Changes in the incidence of tuberculosis in a cohort of HIV-seroconverters before and after the introduction of HAART

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Objective: To analyse incidence and determinants of tuberculosis in HIV-seroconverters before and after the introduction of HAART.

Methods: Data from a multicenter cohort study of 2238 HIV-seroconverters between the 1980s and 2004 were analysed and censored by December 2004. Calendar year at risk intervals were pre-1992, 1992–1996 and 1997–2004. Incident tuberculosis was calculated as cases per 1000 person-years (p-y). Survival analyses using Kaplan–Meier and multivariate Cox regression allowing for late-entry were used. Proportional hazards assumptions were checked with tests based on Schoenfeld residuals.

Results: Overall, 173 (7.7%) patients developed tuberculosis over 23 698 p-y at a rate of 7.3 cases per 1000 p-y [95% confidence interval (CI), 6.3–8.5]. Incident tuberculosis was higher in intravenous drug-users (IDUs), 12.3 per 1000 p-y compared with persons infected sexually, 3.8 per 1000 p-y ($P < 0.001$), and persons with clotting disorders (PCD), 2.7 per 1000 p-y ($P < 0.001$). A decreasing tuberculosis incidence trend was observed from 1995 in all categories. Highest tuberculosis rates, 44 per 1000 p-y, were observed prior to 1997 in IDUs infected with HIV for 11 years. In multivariable analyses women were less likely to develop tuberculosis [relative hazard (RH), 0.62; 95% CI, 0.41–0.96; $P < 0.05$) and IDUs were more likely to develop tuberculosis (RH, 3.0; 95% CI, 1.72–5.26, $P < 0.001$). In the HAART era, the hazard of developing tuberculosis was 70% lower (RH, 0.31; 95% CI, 0.17–0.54; $P < 0.001$). Before 1997, the risk of tuberculosis increased with time since HIV seroconversion, whereas it remained nearly constant in the HAART era.

Conclusions: Since the mid-1990s important decreases in tuberculosis have been observed in HIV-seroconverters that probably reflect the impact of both HAART and tuberculosis control programmes.

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Introduction

HIV is the most powerful risk factor for the development of tuberculosis (TB) in patients infected by *Mycobacterium tuberculosis* [1,2]. HIV increases the risk of TB disease through reactivation of latent infection or by accelerating the progression of recently acquired infection [3–6]. The risk of TB in HIV-infected individuals depends on host factors, such as the degree of immunodeficiency, as well as environmental factors such as the level of exposure to TB infection [6]. Thus, TB control programmes and the advent of highly active antiretroviral therapy (HAART) are likely to have had an impact on the epidemiology of TB in HIV-infected individuals.

Spain has the second highest incidence of AIDS in Western Europe and intravenous drug use (IDU) has been the main route of HIV transmission accounting for 46% of the cumulative number of AIDS cases from 1981 to 2005 [7]. Before the HIV epidemic, Spain had the second highest TB rate of Western Europe in the general population [8]. IDUs, irrespective of their HIV status, are exposed to high levels of TB infection and have higher TB incidence rates [9]. As a result of this, the epidemiological situation in Spain has been characterized by a large overlap of both the HIV and TB epidemics leading to high rates of HIV–TB co-infection [10].

GEMES, the Spanish Multicenter Study Group of HIV Seroconverters, is an established nation-wide cohort of HIV-infected individuals from all transmission categories with well known dates of seroconversion from the 1980s to the present date. The objective of this study is to measure the trends of incident tuberculosis among HIV-seroconverters and to ascertain the temporal relationship between HIV seroconversion and the development of TB disease before and after the introduction of HAART.

Patients and methods

Data from 2238 patients with well documented HIV seroconversion dates from eight cohorts included in GEMES were analysed. Detailed information on the characteristics of these cohorts can be obtained from individual publications [11,12]. The cohorts within GEMES have identified HIV seroconverters either retrospectively or prospectively from the 1980s to the current date and followed them up over time in six Spanish cities within three of the Autonomous Communities (Catalonia, Madrid and Valencian Community) with high HIV/AIDS incidence. A seroconverter was defined as an individual who tested HIV negative prior to the first HIV-positive test with a maximum 3-year interval between test dates or had a documented seroconversion illness. For these individuals, seroconversion was estimated as the mid-point between the last HIV-negative and the first HIV-positive test. Furthermore, all HIV-infected persons with clotting disorders (PCD) from three of the largest Haemophilia Units in Spain were included. Ascertainment of all HIV-1-positive haemophiliacs was complete since records were well kept for medical and legal reasons. Previous HIV-negative tests were only available for some cases, but all individuals born before 1979 were assumed to have become infected by HIV between 1979 and 1985, the date by which all plasma concentrates were heat-treated in Spain. For those born after 1979, date of birth was taken as the date of starting exposure to HIV. Date of HIV seroconversion was estimated through the probability of cumulative distribution of infections using mathematical techniques for interval-censored data from 1979 to 1985. More details on the estimation of the seroconversion dates have been published elsewhere [13].

Information on sociodemographic characteristics (age, sex, transmission category [IDU, men who have sex with men (MSM); heterosexuals, PCD/haemophiliacs]) as well as clinical and immunological data (number and type of AIDS events, antiretroviral treatments prescribed, lymphocyte CD4 cell count, HIV-RNA viral load, vital status and cause of death) were collected.

Each of the cohorts within GEMES follows its patients at the recruiting centres and referral hospitals and follow-up is up-dated yearly.

In case of clinical symptoms suggesting TB, patients were investigated for pulmonary and disseminated disease. Chest X-ray and sputum analysis (Ziehl–Neelsen smear and Jensen–Lowenstein culture) were obtained. In addition, patients with persistent fever and constitutional symptoms were analysed by means of culture for *M. tuberculosis* in blood, urine, faeces and bone marrow samples. Fine needle aspiration to sample fluid from adenopathies was obtained if necessary. In this study TB diagnoses were culture proven in 85% of cases.

Although pulmonary TB was not considered an AIDS-defining condition in Spain until 1994, this diagnosis was recorded in the clinical records and included in these analyses. Additionally, to increase the completeness of the data, cross-checks with local and National AIDS and mortality registers were also performed. For these analyses, cross-checks with the National AIDS Register were performed by December 2004. AIDS was classified as in the 1993 European definition [14].

Statistical analyses

For this analysis, surviving individuals who did not develop TB were censored at 31 December 2004. The outcome of interest was the development of TB disease. The comparison of (uncensored) times from seroconversion to TB among groups was carried out with the non-parametric Kruskall–Wallis test. Tuberculosis incidence

rates were calculated as cases per 1000 person-years and compared by means of the Wald test.

Calendar year at risk was divided into three periods (before 1992, between 1992–1996 and 1997–2004) reflecting the availability of different antiretroviral therapies (ART) before the introduction of highly active antiretroviral therapy (HAART) in Spain in 1996. Between 1992 and 1996 only zidovudine, zalcitabine, lamivudine, stavudine and didanosine were available for the treatment of HIV/AIDS. The proportional hazard model was used to determine the factors associated with the risk of developing TB taking into account the following covariates: gender, exposure category, age at seroconversion, and calendar period. Possible interactions between exposure category, calendar period and the remaining variables were considered, however, since not being significant they were not included in the final model. To check whether the proportional hazards assumptions held, tests based on the Schoenfeld residuals were used. In order to include calendar period in that model (as a time-dependent covariate), each individual contributed to this analysis with as many registers of calendar periods he/she had been at risk. Hence, time to TB onset was treated as left truncated (late entry) if admission to the recruiting centre occurred in a previous period and as right-censored if the individual was alive at the end of the period.

Analyses were performed in R using the libraries eha [15] and epitools [16]. Test results were considered statistically significant if the resulting *P*-value was less than 0.05.

Results

The analyses included 2238 HIV seroconverters (1874 men and 364 women); of whom 423 (18.9%) acquired HIV infection in the HAART era. Overall, 51.9% were IDUs, 27.4% were PCD and 20.6% were infected by sexual transmission of which 14.7% were heterosexuals. The proportion of females among IDUs and heterosexuals were 25.6 and 73.5%, respectively. The median dates of HIV seroconversion were September 1982, June 1993 and May 1996 for PCD, IDUs and for sexual transmission, respectively (Table 1). Median follow-up was 10.1 years [interquartile range (IQR), 6.4–13.7 years) and total follow-up was 23 698 person-years. Lifetime prevalence of ART use was 51.4%; 62% in PCD, 50% in IDUs and 41% in individuals infected through the sexual route. As for lifetime prevalence of HAART use, it was 41% in IDUs, 38% in persons infected sexually and 35% in PCD (Table 1). After 1997, lifetime prevalence was equal to 45.4% (IDUs), 38.5% (sexual transmission), and 74.0% (PCD), respectively.

By December 2004, 173 (7.7%) patients had developed TB (55.5% pulmonary, 35% extra-pulmonary and 10% of them in both locations) giving an overall rate of 7.3 cases per 1000 person-years (p-y) [95% confidence interval (CI), 6.3–8.5]. TB was the first AIDS-defining condition (ADC) in 147 patients (85%), second in 19 cases (11%) and third in six cases (3.5%). Median time from HIV seroconversion to TB disease was 5.6 years (IQR, 3.3–8.0). Median times differed significantly ($P < 0.001$) between PCD (9.9 years; IQR, 7.3–10.8), IDUs (5.2 years; IQR, 3.1–7.4) and sexually transmitted HIV (6.0 years; IQR, 4.9–7.5). Differences between the latter two were not statistically different ($P = 0.33$). The median value of lymphocyte CD4 cell count at TB diagnosis was 80 cells/μl (IQR, 16–254) among the 35 (32.1%) patients for which this information was available before 1997. After the introduction of HAART, the median amounted to 182 cells/μl (IQR, 88–313) among the 38 (59.4%) patients with this information available. The majority (106; 61.2%) of the patients that developed TB had not received any antiretroviral treatment and 135 out of 173 (78%) were IDUs.

Table 1. Characteristics of the study population from GEMES cohorts by exposure category.

	Exposure categories		
	Sexual transmission $N = 462$	Injecting drug users $N = 1162$	Haemophiliacs $N = 614$
Men	412 (89.2)	865 (74.4)	597 (97.2)
Date of seroconversion (median; IQR)	1996.4 (1992.9–2000.5)	1993.5 (1990.7–1996.0)	1982.8 (1982.4–1983.2)
Age at seroconversion (median; IQR)	28.8 (24.8–34.2)	25.3 (22.2–28.9)	16.5 (8.7–25.2)
Median follow-up (years) (median; IQR)	7.8 (4.2–11.2)	9.6 (6.3–12.5)	13.3 (9.2–21.9)
ART during study period	189 (40.9)	580 (50.2)	382 (62.2)
HAART during study period	174 (37.7)	471 (40.8)	216 (35.2)
Tuberculosis disease[a]	14 (3.0)	137 (11.8)	22 (3.6)
Pulmonary	7 (50.0)	72 (52.5)	17 (77.3)
Extrapulmonary	6 (42.9)	49 (35.8)	5 (22.7)
Both	1 (7.1)	16 (11.7)	0 (0.0)
AIDS	45 (9.7)	321 (27.6)	379 (61.7)
Deaths at end of study	21 (4.5)	244 (21.0)	370 (60.3)

ART, antiretroviral treatment; HAART, highly active antiretroviral treatment; IQR, interquartile range.
[a]Pulmonary TB was not considered AIDS in Spain until 1994.

Table 2. Tuberculosis rates [95% confidence interval (CI)] per 1000 person-years according to selected sociodemographic and clinical characteristics.

	Number of seroconverters	Tuberculosis cases[a]	Persons-years (p-y)	Tuberculosis rate × 1000 p-y (95% CI)
Gender				
Male	1874	147	19991.2	7.4 (6.2–8.6)
Female	364	26	3706.9	7.0 (4.6–10.3)
Age at seroconversion				
≤20	620	40	8864.0	4.5 (3.2–6.1)
21–30	1148	106	11056.9	9.6 (7.8–11.6)
≥30	467	26	3742.8	6.9 (4.5–10.2)
Exposure category				
Injecting drug use	1162	135	11001.0	12.3 (10.3–14.5)
Heterosexual and homosexual	462	14	3678.9	3.8 (2.1–6.4)
Clotting disorders/haemophilia	614	24	9018.2	2.7 (1.7–4.0)
Year of seroconversion				
before 1992	1138	121	15228.9	7.9 (6.6–9.5)
1992–1996	676	49	6325.7	7.7 (5.7–10.2)
1997–2004	424	3	2143.6	1.4 (0.3–4.1)
Calendar period[b]				
before 1992	1137	34	6511.9	5.2 (3.6–7.3)
1992–1996	1623	74	5664.9	13.1 (10.3–16.4)
1997–2004	1714	65	11521.3	5.6 (4.4–7.2)
Time since HIV seroconversion (years)[b]				
<4	2238	57	8515.3	6.7 (5.1–8.7)
4–10	1968	89	9509.6	9.4 (7.5–11.5)
>10	1127	27	5673.3	4.8 (3.1–6.9)
Total	2238	173	23698.1	7.3 (6.3–8.5)

[a]Pulmonary TB was not considered an AIDS-defining illness in Spain until 1994.
[b]Sum of numbers of seroconverters exceeds 2238 because patients may contribute to more than one category.

Among the 65 TB cases observed since 1997, 52 (80%) were IDU and 41of the 65 (63%) were not under HAART. The remaining 24 patients developed TB despite having started HAART.

Incident TB was higher in IDU, 12.3 cases per 1000 p-y (95% CI, 10.3–14.5), in comparison with people infected sexually ($P < 0.001$), 3.8 cases per 1000 p-y (95% CI, 2.1–6.4), and PCD ($P < 0.001$), 2.7 cases per 1000 p-y (95% CI, 1.7–4.0). TB rates in calendar periods in the HAART era (5.6 per 1000 p-y) were significantly lower than before 1997 (8.9 per 1000 p-y) (Table 2).

TB rates for all transmission categories were higher in the period prior to the introduction of HAART (Fig. 1). TB rates before and after 1997 were 18.09 and 8.68 cases per 1000 p-y for IDUs, 8.18 and 2.22 cases per 1000 p-y for sexually transmitted HIV and 3.43 and 0 cases per 1000

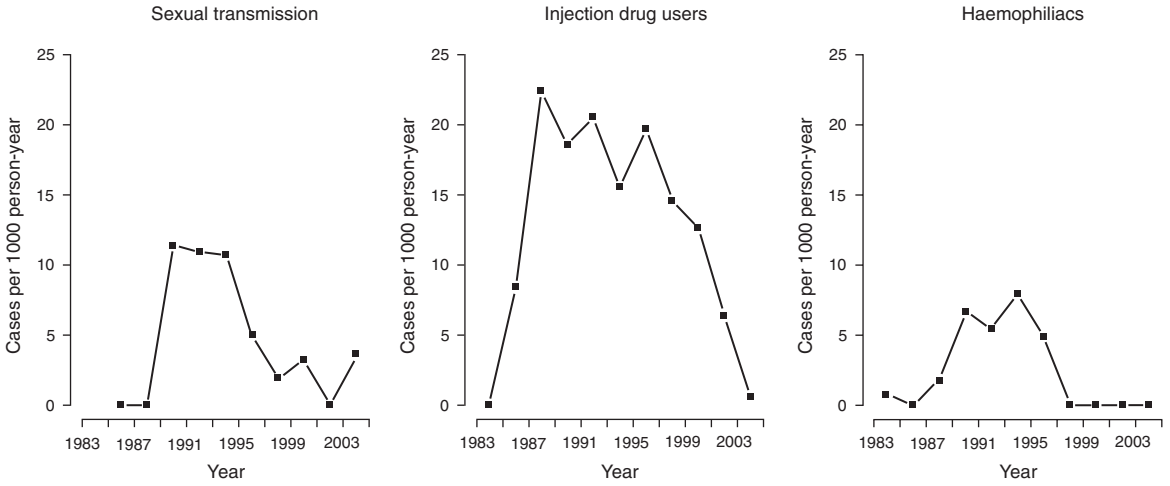

Fig. 1. Incidence rates of tuberculosis in HIV seroconverters according to calendar year for each transmission exposure category.

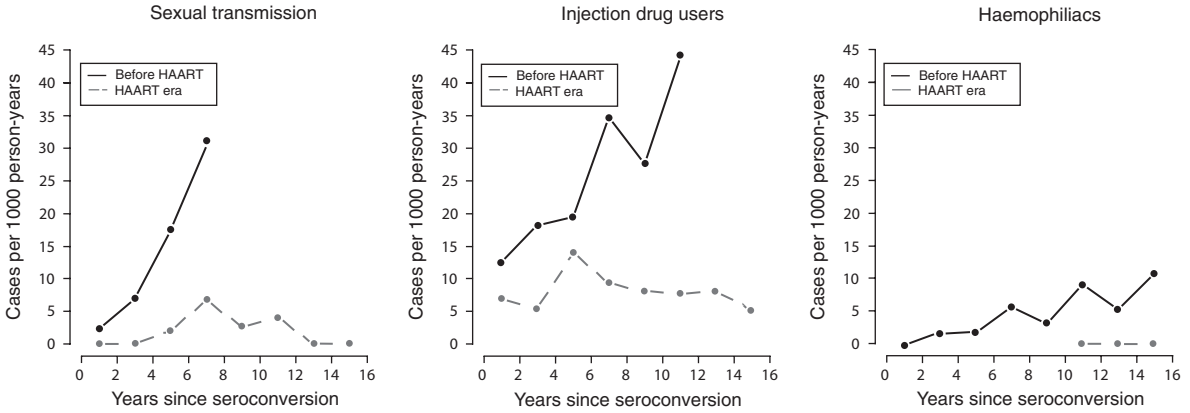

Fig. 2. Incidence rates of tuberculosis according to years since HIV seroconversion before and after HAART introduction (1997) for each transmission exposure category.

p-y for PCD, respectively. The reductions in the hazard of TB for each of the transmission categories were 48, 27 and 100%, respectively.

The duration of HIV infection was a strong determinant of the risk of TB before 1997 but not so much afterwards (Fig. 2). Before 1997, the risk of TB increased in a quasi linear fashion with the time since HIV seroconversion in the three transmission categories. Highest TB rates, 45 per 1000 p-y, were found in IDUs infected for 13 years before the HAART era, and in sexually transmitted HIV patients infected for 8 years, TB rates mounted to 30 per 1000 p-y. After 1997, as well as the remarkable reductions in TB incidence, risk of TB did not increase with longer duration of HIV infection and, in fact, it peaked around the fifth to seventh year in IDU and sexually transmitted HIV to decrease thereafter. It is remarkable that for PCD, no new TB cases were observed after 1997.

The results of the proportional hazards model for the risk of incident TB are shown in Table 3. Women had a 38% lower hazard of TB compared to men [relative hazard (RH), 0.62; 95% CI, 0.41–0.96; $P=0.029$). IDUs showed a three times higher hazard of developing TB (RH, 3.00; 95% CI, 1.72–5.26; $P<0.001$) and PCD had a 60% lower hazard (RH, 0.40; 95% CI, 0.19–0.88; $P=0.023$) in comparison with people infected through sexual transmission. Regarding calendar periods, the hazard of developing TB in the HAART era was 69% lower in comparison with periods before 1997 (RH, 0.31; 95% CI, 0.17–0.54; $P<0.001$). The proportional hazards assumption held reasonably for all ($P>0.15$) but one covariate of the model, calendar period 1992–1996 ($P=0.03$). As the differences between this period and the previous one were highly non-significant ($P=0.962$), however, the preceding conclusions based on the proportional hazards model will not be affected.

Table 3. Adjusted relative hazards (RH) of TB disease in Cox regression models including gender, exposure category, age at HIV seroconversion, and calendar period as covariates.

	RH	95% CI	*P*-value
Gender			
Men	1		
Women	0.62	(0.41–0.96)	0.029
Exposure categories			
Sexual transmission	1		
Injecting drug users	3.00	(1.72–5.26)	<0.001
Clotting disorders/haemophilia	0.40	(0.19–0.88)	0.023
Age at seroconversion (years)			
0–20	1		
21–30	1.25	(0.83–1.87)	0.285
>30	1.38	(0.81–2.36)	0.232
Calendar period			
before 1992	1		
1992–1996	1.01	(0.63–1.62)	0.962
1997–2004	0.31	(0.17–0.54)	<0.001

CI, confidence interval.

Discussion

The present study from nine long-term observational cohorts shows a 69% reduction in the incidence of TB among in HIV-seroconverters from all transmission categories from 1997 onwards. Pulmonary TB was extremely common among HIV individuals, particularly in IDUs who, prior to the introduction of HAART in Spain were at high risk of transmitting and developing TB. Whereas the reduction in TB burden in patients from developing countries taking HAART has been documented [17,18], there are less data available concerning the impact of HAART in western countries [19].

Our results suggest that in the period between 1997 and 2004, improvements in the immune status among those receiving HAART and/or a reduction in the environmental risk of TB transmission must have taken place. Forty percent of patients from GEMES cohorts had been initiated on HAART, so it is likely that antiretroviral therapy may be responsible for a large proportion of the observed reductions in TB, as it has been for other AIDS-defining conditions [11,12]. Our group has previously

described the marked population effectiveness of HAART in reducing AIDS and death in all transmission categories, although lower in IDUs [11,12]. As the decreasing trends in TB were observed just before the introduction of HAART in Spain [8], it is likely that TB control programmes and the more aggressive ascertainment of TB infection particularly in groups at high risk of transmitting and developing TB disease may have also contributed to the observed reductions. It is crucial, for a good understanding of the results, to explore other changes that may affect TB risk, such as public health initiatives. Indeed, TB surveillance highlights reductions in TB incidence in most regions in Spain, and rates of pulmonary TB in the general population decreased from 23 per 100 000 in 1997 to 15 per 100 000 by 2004 [8]. This also includes high prevalence groups such as heroin users in which preventive interventions, substitution therapy with methadone and treatment of TB with directly observed therapy (DOT) programmes have been developed during the last decade [20]. This is particularly true for HIV-infected IDUs, where TB outbreaks have been reported as a result of life conditions and close contact with TB cases [9]. Besides, Díez *et al.* have reported how IDUs in Spain have delays in the initiation of TB treatment and that their outcome after starting treatment is worse than in TB cases from general population [21]. Other groups have described, with molecular epidemiology tools, the contribution of recent transmission of TB in Spain [22,23]. Badri *et al.* has reported large reductions in TB incidence associated to HAART in South Africa and claimed the importance of HAART in TB control in settings with high TB-HIV co-infection rates [17,24]. Castilla *et al.* reported decreases in the incidence of TB following the introduction of HAART in the National AIDS Registry [25] and Moreno *et al.* in a large multicentre hospital-based cohort study from 10 hospitals in Spain, have also found a decreasing trend in TB rates in HIV-infected patients from 1997 onwards [26].

The incidence rates of TB detected in our population are extremely high, 1300 per 100 000 p-y in calendar period 1992–1996. This rate is considerably higher than the rates of 61.35 per 100 000 p-y for the general population aged 25–34 years described by Diez *et al.* [27] in 1997 in a large nation-wide study. For PCDs, TB incidence for the whole period was the lowest at 270 per 100 000. The fact that the median time to TB disease was 10 years for PCD in comparison with 5–6 years in the other transmission categories suggests higher environmental exposure in IDU and persons infected sexually. Van Asten *et al.* [28] and Sonnenberg *et al.* [29] have described increased risks of TB soon after seroconversion in highly exposed seroconverters, IDUs in Amsterdam and gold miners in South-Africa.

Women were less likely to develop TB than men in the adjusted analyses which accounted for the sex differences. In HIV-negative individuals, TB rates were lower in women than in men and this has been attributed to a number of factors that range from lower exposure to TB infection to healthier lifestyles [30]. Furthermore, our group and others have previously reported that HIV-infected women in Spain have better outcomes than men, with lower progression rates to AIDS and death [11,31].

The present study has several limitations that merit discussion. First, TB infection and prophylactic treatment with isoniazid were not routinely obtained; however, it is unlikely that changes over time in treatment with isoniazid may explain the observed trends. Furthermore, assessment of TB disease by culture confirmation was not obtained in 15% of cases and finally, since pulmonary TB was not an AIDS-defining condition in Spain until 1994, we cannot exclude a certain under-reporting of disease in patients lost to follow-up prior to 1994. For those diagnosed after 1994, cross checks with AIDS registers ought to have captured the majority of cases.

To conclude, since the mid-1990s, important decreases in incident TB have been observed in Spain among HIV-infected seroconverters from all transmission categories. These changes are likely to reflect the population effectiveness of HAART and TB control programmes.

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