

RESEARCH ARTICLE

Mortality, Causes of Death and Associated Factors Relate to a Large HIV Population-Based Cohort

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Abstract

Introduction

Antiretroviral therapy has led to a decrease in HIV-related mortality and to the emergence of non-AIDS defining diseases as competing causes of death. This study estimates the HIV mortality rate and their risk factors with regard to different causes in a large city from January 2001 to June 2013.

Materials and Methods

We followed-up 3137 newly diagnosed HIV non-AIDS cases. Causes of death were classified as HIV-related, non-HIV-related and external. We examined the effect of risk factors on survival using mortality rates, Kaplan-Meier plots and Cox models. Finally, we estimated survival for each main cause of death groups through Fine and Gray models.

Mortality Results

182 deaths were found [14.0/1000 person-years of follow-up (py); 95% confidence interval (CI):12.0–16.1/1000 py], 81.3% of them had a known cause of death. Mortality rate by HIV-related causes and non-HIV-related causes was the same (4.9/1000 py; CI:3.7–6.1/1000 py), external was lower [1.7/1000 py; (1.0–2.4/1000 py)].

to the dataset after asking for permission from Patricia Garcia de Olalla or Joan A. Cayla.

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Survival Results

Kaplan-Meier estimate showed worse survival in intravenous drug user (IDU) and heterosexuals than in men having sex with men (MSM). Factors associated with HIV-related causes of death include: IDU male (subHazard Ratio (sHR):3.2; CI:1.5–7.0) and <200 CD4 at diagnosis (sHR:2.7; CI:1.3–5.7) versus \geq 500 CD4. Factors associated with non-HIV-related causes of death include: ageing (sHR:1.5; CI:1.4–1.7) and heterosexual female (sHR:2.8; CI:1.1–7.3) versus MSM. Factors associated with external causes of death were IDU male (sHR:28.7; CI:6.7–123.2) and heterosexual male (sHR:11.8; CI:2.5–56.4) versus MSM.

Conclusion and Recommendation

There are important differences in survival among transmission groups. Improved treatment is especially necessary in IDUs and heterosexual males.

Introduction

The survival of HIV-infected people underwent a remarkable improvement after the introduction of highly active antiretroviral therapy (HAART) in 1996 [1–3]. Since then, the natural history of HIV disease as well as the morbidity and the mortality patterns have changed. HIV-infected people are now experiencing immune reconstitution and prolonged survival as a result of HAART. In parallel, HAART has undergone a great evolution: the new antiretrovirals with milder side-effects have facilitated adherence to treatment and have contributed to an increased life expectancy [4]. Clinical trials and observational studies demonstrate that the introduction of HAART and its continuous implementation have redefined the HIV epidemic [5–8].

The changes in HIV transmission routes have also contributed to epidemic evolution. In the 80s and 90s, the HIV and the heroin epidemics were closely related to one another in Western Europe [9]. Intravenous drug users (IDU) and their partners constituted the greatest number of new infections. The causes of death among IDUs were frequently related to infections, drug dependence, violence and suicide, along with a poor adherence to the medical treatments [8, 10–13].

HIV infection has more recently acquired the features of a sexually transmitted epidemic, where the group with the highest number of infections is men who have sex with men (MSM) [14, 15]. Over previous decades, the higher survival rate of HIV-patients along with the changes in the epidemic transmission route led to the onset of non-HIV-related causes of death, among them: cancer, liver and cardiovascular diseases [1, 16, 17]. The identification and better understanding of these new endpoints may facilitate the development of new clinical guidelines and practices for the management of HIV-infected patients.

Here, we aim to inform the development of new prevention plans by studying a large cohort of HIV-non-AIDS population. Our specific objectives are: 1) to calculate mortality rates according to the causes of death and their association to transmission categories and socio-demographic factors, 2) to determine factors related with worse survival in the defined population-based cohort and 3) to identify factors associated with worse survival in three groups, formed on the basis of cause of death (HIV-related, non-HIV-related and external).

Materials and Methods

Study design, setting and participants

We undertook an observational study in a population-based cohort of HIV-infected patients residing in Barcelona aged 18 years and older. The study spanned the period between 2001 and

30th of June 2013 (the end of follow up). Patients first diagnosed with HIV at the AIDS stage (less than 200 CD4 cells/ μ l and become ill with an opportunistic infection) were not included in the study [18]. The aim was to assess the impact of the risk factors on HIV-related or a non-HIV-related outcome before AIDS was present.

The cases were reported to the surveillance system of the Public Health Agency of Barcelona between 2001 and 2012. Reporting of new cases of HIV infection was voluntary between 2001 and 2009, but became mandatory for physicians from 2010. From then on the Barcelona HIV surveillance system became an active system that collects data provided by doctors, hospital discharges, the sexually transmitted infections registry, and mortality databases regarding patients diagnosed with HIV/AIDS.

Data collection

Clinicians completed a standard data collection form and used nominal identification. Socio-demographic factors considered in the study were: sex, date and country of origin, completed educational level and district household income at diagnosis [index calculated from (1) educational level of the population, (2) work status, (3) the amount of cars, (4) the power of newly bought cars, and (5) second-hand housing prices; it is measured as units standard: low-medium (<100), high (100–159) and very high (>159)] [19], HIV transmission category (IDU, MSM and heterosexual) and clinical data [CD4 cell count/ μ l; viral load (RNA copies/mL), hepatitis B surface antigen (HBsAg) and anti-HCV antibodies, year of HIV diagnosis]. A compound variable was constructed combining sex and HIV transmission. This variable takes into account the confounding of effects between sex and HIV transmission categories, resulting from the fact that the MSM transmission category only includes men, and that there are more IDU males than females [20].

Date of death was collected from the municipal registry of Barcelona city between January 2001 and June 2013. The administrative censoring date was 30th June 2013. Patients with a listing or delisting date in the municipal registry after this date were classified as alive at the end of the study. Subjects were considered lost to follow-up if they did not have a listing or delisting date after the administrative censoring date. Individuals were censored at the midpoint between their last recorded date and the administrative censoring date. Subjects with no registered date of death in the Catalonia region were classified as alive or loss to follow-up. Information on the status of the subjects lost to follow-up due to relocation was unavailable.

Specific cause of death was searched for in the Barcelona city death registry. The causes of death were coded using the International Classification of Diseases 10th revision (ICD-10) [21]. Specific causes of death were classified in three main groups: HIV-related (B20-B24, B44.9, C83.7, C85.9, and tuberculosis codes A15-A19), non-HIV-related (remaining codes not included in HIV-related or external groups) and external (overdose, suicide and accident codes: F19.2, Y19.9 and, X00-X99).

Statistical analysis

Descriptive analysis. Socio-demographic and clinical frequencies were calculated. Median and interquartile ranges (IQR) for continuous variables were obtained (i.e., age, CD4 counts and viral load).

Mortality. Incidence density and mortality rates were calculated as person-years of follow-up (py) per thousand with 95% confidence interval (CI). Mortality rates were obtained for specific and grouped causes of death according to sex, country of origin, educational level, district household income, transmission category (by sex), CD4 counts, and year of HIV diagnosis.

Survival time was estimated as the number of years between the date of HIV diagnosis and the date of death or the end of follow-up. Median and IQR survival times were obtained from

HIV-related, non-HIV-related, and external causes of death. These values were compared using the Kruskal-Wallis non-parametric test to check if there were different survival times according to the causes of death.

Survival analysis. Kaplan-Meier survival analysis was conducted to estimate and to graph survival for various factors (socio-demographic and clinical). Next, survival curves were compared using the log-rank test with the null hypothesis of a common survival curve (P values < 0.05 indicating a statistically significant difference between categories of the variables analysed). Multivariable analysis was carried out using Cox proportional hazards regression model after adjustment for confounding factors (age, country of origin, educational level, HIV transmission category by sex and CD4 counts at HIV diagnosis). The effect of the factors on survival was assessed by hazard ratios (HR) and their CI. Statistically significant factors in the univariable model were included in the multivariable model.

Three multivariable Fine and Gray regressions were fitted to estimate the adjusted effect of factors on the different outcomes (HIV-related, non-HIV-related and external) accounting for competing risks among all groups (the three outcome groups and the group with unidentified cause of death) [22]. These analyses were adjusted for any confounders. Sub-distributional hazard ratios were obtained. Unlike the hazard ratio, sub-distributional hazard represent the ordering and not a numerical value for the ratio [23]. A sensitivity Fine and Gray regression was performed to estimate the effect of the factors associated with unavailable cause of death, following diagnosis for HIV. In addition, a sensitivity analysis was performed to evaluate immortal time bias including only those patients with administrative censoring together with who died after one-year follow-up ($n = 266$). Analyses were performed using SPSS v.22.0 for descriptive analysis and Stata v.13 for Cox, Fine and Gray regression models.

Ethical considerations

HIV is a mandatory notifiable infection for health professionals in compliance with Article 13 of the law 67/2010 (25th May 2010) of the Health Department of Generalitat de Catalunya. All data were treated in a strictly confidential manner according to the ethical principles of the Helsinki Declaration of 1964 revised by the World Medical Organization in Fortaleza, 2013 [24], and Law 15/1999 of Personal Data Protection in Spain [25]. Patient information was anonymized and de-identified prior to analysis.

Results

Study population

We followed-up 3137 new HIV non-AIDS diagnoses which represented 12960 py. 85.9% of the cohort was male (Table 1). 61.0% of the patients were MSM. At diagnosis, the median age was 33 years (IQR 28–40 years), the median CD4 count was 399 cells/ μ L (IQR 245–579 cells/ μ L) and the median viral load was 45500 RNA copies/mL (IQR 11500–148300 RNA copies/mL).

Educational level. There was a significantly higher proportion of IDUs in the group of patients with lower educational level (26.0%) than in the higher education level (1.5%). In addition, patients in the lower educational level group had lower CD4 counts at diagnosis (< 200 CD4 cells/ μ L) than patients in the higher educational level group (19.9% vs. 12.0%). The proportion of immigrants having low educational level (14.5%) was lower than in Spanish-born (21.4%).

Mortality rate and causes of death

182 deaths were identified (14.0/1000 py; CI: 12.0–16.1/1000 py), 148 (81.3%) of them had a known cause of death, and 34 had an unknown cause of death. Educational level was the only

Table 1. Socio-demographic and clinical features of HIV-non-AIDS patients. Barcelona city, 2001–2012.

		HIV-infected individuals	
		n	
Socio-demographic features			
	Sex (%)		
	Male	2694	(85.9)
	Female	443	(14.1)
	Median age (IQR)	3137	33 (28–40)
	Country of origin (%)		
	Spaniards	1732	(55.2)
	Immigrants	1403	(44.7)
	Unknown	2	(0.1)
	Education level completed (%)		
	Illiteracy/Primary	573	(18.3)
	Lower secondary	689	(22.0)
	Upper secondary	887	(28.3)
	University	911	(29.0)
	Unknown	77	(2.5)
	District household income^a (%)		
	Low-medium	1755	(55.9)
	High	1111	(35.4)
	Very high	226	(7.2)
	Unknown	45	(1.4)
	HIV transmission category (%)		
	IDU Male	253	(8.1)
	IDU Female	75	(2.4)
	MSM non-IDU	1913	(61.0)
	Heterosexual Male	378	(12.0)
	Heterosexual Female	347	(11.1)
	Unknown	171	(5.5)
Clinical features^b			
	CD4 (cells/μl)^c(%)		
	<200	478	(15.2)
	200–349	581	(18.5)
	350–499	621	(19.8)
	≥500	899	(28.7)
	Unknown	558	(17.8)
	Viral load (RNA copies/mL)^d (%)		
	<50	69	(2.2)
	50–499	103	(3.3)
	500–29999	898	(28.6)
	≥30000	1408	(44.9)
	Unknown	659	(21.0)
	HbsAg (%)		
	Positive	179	(5.7)
	Negative	2183	(69.6)
	Unknown	775	(24.7)
	Anti-HCV antibodies (%)		
	Positive	351	(11.2)

(Continued)

Table 1. (Continued)

		HIV-infected individuals	
		n	
	Negative	2024	(64.5)
	Unknown	762	(24.3)
	Year of HIV diagnosis		
	2001	168	(5.4)
	2002	221	(7.0)
	2003	211	(6.7)
	2004	247	(7.9)
	2005	234	(7.5)
	2006	274	(8.7)
	2007	251	(8.0)
	2008	280	(8.9)
	2009	257	(8.2)
	2010	340	(10.8)
	2011	333	(10.6)
	2012	321	(10.2)
Total		3137	

IQR, interquartile range; MSM, men who have sex with other men; IDU, injecting drug user; HBsAg, hepatitis B surface antigen and HCV, hepatitis C virus.

^a 2013 Barcelona household income according to district where the person diagnosed with HIV lived.

^b At the time of HIV diagnosis.

^c <200 CD4 counts highlights severe immunodeficiency.

^d <50 RNA copies/mL highlights suppressive control of HIV.

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statistically significant difference between known cause of death group and unknown cause of death group ($P < 0.01$).

There was not a statistical difference in mortality rates among cause of death groups ($P = 0.5$). The mortality rate by HIV-related causes and non-HIV-related causes was the same (4.9/1000 py; CI: 3.7–6.1/1000 py) (Table 2). Individuals who died from external causes presented a lower mortality rate (1.7/1000 py; CI: 1.0–2.4/1000 py). We did not detect any external cause of death among women. In addition, Table 2 also shows crude mortality rates weighted per category in main covariates.

We compared mortality among Spanish-born and immigrants (11.5/1000 py; CI: 9.7–13.4/1000 py vs. 2.4/1000 py; 1.6 py– 3.3/1000 py, respectively). We observed a lower mortality rate for each cause of death in the group of immigrants, when compared to the group of Spanish-born people (Table 2). Moreover, we found that immigrants did not suffer from non-HIV-related causes of death such as other infections, cancer or liver disease (data not shown). The proportion of people, older than 49 years of age, living with HIV, was three times higher in the Spanish-born group than in the immigrant group (12.1% vs. 4.3%, respectively).

In addition, we analysed CD4 counts at HIV diagnosis. We found that mortality rates were higher in patients with CD4 count ≤ 200 cells/ μ L for main causes of death (Table 2). Furthermore, we assessed the influence of educational level on mortality rates. We detected higher mortality rates in individuals with lower educational level (Table 2). Finally, we did not find differences in crude mortality rates for the year of HIV diagnosis among years with voluntary reporting (2001–2009) and compulsory (2010–2012) (Table 2).

Table 2. Mortality rates. Barcelona city, January 2001- June 2013.

Causes of death			All causes		HIV-related		Non-HIV-related		External	
	n	py	deaths	WA (95% CI)	deaths	WA (95% CI)	deaths	WA (95% CI)	deaths	WA (95% CI)
Total	3137	12960	182	14.0 (12.0–16.1)	63	4.9 (3.7–6.1)	63	4.9 (3.7–6.1)	22	1.7 (1.0–2.4)
Socio-demographic features										
Sex										
Male	2694	10769	144	11.5 (9.6–13.3)	52	4.1 (3.0–5.3)	45	3.6 (2.5–4.6)	22	1.8 (1.0–2.5)
Female	443	2190	38	2.5 (1.7–3.2)	11	0.7 (0.3–1.1)	18	1.2 (0.6–1.7)	0	—
Age										
18–29 years	979	4277	21	1.5 (0.9–2.2)	10	0.7 (0.3–1.2)	3	0.2 (0.0–0.5)	4	0.3 (0.0–0.6)
30–39 years	1311	5572	60	4.5 (3.4–5.6)	23	1.7 (1.0–2.4)	13	1.0 (0.4–1.5)	11	0.8 (0.3–1.3)
≥40 years	847	3111	101	8.8 (7.1–10.4)	30	2.6 (1.7–3.5)	47	4.1 (2.9–5.2)	7	0.6 (0.2–1.1)
Country of origin										
Spaniards	1732	7230	151	11.5 (9.7–13.4)	48	3.7 (2.6–4.7)	57	4.4 (3.2–5.5)	20	1.5 (0.9–2.2)
Immigrants	1403	5719	31	2.4 (1.6–3.3)	15	1.2 (0.6–1.8)	6	0.5 (0.1–0.8)	2	0.2 (0.0–0.4)
Unknown	2	12	0	—	0	—	0	—	0	—
Education level completed										
Illiteracy/Primary	573	2652	78	5.4 (4.2–6.5)	30	2.1 (1.3–2.8)	30	2.1 (1.3–2.8)	8	0.6 (0.2–0.9)
Lower secondary	689	2946	36	2.7 (1.8–3.6)	14	1.0 (0.5–1.6)	11	0.8 (0.3–1.3)	10	0.7 (0.3–1.2)
Upper secondary	887	3597	34	2.7 (1.8–3.6)	9	0.7 (0.2–1.2)	12	0.9 (0.4–1.5)	4	0.3 (0.0–0.6)
University	911	3488	17	1.4 (0.7–2.1)	7	0.6 (0.2–1.0)	7	0.6 (0.2–1.0)	0	—
Unknown	77	277	17	1.5 (0.8–2.2)	3	0.3 (0.0–0.6)	3	0.3 (0.0–0.6)	0	—
District household income^a										
Low-medium	1755	7351	113	8.6 (7.0–10.2)	41	3.1 (2.2–4.1)	41	3.1 (2.2–4.1)	12	0.9 (0.4–1.4)
High	1111	4524	47	3.7 (2.6–4.7)	16	1.3 (0.6–1.9)	15	1.2 (0.6–1.8)	9	0.7 (0.2–1.2)
Very high	226	933	14	1.1 (0.5–1.6)	5	0.4 (0.0–0.7)	6	0.5 (0.1–0.8)	1	0.1 (0.0–0.2)
Unknown	45	152	8	0.8 (0.2–1.3)	1	0.1 (0.0–0.3)	1	0.1 (0.0–0.3)	0	—
HIV transmission category										
IDU Male	253	1278	50	3.2 (2.3–4.0)	18	1.1 (0.6–1.7)	13	0.8 (0.4–1.3)	13	0.8 (0.4–1.3)
IDU Female	75	396	14	0.8 (0.4–1.3)	3	0.2 (0.0–0.4)	7	0.4 (0.1–0.7)	0	—
MSM non-IDU	1913	7371	38	3.1 (2.1–4.1)	15	1.2 (0.6–1.9)	12	1.0 (0.4–1.6)	2	0.2 (0.0–0.4)
Heterosexual Male	378	1508	43	3.4 (2.4–4.4)	14	1.1 (0.5–1.7)	16	1.3 (0.7–1.9)	7	0.6 (0.1–1.0)
Heterosexual Female	347	1697	23	1.5 (0.9–2.1)	8	0.5 (0.2–0.9)	10	0.7 (0.2–1.1)	0	—
Unknown	171	709	14	1.1 (0.5–1.6)	5	0.4 (0.0–0.7)	5	0.4 (0.0–0.7)	0	—
Clinical features^b										
CD4 (cells/μl)^c										
<200	478	1971	58	4.5 (3.3–5.6)	28	2.2 (1.4–3.0)	18	1.4 (0.8–2.0)	4	0.3 (0.0–0.6)
200–349	581	2401	28	2.2 (1.4–3.0)	7	0.5 (0.1–0.9)	10	0.8 (0.3–1.2)	5	0.4 (0.0–0.7)
350–499	621	2459	18	1.4 (0.8–2.1)	7	0.6 (0.1–1.0)	6	0.5 (0.1–0.9)	2	0.2 (0.0–0.4)
≥500	899	3627	32	2.5 (1.7–3.4)	11	0.9 (0.4–1.4)	9	0.7 (0.2–1.2)	5	0.4 (0.0–0.7)
Unknown	558	2501	46	3.3 (2.3–4.2)	10	0.7 (0.3–1.2)	20	1.4 (0.8–2.0)	6	0.4 (0.1–0.8)
Year of HIV diagnosis										
2001	168	1039	23	1.2 (0.7–1.7)	7	0.4 (0.1–0.6)	6	0.3 (0.1–0.6)	2	0.1 (0.0–0.2)
2002	221	1745	37	1.5 (1.0–2.0)	14	0.6 (0.3–0.9)	11	0.4 (0.2–0.7)	5	0.2 (0.0–0.4)
2003	211	1519	23	1.0 (0.6–1.4)	9	0.4 (0.1–0.7)	11	0.5 (0.2–0.8)	2	0.1 (0.0–0.2)
2004	247	1568	26	1.3 (0.8–1.8)	8	0.4 (0.1–0.7)	11	0.6 (0.2–0.9)	4	0.2 (0.0–0.4)
2005	234	1375	14	0.8 (0.4–1.2)	3	0.2 (0.0–0.3)	5	0.3 (0.0–0.5)	3	0.2 (0.0–0.3)
2006	274	1452	19	1.1 (0.6–1.7)	4	0.2 (0.0–0.5)	9	0.5 (0.2–0.9)	1	0.1 (0.0–0.2)
2007	251	1115	9	0.6 (0.2–1.1)	4	0.3 (0.0–0.6)	1	0.1 (0.0–0.2)	1	0.1 (0.0–0.2)

(Continued)

Table 2. (Continued)

Causes of death			All causes		HIV-related		Non-HIV-related		External	
	n	py	deaths	WA (95% CI)	deaths	WA (95% CI)	deaths	WA (95% CI)	deaths	WA (95% CI)
2008	280	1027	9	0.8 (0.3–1.3)	5	0.4 (0.1–0.8)	4	0.3 (0.0–0.7)	0	—
2009	257	748	6	0.7 (0.1–1.2)	4	0.4 (0.0–0.9)	2	0.2 (0.0–0.5)	0	—
2010	340	731	8	1.2 (0.4–2.0)	4	0.6 (0.0–1.2)	0	—	2	0.3 (0.0–0.7)
2011	333	445	7	1.7 (0.4–2.9)	1	0.2 (0.0–0.7)	3	0.7 (0.0–1.5)	1	0.2 (0.0–0.7)
2012	321	194	1	0.5 (0.0–1.6)	0	—	0	—	1	0.5 (0.0–1.6)

py, person-years follow-up in a category; WA, Weighted average of crude mortality rate in categories; CI, confidence intervals; MSM, men who have sex with other men; IDU, injecting drug user; HBsAg, hepatitis B surface antigen and HCV, hepatitis C virus.

WA = (deaths/py) x (n/3137); deaths/py = mortality rate

^a 2013 Barcelona household income according to district where the person diagnosed of HIV lived

^b At the time of HIV diagnosis

^c <200 CD4 counts highlights severe immunodeficiency

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Survival outcomes

The cumulative survival probability 12 years after diagnosis was 83.1% (CI 78.1%-87.1%). We found significant differences ($P < 0.01$) in the Kaplan-Meier survival analysis by age at HIV diagnosis, country of origin, educational level, district household income, HIV transmission category (by sex), CD4 and anti-HCV antibodies (Fig 1). Furthermore, we observed a strong inverse relationship between age at HIV diagnosis and CD4 counts (P linear association = 5.4×10^{-21}), i.e. higher age was related to lower CD4 level.

Factors associated with survival

Table 3 show the factors associated with worse survival. Spanish-born patients showed higher mortality than immigrants. We also observed that those with illiteracy or primary education only had worse survival than those having university education, in turn we did not find differences according to district household income among HIV-infected residents at the time of diagnosis.

We evaluated immortal time bias using a Cox proportional hazards model, with patients with administrative censoring and with those who died after one-year of follow-up ($n = 266$). We found a statistically significant association with ageing (HR: 1.1; 95% CI: 1.0–1.2); and IDU male and heterosexual male vs. MSM non-IDU (HR: 1.9; 95% CI: 1.0–3.6 and HR: 2.1; 95% CI: 1.1–3.8, respectively).

We observed that 1092 of 3137 had incomplete data for some of the variables included in the adjusted model (age, country of origin, educational level, district household income, HIV transmission category, CD4 counts, and antibodies anti-HCV). We validated the hazard ratios direction and value with a univariable model excluding the missing values for the adjustment variables. Equal statistically significant hazard ratio was obtained in this comparison for age, educational level, district household income, CD4+ counts, and positive to antibodies anti-HCV. Similar significant hazard ratios were calculated comparing Spanish-born vs. immigrants (HR: 3.5; 95% CI: 2.4–5.1); and HIV transmission category, e.g.: HTS female vs. MSM non-IDU (HR: 2.6; 95% CI: 1.5–4.3). This suggests that missing values in these variables are not modifying the direction of hazard ratios in the models that did not exclude missing values.

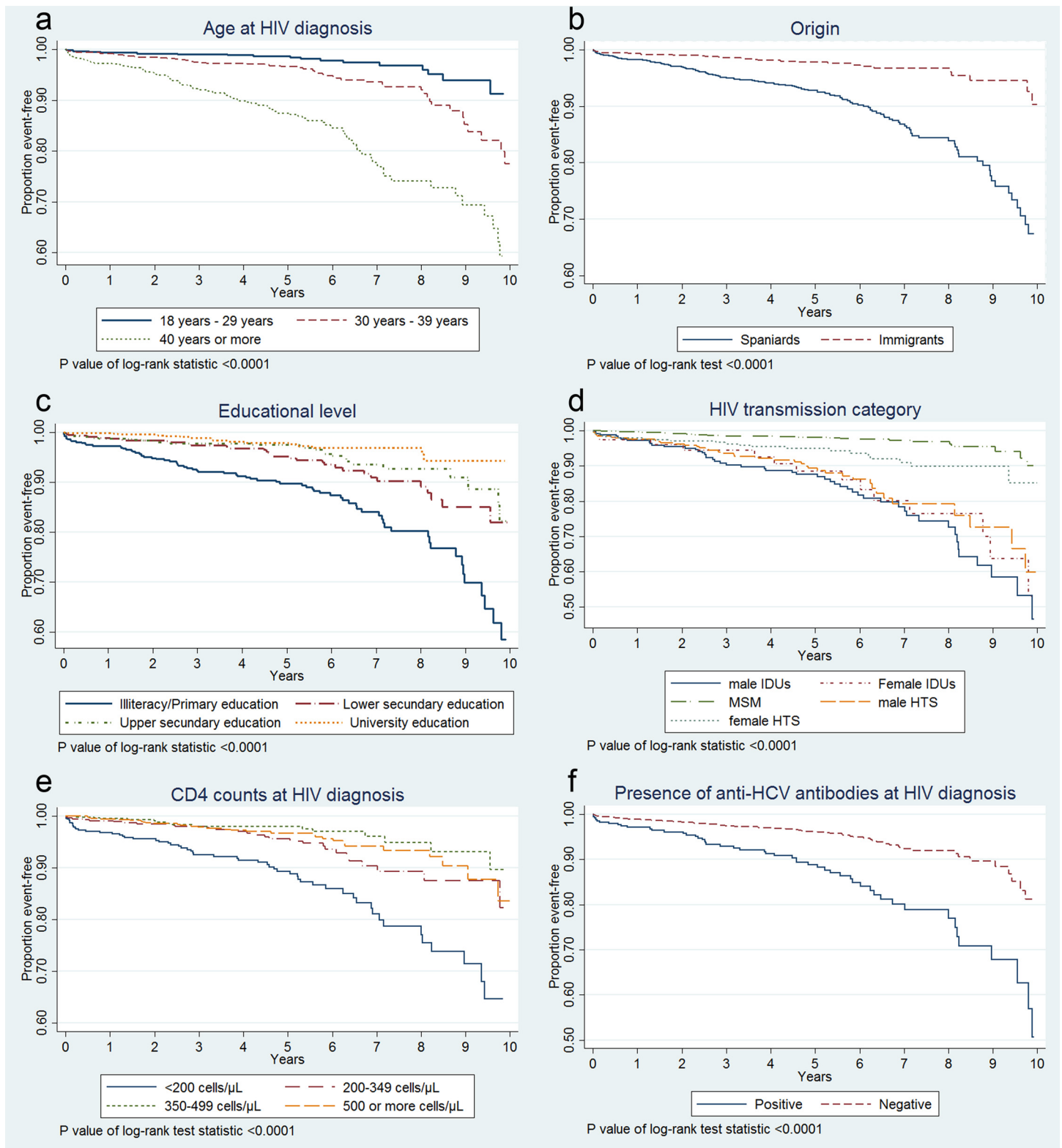


Fig 1. Survival curves according to age, origin, educational level, transmission category, CD4, and anti-HCV antibodies. Survival probabilities according to: (a) age at HIV diagnosis, (b) country of origin, (c) educational level, (d) HIV transmission category and sex (IDUs: injecting drug users, MSM: men who have sex with men, and HTS: heterosexuals), (e) CD4 cell count/ μ L at HIV diagnosis and (f) anti-HCV antibodies at HIV diagnosis.

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Table 3. Cox proportional hazards model showing factors associated with survival in a cohort of 3137 HIV-non-AIDS patients. Barcelona city, January 2001–June 2013.

Independent variables ^a	Univariable analysis			Multivariable analysis ^b		
	Hazard ratio (95% CI)	P value (Wald test)	Hazard ratio (95% CI)	P value (Wald test)		
Age at HIV diagnosis (continuous variable 5-year groups)^b	1.4	(1.3–1.5)	<0.001	1.3	(1.2–1.4)	<0.001
Country of origin (Immigrants)^b						
Spaniards	3.9	(2.7–5.8)	<0.001	2.3	(1.5–3.5)	<0.001
Educational level completed (University education)^b						
Illiteracy/Primary education	6.1	(3.6–10.3)	<0.001	2.2	(1.2–3.9)	0.007
Lower secondary education	2.5	(1.4–4.5)	0.002	1.5	(0.8–2.8)	0.174
Upper secondary education	2.0	(1.1–3.5)	0.023	1.6	(0.9–2.8)	0.143
Unknown	15.1	(7.7–29.7)	<0.001	7.6	(3.8–15.5)	<0.001
District household income^c (Very high)^b						
Low-medium	1.0	(0.6–1.7)	0.959			
High	0.7	(0.4–1.2)	0.214			
Unknown	3.9	(1.6–9.3)	0.002			
HIV transmission category (MSM non-IDU)^b						
IDU Male	7.6	(5.0–11.7)	<0.001	4.1	(2.6–6.5)	<0.001
IDU Female	6.2	(3.3–11.4)	<0.001	3.3	(1.7–6.3)	<0.001
Heterosexual Male	5.8	(3.7–9.0)	<0.001	2.6	(1.6–4.1)	<0.001
Heterosexual Female	2.6	(1.5–4.3)	<0.001	1.7	(1.0–2.9)	0.064
Unknown	3.8	(2.0–6.9)	<0.001	2.3	(1.2–4.3)	0.012
CD4 at diagnosis (≥ 500)						
<200 ^d	3.2	(2.1–5.0)	<0.001	1.7	(1.1–2.7)	0.016
200–349	1.3	(0.8–2.1)	0.336	1.1	(0.6–1.8)	0.790
350–499	0.8	(0.4–1.4)	0.416	0.7	(0.4–1.3)	0.263
Unknown	2.0	(1.3–3.1)	0.003	1.4	(0.9–2.2)	0.183
Viral load at diagnosis (<50)^{b, e}						
50–499	1.4	(0.3–7.7)	0.687			
500–29 999	2.0	(0.5–8.4)	0.328			
$\geq 30\ 000$	1.9	(0.5–7.8)	0.370			
Unknown	3.2	(0.8–13.3)	0.101			
Anti-HCV (Negative)^b						
Positive	3.0	(2.1–4.3)	<0.001			
Unknown	1.2	(0.8–1.7)	0.349			
Year of HIV diagnosis (2007)						
2001	2.2	(1.0–4.8)	0.049			
2002	1.4	(0.7–3.1)	0.352			
2003	1.3	(0.6–2.9)	0.513			
2004	1.7	(0.8–3.6)	0.186			
2005	1.1	(0.5–2.6)	0.790			
2006	1.5	(0.7–3.4)	0.305			
2008	1.1	(0.4–2.8)	0.843			
2009	1.0	(0.3–2.8)	0.973			
2010	1.3	(0.5–3.4)	0.615			
2011	1.7	(0.6–4.8)	0.292			

(Continued)

Table 3. (Continued)

Independent variables ^a	Univariable analysis		Multivariable analysis ^b	
	Hazard ratio (95% CI)	P value (Wald test)	Hazard ratio (95% CI)	P value (Wald test)
2012	0.4 (0.0–3.1)	0.377		

CI, confidence intervals; MSM, men who have sex with other men; IDU, injecting drug user; and HCV, hepatitis C virus.

^a Reference categories are shown in parentheses.

^b P values (Pseudo-Likelihood Ratio test) <0.05 by factors in crude analysis and as a whole in multivariable analysis.

^c 2013 Barcelona household income according to district where the person diagnosed of HIV lived.

^d <200 CD4 counts highlights severe immunodeficiency.

^e <50 RNA copies/mL highlights suppressive control of HIV.

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Prognostic factors

The factors associated with HIV-related causes of death were: older age at HIV diagnosis, 20% increase in death risk corresponding to each 5-year period; low educational level; IDU male and CD4<200 (Table 4). In the unadjusted analysis we observed a higher risk of death by HIV-related causes of death in those with illiteracy or primary education than those with university studies. In addition we observed a non-statistically significant reduction in risk from lower to higher schooling.

Factors associated with non-HIV-related causes of death were: older age at HIV diagnosis, 50% increase in death risk corresponding to each 5-year age period; Spanish-born; IDU female (marginally significant) or heterosexual male and female; and presence of anti-HCV antibodies at HIV diagnosis (Table 4).

Factors associated with external causes of death were: IDU male and heterosexual male (Table 4).

Competing risk hazards models with administrative censoring and patients who had died after one-year of follow-up (n = 266) were performed to evaluate potential immortal time bias. The model of HIV-related causes of death was not statistically significant because of the loss in statistical power. The model of non-HIV-related causes of death found a statistically significant association with ageing (sHR: 1.4; 95% CI: 1.2–1.6); heterosexual female vs. MSM non-IDU (sHR: 3.4; 95% CI: 1.1–10.5) and positive vs negative antibodies anti-HCV (sHR: 5.2; 95% CI: 2.0–13.4). The model of external causes of death found a statistically significant association with ageing, and IDU male and heterosexual male vs. MSM non-IDU (sHR: 18.9; 95% CI: 2.7–129.5 and sHR: 12.5; 95% CI: 1.4–110.8, respectively).

Discussion

Key results

Because of epidemic and treatment changes in the HAART era we expected to find higher mortality rates for non-HIV-related causes of death group than for HIV-related ones [26, 27]. However, we found equal mortality rates. However, the mortality rates for HIV-related causes of death would have been greater than for non-HIV-related if some of the non-HIV-related causes of death were in fact, either directly or indirectly, a consequence of HIV infection (e.g. cancers could be associated with loss of immunity).

Our unit observed in previous studies that all-cause mortality tends to diminish over the time for every transmission category [28, 29]. Nonetheless, the overall rate (14.0/1000 py) was

almost twice that of the general Barcelona city population (range of 7.8–8.3/1000 py for 2009–2013) [30]. We also found higher mortality than the Spanish AIDS research network (10.2/1000 py; CI: 9.1–11.5/1000 py) [17] and slightly greater than a collaborative cohort consisting of 13 cohorts of Europe and North America of patients starting HAART during 1996–2006 (12.1/1000 py; CI: 11.6–12.7/1000 py) [31]. Most likely, the higher mortality found in this population level study is because we included patients with higher risk of death than those considered in active follow-up cohorts. This is because we include patients not receiving HIV medical care as well as those who are [32].

Table 4. Factors associated with HIV-related causes of death, non-HIV-related and external following diagnosis for HIV. Competing risk hazards for univariable and multivariable models. Barcelona city, January 2001–June 2013.

	HIV-related (63 deaths)				non-HIV-related (63 deaths)				external (22 deaths)			
	Crude analysis		Adjusted analysis		Crude analysis		Adjusted analysis		Crude analysis		Adjusted analysis	
	sHR	95% CI	sHR	95% CI	sHR	95% CI	sHR	95% CI	sHR	95% CI	sHR	95% CI
Independent variables^{a, b}												
Age at HIV diagnosis (continuous variable)	1.3^c	(1.2–1.4)	1.2	(1.1–1.3)	1.5	(1.4–1.6)	1.5	(1.4–1.7)	1.1	(0.9–1.3)	1.0	(0.8–1.2)
Country of origin (Immigrants)												
Spaniards	2.5	(1.4–4.4)	1.4	(0.7–2.6)	7.4	(3.2–17.0)	3.8	(1.6–8.8)	7.7	(1.8–32.8)	3.6	(0.8–15.1)
Educational level completed (University)												
Illiteracy/Primary education	5.3	(2.3–12.2)	2.7	(1.1–6.6)	5.1	(2.2–11.6)	1.3	(0.6–3.2)	2.5	(0.7–8.3)	1.0	(0.3–3.5)
Lower secondary education	2.3	(0.9–5.8)	1.6	(0.6–4.1)	1.8	(0.7–4.6)	1.0	(0.4–2.5)	3.0	(1.0–9.5)	1.7	(0.5–5.5)
Upper secondary education	1.2	(0.5–3.4)	1.1	(0.4–2.9)	1.7	(0.7–4.2)	1.2	(0.5–3.0)	1 ^e	–	1 ^e	–
Unknown	5.0	(1.3–19.7)	2.8	(0.7–11.4)	5.1	(1.4–18.5)	1.8	(0.4–8.0)	–	–	–	–
HIV transmission category (MSM non-IDU)												
IDU Male	6.4	(3.3–12.6)	3.2	(1.5–7.0)	5.4	(2.4–11.9)	1.7	(0.7–4.3)	34.3	(7.9–149.0)	28.7	(6.7–123.2)
IDU Female	3.2	(0.9–10.9)	1.8	(0.5–6.5)	8.8	(3.3–23.3)	2.9	(0.9–9.1)	–	–	–	–
Heterosexual Male	4.4	(2.1–9.0)	1.8	(0.8–4.2)	6.2	(2.9–13.2)	2.5	(1.1–6.0)	16.5	(3.4–78.8)	11.8	(2.5–56.4)
Heterosexual Female	2.2	(0.9–5.3)	1.2	(0.5–3.0)	3.3	(1.4–7.8)	2.8	(1.1–7.3)	–	–	–	–
Unknown	3.2	(1.1–9.0)	2.4	(0.8–7.1)	4.1	(1.4–11.6)	1.5	(0.5–4.2)	–	–	–	–
CD4 at diagnosis (≥500)												
<200 ^d	4.4	(2.2–8.8)	2.7	(1.3–5.7)	3.4	(1.5–7.7)	1.6	(0.7–3.9)	1.4	(0.4–5.0)	1.0	(0.2–3.9)
200–349	0.9	(0.4–2.4)	0.8	(0.3–2.2)	1.6	(0.7–4.0)	1.2	(0.5–3.2)	1.5	(0.4–5.2)	1.6	(0.5–5.3)
350–499	0.9	(0.4–2.3)	0.9	(0.4–2.2)	0.9	(0.3–2.6)	0.8	(0.3–2.3)	0.6	(0.1–3.0)	0.8	(0.1–4.6)
Unknown	1.2	(0.5–2.9)	0.8	(0.3–2.0)	3.0	(1.4–6.6)	2.3	(1.0–5.4)	1.7	(0.5–5.5)	1.5	(0.5–5.0)
Anti-HCV (Negative)												
Positive	2.1	(1.2–3.8)	–	–	2.1	(1.2–3.8)	4.3	(1.9–9.8)	3.4	(1.4–8.6)	0.4	(0.1–1.4)
Unknown	0.8	(0.4–1.5)	–	–	0.8	(0.4–1.5)	3.1	(1.4–6.5)	0.7	(0.2–2.3)	0.4	(0.1–1.2)

sHR, subHazard Ratio; CI, confidence intervals; MSM, men who have sex with other men; IDU, injecting drug user; and HCV, hepatitis C virus.

^a All the models have been adjusted by age, origin, educational level, HIV transmission, and CD4 counts. A sensitivity analysis was carried out from non-available causes of death. The only associated factor was being older at HIV diagnosis, with each 5 years the death risk increased by 30%.

^b Reference categories are shown in parenthesis.

^c Bold numbers highlight *P* values < 0.05 for Wald test.

^d <200 CD4 counts highlights severe immunodeficiency.

^e Upper secondary education as reference category.

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Differences among transmission groups continue to be important. IDUs are a vulnerable group with respect to death from HIV-related and external causes, although they make up only 10% of new HIV-non-AIDS diagnoses [33]. The IDU males had a higher mortality rate by external causes of death. MSM had higher weighted crude mortality rates for both HIV-related and non-HIV-related causes of death, as well as heterosexual males. In the adjusted model we found higher mortality in heterosexual males and IDUs (male and female) than MSM. These groups have been found to be more likely to be diagnosed at a later stage, related to receiving care for their condition [33, 34].

Factors associated with survival

Age and CD4 counts. Besides lower CD4 counts, the patients with worse survival were also older. Being older at HIV diagnosis was associated with higher mortality by HIV-related and non-HIV-related causes of death. This risk of death was higher for non-HIV-related causes of death suggesting that HIV infection accelerates the progression of other comorbidities. Therefore, classification of some ICD-10 codes as non-HIV-related causes of death might need revision. In addition, other co-morbidities such as hepatitis B or C, and a longer delay in the diagnosis increase the deterioration caused by HIV infection [35–39]. Older heterosexual males are likely less aware about their HIV exposure and IDUs take less care of themselves delaying the initiation of their treatments. Other studies have linked to higher age at HIV diagnosis with worse survival rates [40, 41]. The burden of disease in HIV-infected subjects is thus heavier in older patients [42, 43]. In fact, CD4 cell count at HIV diagnosis is the predominant prognostic factor for survival [33, 44]. Baseline counts of CD4 are more relevant for HIV-related causes of death. This observation has important implications for the clinical management of the patients [44].

Survival in Spanish-born and immigrants. The better survival in immigrants, observed in this study, may be due to follow-up losses in those who left their residence in Barcelona [29]. This may be a source of informational bias in our study [45]. It has been found, with respect to other illnesses, that patients with deteriorating condition have the tendency to go back to their countries [46, 47]. We did not find a statistically significant difference in HIV-related causes of death while we did find a difference in non-HIV-related causes. Less deterioration in their condition might be behind the loss of follow-up of these patients. Difference in mortality between immigrants and Spanish-born is also observed in general population. For example, in 2013 0.22% of deaths were registered among immigrants and 0.97% of deaths among Spanish-born, both living in Barcelona [30]. In addition, 16.2% of immigrants were delisted because of leaving Barcelona and Spain [30]. Taking into account that deaths in Spanish category were 8.7% of our study population and assuming the same mortality proportion for immigrants, we might not detect 1.4% of deaths among immigrants group. Although this number would be lower as immigrants were shown to be statistically significantly younger than Spaniards.

Educational level. Educational level is an approximation to socio-economic status reflecting social health inequalities. Lower educational level has been related to higher mortality rates from most causes in high-income countries [15, 48]. This gap also occurs among our HIV patients, receiving universal health care [15, 49]. Low educational level may be associated with poor adherence to HAART and worse self-care [50, 51]. Individuals with HIV may have more prevalent risk behaviours [52], for example, this has been observed with smoking [53]. Therefore, low educational level has been related to life-style mortality (non-HIV-related and external causes) but does not derive from HIV-related risk factors, i.e. low educational level is not associated with late presentation of HIV, time to HAART initiation, or HAART response [54]. Despite this we found association among the lowest educational level, the lowest baseline CD4 and to be UDI male with HIV-related causes of death.

Survival and district household income. We did not find worse survival according to household income which might be because of the universal access to HAART provided by the Spanish national health system. Other authors have, however, found socioeconomic status, including work status to be an important prognostic risk factor [55–57]. We expected to find worse survival in low-medium household income districts which our Unit had previously observed using other neighbourhood deprivation indicators [29]. Indeed, a national survey in Spain showed that employment among HIV patients was low (45.8%) [58]. Additionally, some of responders in this survey were living in prison (3.0%), other closed facilities (5.0%) or were homeless (1.8%).

IDU and heterosexual survival. We found that being IDU was a risk factor for death, which further confirmed the results of previous studies carried out in our unit [28, 29]. IDU male presented higher risk of death by HIV-related causes while IDU female presented higher risk by non-HIV-related causes. Heterosexuals, male and female, also had higher risk of death from non-HIV-related causes than MSM. Our findings may relate to worse HAART adherence [59, 60], or to later HAART initiation [33, 34]. IDUs are diagnosed at an early stage of their infection, but they delay HAART initiation by one or two years. During this time the reduction in their CD4 counts has already reduced their life expectancy [39, 61–64]. Support programmes are therefore necessary to encourage early HAART initiation in this group. On the other hand, heterosexual males have impaired survival due to late diagnosis [29, 39]. For this group, actions aimed at increasing the awareness of and accessibility to HIV testing should be conducted. On the other hand, female risk of death is associated more with non-HIV-related causes which might be because of earlier HAART initiation avoiding HIV-related infections.

Limitations

The main limitation of our study was that outcome identification required using secondary data sources. The municipal registry and death registry used only captured deaths in the city of Barcelona. We could not identify deaths which occurred outside of the Catalonia region. Despite this, we have found a slightly higher proportion of deaths (5.8%) than a) a Swiss cohort with patients starting HAART during 2005–2009 (5.1%) [27], b) a collaborative cohort composed of HIV-1-infected patients following HAART between 1996 and 2006 from 13 cohorts of Europe and North America (4.8%) [31], and c) Spanish cohorts of the AIDS Research Network from 1997 to 2010 (3.6%) [17]. General population of the municipal registry show a migratory movement surrounding 3% between 2009 and 2013. Assuming a roughly similar behaviour of HIV population without to have into account age, origin and transmission HIV category this might mean the no detection of 8.7% of deaths among a hypothetical 3% of patients leaving Barcelona in our study population resulting in not detecting 6 deaths. Although loss to follow-up would be lower because access to HAART is free in Spain and Barcelona has some of the best high quality hospitals of the country.

We did not find a specific cause of death in 18.7% cases. A sensitivity analysis did not show statistically significant difference between patients with known and unknown specific cause of death when comparing by sex, age, country of origin, household income, HIV transmission category, CD4 counts, viral load and presence of antibodies anti-HCV. Thus, we may not expect changes interpreting Fine and Gray model results if we knew the specific cause for all deaths. Nevertheless, we found difference in educational level. Patients with unavailable cause of death had lower proportion of lower studies completed and higher percentage with unknown educational level. Assuming patients with unknown educational level were patients with low educational level we also might not expect changes interpreting Fine and Gray models.

The evolution of the HIV infection depends on early HAART initiation and good adherence to treatment. Firstly, the criteria for HAART initiation have not always been the same; they

change over our study period. For example, the criteria for HAART initiation in 2013 in Spain was having a CD4 count of < 500 cells/ μ L in asymptomatic patients or presenting an AIDS-defining illness [65]. This means that for our survey and according to CD4 counts, 1680 patients (65.1% of the subjects with CD4 known values) should have started HAART shortly after knowing their HIV diagnosis. However, they did not in fact initiate HAART because the criteria suggested by guidelines in 2002 was to start with CD4 counts < 200 cells/ μ L and in 2009 was to start with CD4 counts < 350 cells/ μ L [66, 67]. Further, our database did not include data about HAART regimen and adherence. These variables would have helped us to better understand patient histories.

Conclusion

There are important differences in survival among transmission groups during study period. The analysis of causes of death in HIV patients has shown that, until 2013, the availability of HAART has not benefited all groups equally, probably because IDUs and heterosexual males start HIV treatment later. It seems that these groups have severe immunodeficiency by the time they approach healthcare services for treatment. Awareness programs and incentives are needed to improve this situation.

Another suggestion, which can be made with respect to the results of this study, is that elderly HIV-patients may require more focused clinical care that extends beyond HIV treatment.

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Author Contributions

Conceived and designed the experiments: CG PGdO JAC. Analyzed the data: CG. Wrote the paper: CG PGdO JAC. Collected data from municipal registry and death registry, generated dataset, debugged data: CG. Drafted the manuscript, interpreted data: CG PGdO JAC. Collected data and notified the cases: JMM IO HK MJB VH PD JMG ER MG AM Barcelona HIV/AIDS Working Group. Reviewed and approved the final manuscript: CG PGdO JMM IO HK MJB VH PD JMG ER MG AM JAC.

References

1. ART Cohort Collaboration. Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. *Lancet* 2008; 372(9635):293–99. doi: [10.1016/S0140-6736\(08\)61113-7](https://doi.org/10.1016/S0140-6736(08)61113-7) PMID: [18657708](https://pubmed.ncbi.nlm.nih.gov/18657708/)

2. May MT, Ingle SM. Life expectancy of HIV-positive adults: a review. *Sex Health* 2011; 8(4):526–33. doi: [10.1071/SH11046](https://doi.org/10.1071/SH11046) PMID: [22127039](https://pubmed.ncbi.nlm.nih.gov/22127039/)
3. Nakagawa F, May M, Phillips A. Life expectancy living with HIV: recent estimates and future implications. *Curr.Opin.Infect.Dis.* 2013; 26(1):17–25. doi: [10.1097/QCO.0b013e32835ba6b1](https://doi.org/10.1097/QCO.0b013e32835ba6b1) PMID: [23221765](https://pubmed.ncbi.nlm.nih.gov/23221765/)
4. Corless IB, Wantland D, Kirksey KM, Nicholas PK, Human S, Arudo J, et al. Exploring the contribution of general self-efficacy to the use of self-care symptom management strategies by people living with HIV infection. *AIDS Patient.Care STDS.* 2012; 26(6):335–43. doi: [10.1089/apc.2011.0404](https://doi.org/10.1089/apc.2011.0404) PMID: [22612448](https://pubmed.ncbi.nlm.nih.gov/22612448/)
5. Monge S, Jarrín I, Pérez-Hoyos S, Ferreros I, García de Olalla P, Muga R, et al. Educational level and HIV disease progression before and after the introduction of HAART: a cohort study in 989 HIV seroconverters in Spain. *Sex Transm.Infect.* 2011; 87(7):571–76. doi: [10.1136/sextrans-2011-050125](https://doi.org/10.1136/sextrans-2011-050125) PMID: [21968461](https://pubmed.ncbi.nlm.nih.gov/21968461/)
6. Porter K, Babiker A, Bhaskaran K, Darbyshire J, Pezzotti P, Porter K, et al. Determinants of survival following HIV-1 seroconversion after the introduction of HAART. *Lancet* 2003; 362(9392):1267–74. PMID: [14575971](https://pubmed.ncbi.nlm.nih.gov/14575971/)
7. Wada N, Jacobson LP, Cohen M, French A, Phair J, Muñoz A. Cause-specific life expectancies after 35 years of age for human immunodeficiency syndrome-infected and human immunodeficiency syndrome-negative individuals followed simultaneously in long-term cohort studies, 1984–2008. *Am.J.Epidemiol.* 2013; 177(2):116–25. doi: [10.1093/aje/kws321](https://doi.org/10.1093/aje/kws321) PMID: [23287403](https://pubmed.ncbi.nlm.nih.gov/23287403/)
8. García de Olalla P, Knobel H, Carmona A, Guelar A, López-Colomé JL, Caylà JA. Impact of adherence and highly active antiretroviral therapy on survival in HIV-infected patients. *J Acquir Immune Defic Syndr.* 2002; 30(1):105–10. PMID: [12048370](https://pubmed.ncbi.nlm.nih.gov/12048370/)
9. Office for Official Publications of the European Communities. Annual report on the state of the drugs problem in the European Union 2000. Luxembourg, 2000.
10. Brion JM, Rose CD, Nicholas PK, Sloane R, Corless IB, Lindgren TG, et al. Unhealthy substance-use behaviors as symptom-related self-care in persons with HIV/AIDS. *Nurs.Health Sci.* 2011; 13(1):16–26. doi: [10.1111/j.1442-2018.2010.00572.x](https://doi.org/10.1111/j.1442-2018.2010.00572.x) PMID: [21352430](https://pubmed.ncbi.nlm.nih.gov/21352430/)
11. Grigoryan A, Hall HI, Durant T, Wei X. Late HIV diagnosis and determinants of progression to AIDS or death after HIV diagnosis among injection drug users, 33 US States, 1996–2004. *PLoS.One.* 2009; 4(2):e4445. doi: [10.1371/journal.pone.0004445](https://doi.org/10.1371/journal.pone.0004445) PMID: [19214229](https://pubmed.ncbi.nlm.nih.gov/19214229/)
12. Langebeek N, Gisolf EH, Reiss P, Vervoort SC, Hafsteinsdóttir TB, Richter C, et al. Predictors and correlates of adherence to combination antiretroviral therapy (ART) for chronic HIV infection: a meta-analysis. *BMC.Med.* 2014; 12(1):142.
13. Murray M, Hogg RS, Lima VD, May MT, Moore DM, Abgrall S, et al. The effect of injecting drug use history on disease progression and death among HIV-positive individuals initiating combination antiretroviral therapy: collaborative cohort analysis. *HIV.Med.* 2012; 13(2):89–97. doi: [10.1111/j.1468-1293.2011.00940.x](https://doi.org/10.1111/j.1468-1293.2011.00940.x) PMID: [21819529](https://pubmed.ncbi.nlm.nih.gov/21819529/)
14. Beyrer C, Sullivan P, Sanchez J, Baral SD, Collins C, Wirtz AL, et al. The increase in global HIV epidemics in MSM. *AIDS* 2013; 27(17):2665–78. doi: [10.1097/01.aids.0000432449.30239.fe](https://doi.org/10.1097/01.aids.0000432449.30239.fe) PMID: [23842129](https://pubmed.ncbi.nlm.nih.gov/23842129/)
15. Marí-Dell'Olmo M, Rodríguez-Sanz M, García de Olalla P, Pasarín MI, Brugal MT, Caylà JA, et al. Individual and community-level effects in the socioeconomic inequalities of AIDS-related mortality in an urban area of southern Europe. *J Epidemiol Community Health.* 2007 Mar; 61(3):232–40. PMID: [17325402](https://pubmed.ncbi.nlm.nih.gov/17325402/)
16. Aldaz P, Moreno-Iribas C, Egúés N, Irisarri F, Floristan Y, Sola-Boneta J, et al. Mortality by causes in HIV-infected adults: comparison with the general population. *BMC.Public Health* 2011; 11:300. doi: [10.1186/1471-2458-11-300](https://doi.org/10.1186/1471-2458-11-300) PMID: [21569323](https://pubmed.ncbi.nlm.nih.gov/21569323/)
17. Hernando V, Alejos B, Monge S, Berenguer J, Anta L, Vinuesa D, et al. All-cause mortality in the cohorts of the Spanish AIDS Research Network (RIS) compared with the general population: 1997–2010. *BMC.Infect.Dis.* 2013; 13:382. doi: [10.1186/1471-2334-13-382](https://doi.org/10.1186/1471-2334-13-382) PMID: [23961924](https://pubmed.ncbi.nlm.nih.gov/23961924/)
18. Centers for Disease Control and Prevention (CDC). Revised surveillance case definition for HIV infection—United States, 2014. *MMWR Recomm Rep.* 2014 Apr 11; 63(RR-03):1–10. PMID: [24717910](https://pubmed.ncbi.nlm.nih.gov/24717910/)
19. Barcelona council, technical programming office. Distribució Territorial de la Renda Familiar Disponible per Càpita a Barcelona (2013). December 2014. <http://barcelonaeconomia.bcn.cat>. Accessed 25 April 2015.
20. Cescon A, Patterson S, Chan K, Palmer AK, Margolese S, Burchell AN, et al. Gender differences in clinical outcomes among HIV-positive individuals on antiretroviral therapy in Canada: a multisite cohort study. *PLoS.One.* 2013; 8(12):e83649. doi: [10.1371/journal.pone.0083649](https://doi.org/10.1371/journal.pone.0083649) PMID: [24391803](https://pubmed.ncbi.nlm.nih.gov/24391803/)

21. World Health Organization. International Statistical Classification of Diseases and Health Related Problems. 10th revision. 2014. <http://apps.who.int/classifications/icd10/browse/2015/en>. Accessed 2 Feb 2015.
22. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Statist Assoc* 1999; 94:496–509.
23. Andersen PK, Geskus RB, de Witte T, Putter H. Competing Risks in Epidemiology: Possibilities and Pitfalls. *Int J Epidemiol* 41, no. 3 (2012): 861–70. doi: [10.1093/ije/dyr213](https://doi.org/10.1093/ije/dyr213) PMID: [22253319](https://pubmed.ncbi.nlm.nih.gov/22253319/)
24. World Medical Association. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. 2013. Fortaleza.
25. Spanish Law 15/1999 of December 13 on the Protection of Personal Data. 298, 43088–43099. 13-12-1999. Madrid, BOE.
26. Miller CJ, Baker JV, Bormann AM, Erlandson KM, Huppler Hullsiek K, Justice AC, et al. Adjudicated morbidity and mortality outcomes by age among individuals with HIV infection on suppressive antiretroviral therapy. *PLoS One*. 2014; 9(4):e95061. doi: [10.1371/journal.pone.0095061](https://doi.org/10.1371/journal.pone.0095061) PMID: [24728071](https://pubmed.ncbi.nlm.nih.gov/24728071/)
27. Weber R, Ruppik M, Rickenbach M, Spoerri A, Furrer H, Battegay M, et al. Decreasing mortality and changing patterns of causes of death in the Swiss HIV Cohort Study. *HIV Med*. 2013; 14(4):195–207. doi: [10.1111/j.1468-1293.2012.01051.x](https://doi.org/10.1111/j.1468-1293.2012.01051.x) PMID: [22998068](https://pubmed.ncbi.nlm.nih.gov/22998068/)
28. Rius C, Binefa G, Montoliu A, Esteve A, Ribas G, Gispert R, et al. [Survival changes among AIDS cases in Catalonia, Spain (1981–2001)]. *Med.Clin.(Barc.)* 2006; 127(5):167–71.
29. Novoa AM, García de Olalla P, Clos R, Orcau A, Rodríguez-Sanz M, Caylà JA. Increase in the non-HIV-related deaths among AIDS cases in the HAART era. *Curr.HIV.Res.* 2008; 6(1):77–81. PMID: [18288979](https://pubmed.ncbi.nlm.nih.gov/18288979/)
30. Catalonia government. Official statistics website of Catalonia. <http://www.idescat.cat>. Accessed 4th October 2015.
31. The Antiretroviral Cohort Collaboration. Causes of death in HIV-1-infected patients treated with antiretroviral therapy, 1996–2006: collaborative analysis of 13 HIV cohort studies. *Clin.Infect.Dis.* 2010; 50(10):1387–96. doi: [10.1086/652283](https://doi.org/10.1086/652283) PMID: [20380565](https://pubmed.ncbi.nlm.nih.gov/20380565/)
32. Gardner EM, McLees MP, Steiner JF, del Rio C, Burman WJ. The Spectrum of Engagement in HIV Care and its Relevance to Test-and-Treat Strategies for Prevention of HIV Infection. *Clin Infect Dis*. 2011 Mar 15; 52(6): 793–800. doi: [10.1093/cid/ciq243](https://doi.org/10.1093/cid/ciq243) PMID: [21367734](https://pubmed.ncbi.nlm.nih.gov/21367734/)
33. García de Olalla P, Manzano C, Sambeat MA, Ocaña I, Knobel H, Humet V, et al. Epidemiological characteristics and predictors of late presentation of HIV infection in Barcelona (Spain) during the period 2001–2009. *AIDS Res Ther*. 2011 Jul 6; 8(1):22. doi: [10.1186/1742-6405-8-22](https://doi.org/10.1186/1742-6405-8-22) PMID: [21729332](https://pubmed.ncbi.nlm.nih.gov/21729332/)
34. Oliva J, Galindo S, Vives N, Arrillaga A, Izquierdo A, Nicolau A, et al. Delayed diagnosis of HIV infection in Spain. *Enferm Infecc Microbiol Clin* 2010; 28:583–589. doi: [10.1016/j.eimc.2010.02.013](https://doi.org/10.1016/j.eimc.2010.02.013) PMID: [20541845](https://pubmed.ncbi.nlm.nih.gov/20541845/)
35. Mensforth S, Goodall L, Bodasing N, Coultas J. Late diagnosis among our ageing HIV population: a cohort study. *J.Int.AIDS Soc.* 2014; 17(4 Suppl 3):19692. doi: [10.7448/IAS.17.4.19692](https://doi.org/10.7448/IAS.17.4.19692) PMID: [25397442](https://pubmed.ncbi.nlm.nih.gov/25397442/)
36. Peters L, Neuhaus J, Duprez D, Neaton JD, Tracy R, Klein MB, et al. Biomarkers of inflammation, coagulation and microbial translocation in HIV/HCV co-infected patients in the SMART study. *J.Clin.Virol.* 2014; 60(3):295–300. doi: [10.1016/j.jcv.2014.03.017](https://doi.org/10.1016/j.jcv.2014.03.017) PMID: [24793968](https://pubmed.ncbi.nlm.nih.gov/24793968/)
37. Phung BC, Sogni P, Launay O. Hepatitis B and human immunodeficiency virus co-infection. *World J. Gastroenterol.* 2014; 20(46):17360–17367. doi: [10.3748/wjg.v20.i46.17360](https://doi.org/10.3748/wjg.v20.i46.17360) PMID: [25516647](https://pubmed.ncbi.nlm.nih.gov/25516647/)
38. Gras L, de Wolf F, Smit C, Prins M, van der Meer JT, Vanhommerig JW, et al. Changes in HIV RNA and CD4 cell count following acute HCV infection in chronically HIV-infected individuals. *J.Acquir.Immune.Defic.Syndr.* 2015 Apr 15; 68(5):536–42. doi: [10.1097/QAI.0000000000000514](https://doi.org/10.1097/QAI.0000000000000514) PMID: [25559595](https://pubmed.ncbi.nlm.nih.gov/25559595/)
39. Gess M, Forton D. Effect of hepatitis C virus on the central nervous system of HIV-infected individuals. *Virus Adaptation and Treatment* 2012; 4:93–106.
40. Jiang H, Xie N, Cao B, Tan L, Fan Y, Zhang F, et al. Determinants of progression to AIDS and death following HIV diagnosis: a retrospective cohort study in Wuhan, China. *PLoS One*. 2013; 8(12):e83078. doi: [10.1371/journal.pone.0083078](https://doi.org/10.1371/journal.pone.0083078) PMID: [24376638](https://pubmed.ncbi.nlm.nih.gov/24376638/)
41. Bakanda C, Birungi J, Mwesigwa R, Ford N, Cooper CL, Au-Yeung C, et al. Association of aging and survival in a large HIV-infected cohort on antiretroviral therapy. *AIDS* 2011; 25(5):701–5. doi: [10.1097/QAD.0b013e3283437ed7](https://doi.org/10.1097/QAD.0b013e3283437ed7) PMID: [21160412](https://pubmed.ncbi.nlm.nih.gov/21160412/)
42. Greene M, Justice AC, Lampiris HW, Valcour V. Management of human immunodeficiency virus infection in advanced age. *JAMA* 2013; 309(13):1397–405. doi: [10.1001/jama.2013.2963](https://doi.org/10.1001/jama.2013.2963) PMID: [23549585](https://pubmed.ncbi.nlm.nih.gov/23549585/)

43. Balderson BH, Grothaus L, Harrison RG, McCoy K, Mahoney C, Catz S. Chronic illness burden and quality of life in an aging HIV population. *AIDS Care* 2013; 25(4):451–58. doi: [10.1080/09540121.2012.712669](https://doi.org/10.1080/09540121.2012.712669) PMID: [22894702](https://pubmed.ncbi.nlm.nih.gov/22894702/)
44. Egger M, May M, Chêne G, Phillips AN, Ledergerber B, Dabis F, et al. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. *Lancet* 2002; 360(9327):119–29. PMID: [12126821](https://pubmed.ncbi.nlm.nih.gov/12126821/)
45. Lanoy E, Mary-Krause M, Tattevin P, Dray-Spira R, Duvivier C, Fischer P, et al. Predictors identified for losses to follow-up among HIV-seropositive patients. *J.Clin.Epidemiol.* 2006; 59(8):829–35. PMID: [16828676](https://pubmed.ncbi.nlm.nih.gov/16828676/)
46. Pablos-Mendez A. Mortality among Hispanics. *JAMA* 1994; 271(16):1237.
47. Abraido-Lanza AF, Dohrenwend BP, Ng-Mak DS, Turner JB. The Latino mortality paradox: a test of the "salmon bias" and healthy migrant hypotheses. *Am.J.Public Health* 1999; 89(10):1543–48. PMID: [10511837](https://pubmed.ncbi.nlm.nih.gov/10511837/)
48. Huisman M, Kunst AE, Bopp M, Borgan JK, Borrell C, Costa G, et al. Educational inequalities in cause-specific mortality in middle-aged and older men and women in eight western European populations. *Lancet* 2005; 365(9458):493–500. PMID: [15705459](https://pubmed.ncbi.nlm.nih.gov/15705459/)
49. Sobrino-Vegas P, Rodríguez-Urrego J, Berenguer J, Caro-Murillo AM, Blanco JR, Viciano P, et al. Educational gradient in HIV diagnosis delay, mortality, antiretroviral treatment initiation and response in a country with universal health care. *Antivir.Ther.* 2012; 17(1):1–8. doi: [10.3851/IMP1939](https://doi.org/10.3851/IMP1939) PMID: [22267463](https://pubmed.ncbi.nlm.nih.gov/22267463/)
50. Borrell C, Rodríguez-Sanz M, Pasarín MI, Brugal MT, García-de-Olalla P, Mari-Dell'Olmo M, et al. AIDS mortality before and after the introduction of highly active antiretroviral therapy: does it vary with socio-economic group in a country with a National Health System? *Eur.J.Public Health* 2006; 16(6):601–8. PMID: [16698886](https://pubmed.ncbi.nlm.nih.gov/16698886/)
51. Pavlova-McCalla E, Trepka MJ, Ramirez G, Niyonsenga T. Socioeconomic Status and Survival of People with Human Immunodeficiency Virus Infection before and after the Introduction of Highly Active Antiretroviral Therapy: A Systematic Literature Review. *J.AIDS Clin.Res.* 2012; 3(6).
52. Louie JK, Hsu LC, Osmond DH, Katz MH, Schwarcz SK. Trends in causes of death among persons with acquired immunodeficiency syndrome in the era of highly active antiretroviral therapy, San Francisco, 1994–1998. *J Infect Dis* 2002; 186: 1023–7. PMID: [12232845](https://pubmed.ncbi.nlm.nih.gov/12232845/)
53. Niaura R, Shadel WG, Morrow K, Tashima K, Flanigan T, Abrams DB. Human immunodeficiency virus infection, AIDS, and smoking cessation: the time is now. *Clin Infect Dis* 2000; 31: 808–12. PMID: [11017836](https://pubmed.ncbi.nlm.nih.gov/11017836/)
54. Legarth R, Omland LH, Kronborg G, Larsen CS, Pedersen C, Gerstoft J, Obel N. Educational Attainment and Risk of HIV Infection, Response to Antiretroviral Treatment, and Mortality in HIV-Infected Patients. *Aids* 28, no. 3 (2014): 387–96. doi: [10.1097/QAD.000000000000032](https://doi.org/10.1097/QAD.000000000000032) PMID: [24670524](https://pubmed.ncbi.nlm.nih.gov/24670524/)
55. Delpierre C, Cuzin L, Lauwers-Cances V, Datta GD, Berkman L, Lang T. Unemployment as a risk factor for AIDS and death for HIV-infected patients in the era of highly active antiretroviral therapy. *Sex Transm Infect* 2008; 84:183–186. doi: [10.1136/sti.2007.027961](https://doi.org/10.1136/sti.2007.027961) PMID: [18192292](https://pubmed.ncbi.nlm.nih.gov/18192292/)
56. Dray-Spira R, Gueguen A, Ravaut JF, Lert F. Socioeconomic differences in the impact of HIV infection on workforce participation in France in the era of highly active antiretroviral therapy. *Am J Pub Health* 2007; 97:552–558.
57. Legarth R, Omland LH, Kronborg G, Larsen CS, Pedersen C, Pedersen G, Dragsted UB, Gerstoft J, Obel N. Employment Status in Persons with and without HIV Infection in Denmark: 1996–2011. *Aids* 28, no. 10 (2014): 1489–98. doi: [10.1097/QAD.0000000000000257](https://doi.org/10.1097/QAD.0000000000000257) PMID: [24732775](https://pubmed.ncbi.nlm.nih.gov/24732775/)
58. Diez M, Diaz A, Garriga C, Pons M, Ten A, Marcos H, et al. A low-cost, sustainable, second generation system for surveillance of people living with HIV in Spain: 10-year trends in behavioural and clinical indicators, 2002 to 2011. *Euro Surveill.* 2014 May 22; 19(20).
59. Collazos J, Asensi V, Carton JA. Association of HIV transmission categories with sociodemographic, viroimmunological and clinical parameters of HIV-infected patients. *Epidemiol.Infect.* 2010; 138(7):1016–24. doi: [10.1017/S0950268809991282](https://doi.org/10.1017/S0950268809991282) PMID: [19941690](https://pubmed.ncbi.nlm.nih.gov/19941690/)
60. Ortego C, Huedo-Medina TB, Llorca J, Sevilla L, Santos P, Rodríguez E, et al. Adherence to highly active antiretroviral therapy (HAART): a meta-analysis. *AIDS Behav.* 2011; 15(7):1381–96. doi: [10.1007/s10461-011-9942-x](https://doi.org/10.1007/s10461-011-9942-x) PMID: [21468660](https://pubmed.ncbi.nlm.nih.gov/21468660/)
61. Vives N, Carnicer-Pont D, García de Olalla P, Camps N, Esteve A, Casabona J; HIV and STI Surveillance Group. Factors associated with late presentation of HIV infection in Catalonia, Spain. *Int J STD AIDS.* 2012; 23(7):475–80. PMID: [22844000](https://pubmed.ncbi.nlm.nih.gov/22844000/)
62. García de Olalla P, Reyes JM, Caylà JA. [Delay in diagnosis of HIV infection]. *Rev Esp Sanid Penit.* 2012; 14(1):28–35. doi: [10.1590/S1575-06202012000100005](https://doi.org/10.1590/S1575-06202012000100005) PMID: [22437906](https://pubmed.ncbi.nlm.nih.gov/22437906/)

63. García de Olalla P, Caylà JA. [Is it possible to reduce the delay in diagnosing HIV infection?]. *Enferm Infecc Microbiol Clin*. 2010 Nov; 28(9):580–2. doi: [10.1016/j.eimc.2010.07.004](https://doi.org/10.1016/j.eimc.2010.07.004) PMID: [20828881](https://pubmed.ncbi.nlm.nih.gov/20828881/)
64. Spillane H, Nicholas S, Tang Z, Szumilin E, Balkan S, Pujades-Rodriguez M. Incidence, risk factors and causes of death in an HIV care programme with a large proportion of injecting drug users. *Trop. Med.Int.Health* 2012; 17(10):1255–63. doi: [10.1111/j.1365-3156.2012.03056.x](https://doi.org/10.1111/j.1365-3156.2012.03056.x) PMID: [22863110](https://pubmed.ncbi.nlm.nih.gov/22863110/)
65. Panel of Experts of GESIDA and Spanish Secretariat for the National Plan on AIDS. [Consensus Statement by GeSIDA/National AIDS Plan Secretariat on antiretroviral treatment in adults infected by the human immunodeficiency virus (Updated January 2013)]. *Enferm.Infecc.Microbiol.Clin*. 2013; 31(9):602. doi: [10.1016/j.eimc.2013.04.009](https://doi.org/10.1016/j.eimc.2013.04.009) PMID: [24161378](https://pubmed.ncbi.nlm.nih.gov/24161378/)
66. Rubio R, Berenguer J, Miró JM, Antela A, Iribarren JA, González J, et al. [Recommendations of the Spanish AIDS Study Group (GESIDA) and the National Aids Plan (PNS) for antiretroviral treatment in adult patients with human immunodeficiency virus infection in 2002]. *Enferm.Infecc.Microbiol.Clin*. 2002; 20(6):244–303. PMID: [12084354](https://pubmed.ncbi.nlm.nih.gov/12084354/)
67. Panel of Experts of GESIDA and Spanish Secretariat for the National Plan on AIDS. [Recommendations from the GESIDA/Spanish AIDS Plan regarding antiretroviral treatment in adults with human immunodeficiency virus infection (update February 2009)]. *Enferm.Infecc.Microbiol.Clin*. 2009; 27(4):222–35. doi: [10.1016/j.eimc.2008.11.002](https://doi.org/10.1016/j.eimc.2008.11.002) PMID: [19246124](https://pubmed.ncbi.nlm.nih.gov/19246124/)