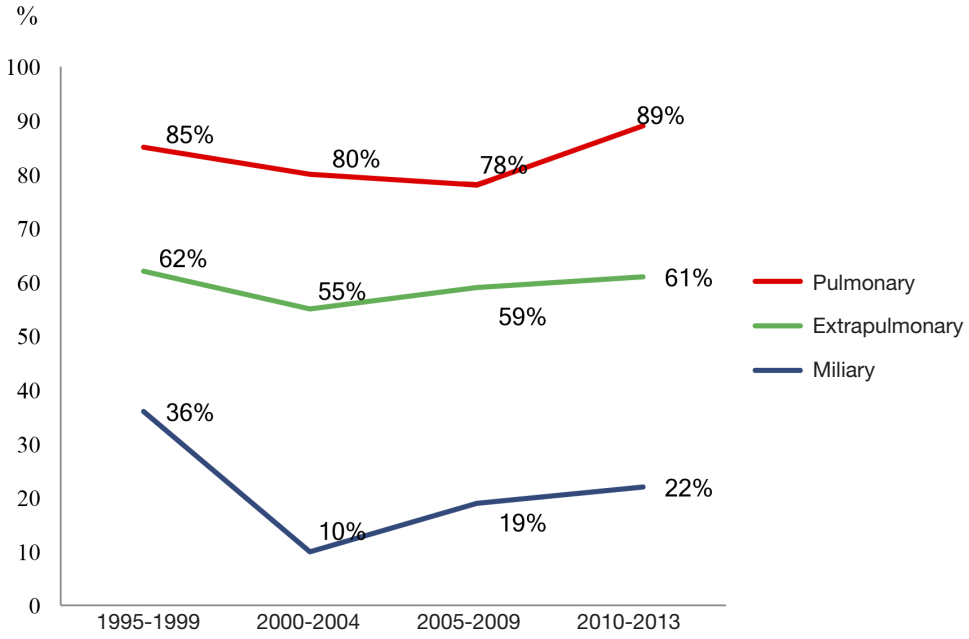

Figure 2 | **Changes in clinical involvement of tuberculosis in HIV infected patients from 1995 to 2013.**

Outcomes. TB-treatment was administered in 291 (98%) episodes. The remaining patients did not receive treatment due to early death during the episode or loss of follow-up. In patients with isolated pulmonary involvement, the median duration of treatment was 9 (IQR = 6-12) months which was not different to that in patients with extrapulmonary or disseminated disease [median 10 (IQR = 6-12) months]. A 4-drug regimen was the therapy most commonly used, with higher implementation at the end of the study period without significant differences (82% from 1995-1999 to 95% in 2010-2013, P = 0.43). A completed treatment was documented in 182 (71%) patients, 44 (17%) discontinued the therapy and 71 (24%) were lost during follow up.

Table 2 Yield of different diagnostic laboratory methods for the diagnosis of tuberculosis in human immunodeficiency virus infected patients

Specimen	Stain	Culture	PCR	ADA[a]
Sputum	154/272 (57)	205/272 (75)	37/45 (82)	-
Urine	23/129 (18)	47/128 (43)	19/49(39)	-
Pleural fluid	1/14 (7)	6/14 (43)	-	37 (33-67)
Ascitic fluid	1/3 (33)	1/2 (50)	-	37 (37-51)
Cerebrospinal fluid	1/18 (6)	4/19 (21)	-	11 (7-12.5)
Lymph node[b]	58/72 (81)	61/70 (87)	19/23 (83)	-
Bone marrow[c]	6/18 (25%)	9/24 (37)	-	
Blood	-	6/15 (40)	-	-

Data are expressed as number positive / number tested (%). PCR = polymerase chain reaction.
ADA = adenosine deaminase.
[a]Data are expressed in median (interquartile range). [b]Lymph tissue evaluated by fine needle aspiration.
[c]Bone marrow examination.

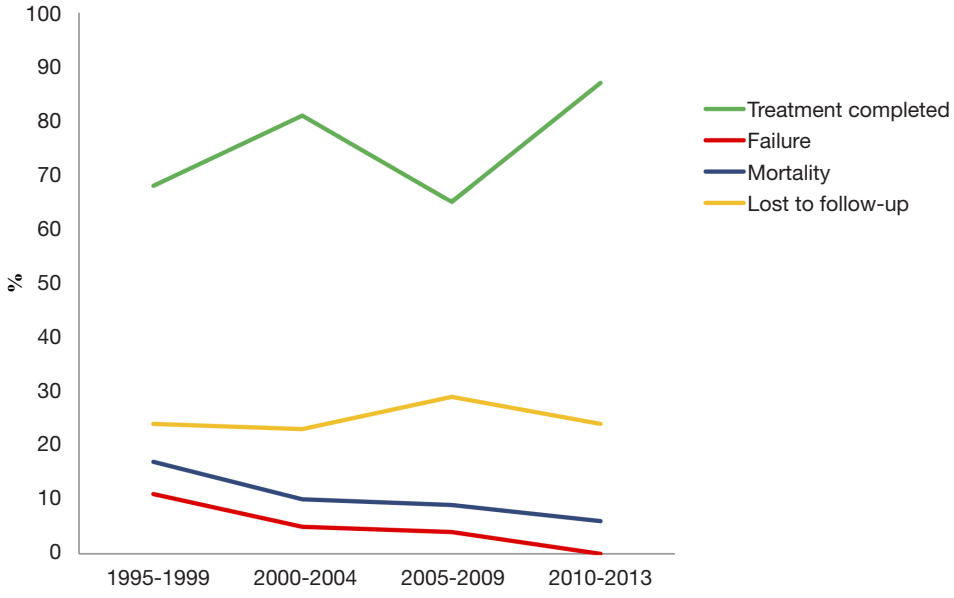

Figure 3 Trends in TB treatment outcomes from 1995 to 2013.

A total of 57 (19%) patients deceased, and death could be directly attributed to TB in 43 cases (14%). Throughout the study period a trend to a better success rate of completed treatment was observed (68% vs. 87%, p =0.1), while mortality ($p < 0.05$) and treatment failure ($p < 0.05$) decreased. The number of patients lost during follow-up remained stable (figure 3).

After adjusting for all variables, treatment failure (OR: 11.7 CI 95%; 3.12-44.1) and miliary TB (OR: 2.8; CI 95% 1.09-7.3) were independently associated with mortality related with TB. The longer duration of treatment was identified as a protective factor (OR: 0.7; CI 95% 0.6-0.8).

DISCUSSION

This study reports the changes in TB in HIV infected patients during two decades in a tertiary center. We have confirmed the importance of HIV infection as a risk factor

for TB. Although the number of patients with TB and HIV infection had decreased during the study period, HIV continues to be an important risk factor for TB even after the advent of combination antiretroviral therapy. At the beginning of the 90's, Spain was a high incidence country in TB with highest rate of AIDS [5]. Over the last few years, important changes have taken place in our country which explains the observed decline in TB-HIV coinfection [6-9].

Most studies have consistently showed a reciprocal interaction among the two diseases which leads to a significant impact [10]. The great spread of the HIV epidemic in the 90's contributed to the resurging global epidemic [11]. Although there has been a fall in Western countries which could be attributable to the introduction of HAART as well as the new development of other policy health control measures [6], it still remains high in other settings. In Europe, Eastern-countries such as ex-former Soviet Union countries or Portugal have the highest incidence rates for coinfection of TB and HIV [12].

Interestingly, our data shows a very low percentage of patients with HAART at TB diagnosis. This issue could be due to the increase of both diagnoses at once.

We have observed no changes in the frequency of overall pulmonary and extrapulmonary involvement in HIV infected patients with TB, but a marked decline in miliary forms. The latter is possibly related to the better control of AIDS with less severe immunodeficiency during shorter periods of time. In fact, the few cases of military TB were diagnosed in patients with very low CD4 counts, as commented in previous reports [9, 13].

Our microbiological results are similar to those of other large cohorts [14], with no relevant changes regarding the diagnostic yield regardless the specimen tested and the methods used. It is true, however, that PCR in sputum led to an increase in the accuracy of diagnosis of almost 10%. Similarly, PCR has been used successfully in patients with several extrapulmonary and pulmonary forms [15-17], and it was helpful in some cases to detect earlier resistant isolates [18].

It is worthy to highlight the low percentage of primary isoniazid resistance in our cohort (3%). This could explain a higher use of the three drugs regimen at the beginning of the study. The American Thoracic Society consensus guidelines [19], as well as the Spanish Health Authorities recommendation [20] have established the four-drug regimen as the recommended starting regimen which is the preferable scheme in our cohort at the end of the study.

Our data show better outcomes throughout the study in completed treatment and mortality. Although mortality is still high in HIV infected patients, it has changed dramatically with respect to the 90's, where AIDS related infections and delayed treatment for TB were the main causes of death [21]. Moreover, HIV is a well- known risk factor for TB mortality in low-income countries [22,23], and has also been a matter of interest in Western countries [24]. In our patients, the failure of treatment and the miliary forms were independently related with death attributable to TB. Several factors could help to mitigate the risk for death in this population. For instance, in a prospective study in Brazil, an earlier timing for HAART initiation was associated to a lower risk for death related to TB [25], as shown in clinical trials [26, 27].

We are aware of the limitations of our study. Those related with the retrospective nature of the analysis are most significant, specially the lack of some important information. In this sense, we could not provide any results on the impact of the timing of initiation of antiretroviral therapy on overall and TB related mortality, as well as on the development of IRIS. It is very likely that an association be found, as shown in clinical trials mentioned earlier [26,27]. We could only show an indirect association between the availability of combination ART and the decrease on the incidence and mortality of HIV-associated TB. The goal of our study was to describe the changes of TB in HIV infected patients during a long period, and hope that we have succeeded with the picture described, despite the admitted limitations.

In summary, our study shows that HIV and TB coinfection has steadily decreased during the last two decades. Miliary TB has been significantly reduced, although other extrapulmonary and pulmonary forms have remained stable. Although the burden of HIV infection in patients with TB is still relevant, a better prognosis has been observed over the years with lower rate of mortality and lost to follow-up.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

FUNDING

None to declare

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