The influence of HIV infection on the natural history of hepatocellular carcinoma: results from a global multi-cohort study.

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Abstract

Purpose. Conflicting evidence indicates HIV-seropositivity to influence the outcome of patients with hepatocellular carcinoma (HCC), a leading cause of mortality in people with HIV. We aimed to verify whether HIV affected the overall survival (OS) of patients with HCC independent of treatment and geographic origin.

Patients and Methods: We designed an international multi-cohort study of HCC patients who did not receive any anticancer treatment accrued from four continents. We estimated the effect of HIV-seropositivity on patients' OS while accounting for common prognostic factors and demographic characteristics in uni- and multi-variable models.

Results: A total of 1588 patients were recruited, 132 of whom were HIV-positive. Most patients clustered within Barcelona Clinic Liver Cancer (BCLC) C/D criteria (n=1168, 74%), Child-Turcotte-Pugh (CTP) Class B (median score 7, IQR 3). At HCC diagnosis the majority of HIV-positive patients (n=65, 64%) had been on anti-retrovirals for a median duration of 8.3 years (IQR 8.59) and had median CD4+ cell counts of 256 (IQR 284) with undetectable HIV RNA (n=68, 52%). OS significantly reduced throughout BCLC stages 0-D (16, 12, 7.5, 3.1 and 3 months, p<0.001). Median OS of HIV-positive patients was half that of HIV-uninfected counterparts: 2.2 months, (bootstrap 95%CI 1.2-3.1) versus 4.1 months (95%CI 3.6-4.4). In adjusted analyses HIV-seropositivity increased the hazard of death by 24% (p=0.0333) independent of BCLC (p<0.0001), CTP (p<0.0001), alpha-fetoprotein (AFP) (p<0.0001), geographical origin (p<0.0001) and male gender (p=0.0016). Predictors of worse OS in HIV-positive patients included CTP (p=0.0071) and AFP (p<0.0001).

Conclusions. Despite adequate antiretroviral treatment, HIV-seropositivity is associated with decreased survival in HCC independent of stage, anti-cancer treatment and geographical origin. Mechanistic studies investigating the immuno-biology of HIV-associated HCC are urgently required.

Introduction.

Hepatocellular carcinoma (HCC) accounts as the third cause of cancer-related mortality on a global scale[1]. As a recognised complication of liver cirrhosis, which pre-dates the onset of cancer in >80% of the incident cases, HCC is characterised by a wide prognostic heterogeneity due to the mutual influence of aetiology and severity of underlying chronic liver disease over the extent of spread of malignancy[2].

In people living with HIV (PLHIV), the high prevalence of co-infection with Hepatitis B (HBV) and C virus (HCV) has made HCC a rapidly increasing cause of morbidity and mortality, currently accounting for 40% of liver-related deaths[3].

The relative contribution of HIV to the pathogenesis and prognosis of HCC has been the focus of intense debate. Mechanistic evidence suggests HIV-mediated impairment of anti-viral CD4+ and CD8+ T-cell responses to facilitate an accelerated progression of chronic liver disease to fibrosis and, ultimately, malignancy[4].

However, more recent evidence has highlighted how other factors including increased oxidative stress from combination anti-retroviral therapy (cART)[5], HIV-associated gut dysbiosis[6] and the high prevalence of excessive alcohol intake in this patient population[7] might exert a synergistic carcinogenic role independently from the achievement of immune-reconstitution by optimal HIV control.

Clinical studies have been fairly inconsistent in addressing the question whether HIV infection might worsen the clinical course of HCC, with some providing null results and other suggesting evidence of association [8-10]. The reasons behind the non-uniform conclusions as to this prognostic relationship are to be found in the low quality of the available evidence, mostly flawed by the retrospective, single-center design of the majority of studies [11]. An important source of bias that affects the currently available evidence emerges from the geographic diversity in the provision of active anti-cancer treatment, which can in turn lead to profound differences in clinical outcomes.

The marked heterogeneity in survival outcomes within each Barcelona Clinic Liver Cancer (BCLC) stage is a renowned feature of HCC[12,13] and is even broader in PLHIV, where attainment of long-term HIV control, polypharmacy, socio-economic factors might influence access and eligibility to treatment, hence influencing patients' survival independently from any true immuno-biologic effect of HIV[14].

Understanding whether HIV carries an independent effect on the clinical history of HCC irrespective of treatment is an important unmet need, with significant ramifications in the definition of screening, diagnosis and treatment algorithms personalised to the needs and clinical features of this patient population[11].

To address this issue and overcome the limitations of previous studies, we designed this large collaborative global study including patients from cohorts from four continents. As a primary objective, we aimed to estimate the effect of HIV status on overall survival of untreated HCC patients while accounting for common prognostic factors. As a secondary objective, we also aimed to characterise prognostic factors among a subset of HIV+ patients with untreated HCC.

Methods.

Study population.

We established a global consortium of tertiary referral centers located in North and South America, Europe and to access prospectively collected cohorts of HIV+/HIV- patients with untreated HCC (**Figure 1**). This multicenter effort led to accumulation of >1500 patients, resulting in the largest observational investigation on the effect of HIV infection among HCC patients conducted to date.

Consecutive patient data were collected as part of routine clinical care. Patients were not selected amongst clinical trial participants. HCC diagnosis was based on imaging or by histologic criteria according to international guidelines[15].

The Liver Cancer in HIV dataset study identified all patients diagnosed with HCC on a background of HIV infection in 44 referral centers providing specialist multidisciplinary care for HIV and HCC across 9 countries including United States, Canada, Brazil, Argentina, Germany, Spain, United Kingdom, Italy and Australia. Consecutive referrals in the period between 1992 and 2016 were collected in a joint database as part of an international research consortium using electronic case report forms. At the last database update a total of 387 HIV-positive patients were identified[16], which were merged with a separate dataset of 226 HIV-negative controls from four centers in the United States[17].

The ITA.LI.CA. dataset is a multi-center Italian collaborative network that has, to date, curated and prospectively maintained a dataset of >5000 consecutive patients evaluated in 24 tertiary referral centers for the assessment and treatment of HCC between years 1988-2012. Before statistical analysis, consistency of data was checked by the ITA.LI.CA. study coordinator (F.T.). Outcomes pertaining to a proportion of the patients presented here have been published in 2015[18].

As a separate cohort, we assessed for eligibility 1470 patients with HCC that were diagnosed at the Department of Gastroenterology and Hepatology in the Singapore General Hospital, a tertiary-level medical center in Singapore and listed into a prospectively-maintained HCC registry since January 1988[19].

The primary clinical endpoint of the study was overall survival (OS), calculated from the date of diagnosis to the date of death and/or last follow-up. Out of the initial sample of 1590 patients, we removed 1 patient with unclear follow-up status and 1 patient with age <18 years.

In the full dataset of 1588 patients, we reconstructed demographic data, complete blood count including liver function tests, alpha-fetoprotein (AFP) and the international normalised ratio value for prothrombin time from electronic medical records. We staged patients using computerized tomography and/or magnetic resonance imaging as clinically required to derive the number of focal hepatic lesions and maximum tumour diameter detected during contrast enhancement. Computation of Child-Turcotte-Pugh (CTP) functional class and Barcelona Clinic Liver Cancer (BCLC) stage followed standard pre-published methodology[20].

We further characterised the subset of 132 HIV+ patients in terms of prior antiretroviral treatment, time from HCC to cART initiation, CD4+ T cells count, HIV viral load, hepatitis antigens and antibodies, and risk factors for HIV infection.

The study protocol was approved by the Institutional Review Board/Ethics Committee in each participating institution and was conducted in accordance with the Declaration of Helsinki.

Statistical analysis.

We explored the distribution of continuous variables with median and interquartile range (IQR) and tested differences in medians using Mann-Whitney tests. We tabulated categorical variables factors and tested for differences in proportions using Pearson's Chi-squared tests. We plotted the overall survival (OS) function by HIV status and potential prognostic factors using Kaplan-Meier curves. Prior to conducting Cox regression analyses, we checked the proportional-hazards assumption with log-likelihood ratio tests of relevant predictors over time-bands. There was no evidence of violation at the 5% level of the proportional-hazards assumption for the main predictor (HIV status, p=0.0870). For the predictors for which the proportional hazards assumption did not hold, we explicitly accounted for time-dependent effects by adding interaction terms with time-bands in all regression analyses[21].

We imputed missing data using a multiple imputation with chained equations (MICE) approach, assuming that the data were missing at random. Details are included in the **Supplementary Methods**.

To select the main prognostic factors, we applied a backward selection algorithm on the imputed datasets. Details are available in the **Supplementary Methods**. In the full dataset (N=1588), backward selection identified the following prognostic factors associated with OS at the 5% significance level:

HIV, gender, BCLC stage, CTP class, log-transformed AFP, and continent (a binary variable coded as North/South America and Asia vs. Europe). In the subset of HIV+ patients (N=132), two prognostic factors were associated with OS: CTP class, log-transformed AFP. We re-added common prognostic factor for HCC and HIV so that the final model for the subset of HIV+ patients included: CTP class, log-transformed AFP, BCLC stage, HIV viral load, and CD4+ concentration.

All reported p-values are two-sided. We carried out all analyses in R v.3.4.4 with the following packages: survival v.2.42, mice v. 3.0.0, and ggplot2 v.2.2.1.

Results.

Baseline Patient Characteristics.

A total of 132 HIV+ and 1456 HIV- patients were included in the study. Baseline features of the study cohort, stratified according to HIV-seropositivity are presented in **Table 1**. HIV+ patients were on average younger (52.9 versus 66 years, p<0.0001), more commonly male (94.7 versus 81.9%, p=0.0003) and had higher prevalence of HCV-related chronic liver disease (78 versus 37.1%, p<0.0001) compared to HIV- patients. Staging by BCLC criteria (p=0.93) and liver functional reserve by CTP class (p=0.34) were not different across groups, however HIV+ patients had lower albumin (31 versus 29 g/L, p<0.0001) and higher ALT (56 vs 47, p=0.0014) and AST (128 versus 95, p=0.0005). The majority of patients had a performance status (PS) of 1 (n=534, 33.6%) and classified within CTP class B criteria across HIV- and HIV+ (median score 7, IQR 3). None of the HCV-infected patients from the ITA.LI.CA. or Singapore General Hospital datasets had previously been successfully treated for HCV. In terms of anti-HBV treatment, 17 HBV-carriers (2.4%) from Singapore and the entirety of Italian patients with chronic HBV (16% of the ITA.LI.CA. dataset) were on nucleoside analogue therapy.

In the HIV+ subset, 103 patients (78%) had HCV infection. Three patients had received treatment (2.9%) with interferon-based regimens prior to HCC diagnosis, all of whom were non-responders. HCV genotype was available in 53 patients (40.2%), 45 of whom (84.9%) were of genotype 1, 3 (5.7%) of genotype 2, and 5 (9.4%) of genotype 3. HCV RNA levels at the time of HCC diagnosis were available in 60 patients (45.5%), with 52 (86.7%) showing evidence of detectable viraemia. In the 33 patients with HBV-associated HCC (25%), 12 (36.4%) had evidence of detectable HBV DNA levels at HCC diagnosis, and 13 had received treatment for HBV infection (39.4%). The majority of HIV patients were

on cART (n=85, 64.4%) with a median duration of treatment of 8.3 years prior to the diagnosis of HCC. Most patients had undetectable HIV viral load at the time of HCC diagnosis (n=68, 51.5%) and CD4 counts >200 cells/mm3 (n=71, 53.8%). The most prevalent risk factor for HIV infection was intravenous drug use (50%).

The relationship between HIV and the survival of HCC.

Kaplan Meier curves (**Figure 2**) revealed strong evidence of unadjusted lower survival for HIV+ patients compared to HIV- participants in the full sample of 1588 HCC patients (log-rank p<0.0001). Median OS for the whole study cohort was 4 months (bootstrap 95% confidence interval [CI] 3.8 to 4.1 months) with Barcelona Clinic Liver Cancer (BCLC) stage-specific median survivals of 16, 12, 7.5, 3.1 and 3 months for stages 0, A, B, C, and D respectively. At the time of analysis, 1428 patients (89.9%) had died. In the HIV+ cohort, no deaths could be attributed to HIV infection.

After adjustment for common prognostic factors and multiple imputation of missing data, HIV infection was independently associated to 24% greater hazard of death (95%CI 2% to 52%; p=0.0333) (**Table 2A**). Other prognostic factors independently associated with OS were male gender (p=0.0016), BCLC stage, CTP class, alpha-fetoprotein (AFP), and geographical origin (all p<0.0001). As expected, the adjusted effects estimated in complete-case analyses (**Supplementary Table 1**) were similar in size and direction compared to multiple imputation analysis.

Prognostic factors for overall survival in HIV-associated HCC.

In the subset of 132 patients with HIV infection, multivariable analyses after multiple imputation revealed two prognostic factors independently associated to OS: AFP (Hazard ratio [HR] 1.18; 95%CI 1.09 to 1.28; p<0.0001) and CTP class C vs A (HR 2.78; 1.31 to 5.91; p=0.0079). There was no evidence of association with OS for BCLC stage, CTP class B vs A, CD4 count, viral load and geographical origin. These results were in line with those obtained from complete-case analyses (**Supplementary Table 1**).

Discussion.

Existing epidemiologic evidence suggests a seven-fold increase in the incidence of HCC for people affected by HIV and hepatitis compared to HIV-negative controls[22]. The excess risk associated with HIV infection has traditionally been explained by the immune-modulatory effects of the virus, leading to a faster progression of liver fibrosis[23]. Consistent with this view, people with HIV-associated HCC present at younger age, suggesting a shorter latency of hepatocarcinogenesis compared to their HIV-negative counterparts[8].

Once cancer is diagnosed, however, the question as to whether HCC arising in the context of HIV is biologically different from other aetiologies has remained unanswered. In absence of evidence to demonstrate potential differences at a molecular level, clinical studies evaluating HIV for its prognostic role in HCC have led to controversial conclusions, with some studies demonstrating HIV to adversely influence the clinical behaviour of HCC and others refuting this prognostic association[8-10].

In this global collaborative study, the largest to have evaluated the clinico-pathologic impact of HIV in patients with HCC to date, we demonstrate that HIV infection adversely influences the clinical course of HCC, leading to a 24% increase in the hazard of death in patients who did not receive any active anticancer treatment.

A precise estimate of the relationship between HIV and survival in patients with untreated HCC is essential to gain insight into the natural history of the disease and establish basic information on patients' prognosis that can be used as a point of reference for future mechanistic and clinical studies.

By specifically selecting patients who did not receive any effective anti-cancer treatment for HCC we intended to rule out the documented confounding effect imposed by the heterogeneity in the provision of radical and palliative therapies for HCC and improve the quality of evidence supporting the clinical characterisation of HIV-associated HCC.

A number of previously published studies have confirmed the provision of treatment as a key prognostic factor in this population. In the cross-sectional study by Puoti, one of the first to document a negative prognostic role of HIV in HCC, 60% of the patients with HIV-associated HCC received best supportive care compared to 38% of HIV-negative controls (p=0.02), highlighting that the adverse prognostic role of HIV-status might have been at least in part dictated by the diverse treatment allocation across groups[9]. Treatment imbalance is also a feature of the study by Berretta et al., where a significantly

higher proportion of HIV+ patients received active anti-cancer treatment (85.6 versus 65.4%, p<0.001). However, despite the high proportion of patients qualifying for radical treatments in both groups (44.5 versus 51.1%, p=0.66), HIV+ patients appear disadvantaged by the inferior likelihood of receiving treatment at recurrence (61% versus 86.2%, p<0.001)[8].

Our study cohort, which includes patients from multiple centers across Europe, America and Asia, reproduced a balanced distribution of liver functional reserve classes and BCLC stages, showing – perhaps unsurprisingly – that the majority of patients who, based on the opinion of the treating multidisciplinary team, qualified solely for best supportive care clustered within the more advanced BCLC stages of the disease (C-D stage, ~75%).

In attempting to determine the prognostic interaction between HIV and basic clinico-pathologic features of HCC, we produced a multivariable regression model of survival to adjust our prognostic estimate for key confounders including BCLC stage, CTP class, gender, AFP levels and geographical origin of the patients.

Interestingly, our results show that the effect of HIV-seropositivity impacts on the survival of patients with HCC independently from a number of established staging parameters, including BCLC stage. We also document the protective effect of female gender[18,24] in the prognosis of advanced HCC as well as the inherent differences in survival amongst Western and Eastern populations, results that find a strong resonance in the HCC literature[25]. Taken together, these results suggest our patient cohort to be fully representative of the broader, contemporary population of HCC patients.

From an HIV infection standpoint, it is worthy of note that the majority of the HIV+ patients considered for this study were HCV-co-infected, on cART at the time of diagnosis and had evidence of acceptable CD4 counts and suppressed HIV RNA levels.

This is in line with previous evidence reporting on incidence and mortality of HCC in PLHIV, where HCV co-infection is demonstrated as the predominant risk factor for HCC[26], causing a significant increase in liver-related mortality even in patients achieving long-term control of the underlying HIV infection[3]. In our study, patients had been on cART for a median duration of 8.3 years. However by choosing cART initiation as a measure of duration of HIV infection, we are likely to have underestimated the true latency period between HIV/hepatitis co-infection and HCC diagnosis, which typically ranges between 10 to 20 years[27].

As a likely consequence of good HIV control, none of the parameters indicative of severity of HIV infection (CD4 counts, HIV RNA) revealed prognostic in our cohort of HIV-associated HCC patients, where liver functional reserve and AFP levels emerged as the only indicators of worse survival.

We believe this finding to have important ramifications with regards to the clinical management of patients with HIV-associated HCC. Clinical practice has evolved to support an equal-access, unbiased environment for the management of HCC in PLHIV[10]. Whilst patients with HCC and well-controlled HIV should face no barriers in the provision of active anti-cancer treatment compared to HIV-negative individuals, the inferior probability of survival that accompanies patients with HIV deserves to be taken into account in patient counselling and therapeutic decision making.

Our study emphasizes the predominance of tumour-related factors and hepatic reserve over parameters relating to the severity of HIV infection in influencing survival. A number of studies have highlighted the complex and multi-factorial pathogenesis of accelerated hepatic fibrosis in patients with HIV and hepatitis. Besides viral factors, enhanced gut permeability, metabolic dysregulation of the liver microenvironment and high prevalence of concurrent liver-specific *noxae* including alcohol and drug use are factors that might explain the accelerated course of the disease[28]. In our study, HIV+ patients were significantly younger at HCC diagnosis and had evidence of poorer synthetic function and more severe portal hypertension as demonstrated by higher ALT/AST levels and lower albumin and platelet counts, to suggest that the prognostic imbalance observed might be due to the superadded burden of HIV infection in deteriorating patients' liver functional reserve[16]. In keeping with this view, the balance in tumour staging features and circulating AFP levels observed across the two patient subgroups further emphasises the pathophysiologic relevance of the cirrhotic microenvironment over tumour-specific features in justifying the excess mortality seen in HIV-associated HCC[29], where decompensation of cirrhosis is more common and predicts for significantly shorter survival[30].

It has been shown that, particularly in the context of HIV/HCV co-infection, patients with HCC often present with infiltrative pattern of growth associated with a faster course of progression and inferior likelihood of long-term survival[27]. Whether HIV infection pre-conditions the efficiency of anti-tumour immunity, an emerging prognostic and therapeutically actionable domain in the progression of HCC, is presently unknown and should be investigated in future studies.

Our study acknowledges a number of limitations. Firstly, the patient population selected for this study is mostly represented by patients with advanced-stage HCC, whose natural history is fundamentally different from that of patients with early-stage disease[31]. However, as far as the primary endpoint of the study is concerned, BCLC stage was balanced across cases and controls and was accounted for in all survival analyses. Interestingly, the survival outcomes reported in the present study consolidate recent evidence suggesting that the prognosis of untreated early and intermediate-stage HCC might be significantly poorer than previously reported. In the study by Khalaf et al., median OS for BCLC 0/A and B patients was in fact 13 and 9 months respectively[32], significantly shorter than the 38-, 25- and 10-months median survival probability previously reported by Giannini et al. for BCLC 0, A and B stages of HCC[18].

Such wide inter-study variability is perhaps unsurprising if one considers the number of potential un-reported factors that might have influenced survival estimation including co-morbidities, socio-economic factors and patients' preferences. However, these factors are difficult if not impossible to fully account for in a global multi-center study where access to care, availability and adherence to screening programs as well as quality of palliative care support after diagnosis can be highly heterogeneous across healthcare systems. As we are unable to reconstruct the clinical reasons as to why patients did not receive effective anti-cancer treatment, we cannot adjust our analysis for the competing effect of liver-unrelated comorbidities as predictors of survival. In particular, due to limitations in sample size, we were unable to study the prognostic contribution of efficacious treatment for HCV or HBV on the survival estimates we reported, a point that should be addressed in future studies. However, the effect of comorbidities and prior anti-viral treatment is likely to be fairly small in reality, as the dominant factor affecting the mortality of this patient population is tumour progression[33].

In addition, whilst our study was affected by missing data, we explicitly addressed this issue using multiple imputation, an approach that relies on the assumption that the data are missing at random (MAR), i.e. that — given the observed data — the missingness mechanism does not depend on unobserved data. The considerable amount of information available in the dataset (>80 variables) and the large sample size (>1500 patients) enabled us to include in the imputation models all variables associated with missingness, rendering the MAR assumption likely to hold.

To conclude, in our global, multi-center collaborative study, we have provided confirmatory evidence to show that HIV infection is associated with worse prognosis in a population of patients with HCC where

survival estimates were not biased by the effect of treatment. The median overall survival of HIV-associated HCC patients of 2.2 months reflects the natural history of untreated patients seen in routine clinical practice and should be considered a reference point for future studies. Follow-on research should explore the prognostic impact of socio-economic factors and co-morbid conditions, two aspects that might be unevenly distributed across HIV-positive and negative patients and might relate to the difference in survival observed in our study. In parallel, mechanistic studies on clinical samples evaluating the immunopathologic features of HIV-associated HCC in comparison with HIV-negative controls are urgently required.

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Conflict of interest statement

The authors do not declare conflicts of interest with regard to this manuscript.

Authors' contributions

Study concept and design: DJP, EA, NB.

Acquisition of data: DJP, TYC, FT, BM, MA, MH, ADP, NM, HP, MJ, EC, LK, JP, MN, FF, GLR, AA, MY, TCK, MB, EGG, NB.

Analysis and interpretation of data: DJP, EA, NB, MB, ADP.

Drafting of the manuscript: DJP, EA, NB.

Critical revision of the manuscript for important intellectual content: all authors.

Obtained funding: DJP, MB.

Administrative, technical, or material support: DJP, NB, MB

Study supervision: DJP, EA, NB.

Figure Legends.

Figure 1. CONSORT diagram illustrating patient accrual to the study.

Figure 2. Kaplan-Meier curves illustrating the prognostic relationship of HIV-seropositivity in influencing the overall survival of patients with untreated HCC.

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Table 1. Clinicopathologic features of patients with untreated hepatocellular carcinoma.

Characteristics	HIV- (N=1456)	HIV+ (N=132)	P-value
Age (years), median (IQR)	66.0 (15.79)	52.9 (10.88)	<0.0001 [a]
Male gender, N (%)	1193 (81.9)	125 (94.7)	0.0003 [b]
Continent: Americas/Asia vs Europe, N (%)	861 (59.1)	97 (73.5)	0.0017 [b]
Viral aetiology of liver disease, N (%)			
HBV	665 (45.7)	33 (25.0)	<0.0001 [b]
HCV	540 (37.1)	103 (78.0)	<0.0001 [b]
Alcohol aetiology of liver disease, N (%)	370 (25.4)	45 (34.1)	0.9112 [b]
AFP (ug/L), median (IQR)	351.0 (6878.00)	660.0 (9949.50)	0.1116 [a]
Albumin (g/L), median (IQR)	31.0 (9.00)	29.0 (10.00)	<0.0001 [a]
Bilirubin (umol/L), median (IQR)	27.4 (32.49)	26.4 (42.75)	0.7675 [a]
ALT (IU/L), median (IQR)	47.0 (44.00)	56.0 (56.75)	0.0014 [a]
AST (IU/L), median (IQR)	95.0 (100.00)	128.0 (111.00)	0.0005 [a]
Platelets (10^9cells/L), median (IQR)	149.0 (138.75)	129.0 (149.50)	0.0675 [a]
Performance status, N (%)			
0	431 (29.6)	55 (41.7)	0.0048
1	502 (34.5)	32 (24.2)	
2	292 (20.1)	18 (13.6)	
3	116 (8.0)	14 (10.6)	
4	29 (2.0)	5 (3.8)	
BCLC stage, N (%)			
0	15 (1.0)	2 (1.5)	0.9323 [b]
A	158 (10.9)	17 (12.9)	
B	191 (13.1)	16 (12.1)	
C	699 (48.0)	62 (47.0)	
D	372 (25.5)	35 (26.5)	
CTP score, median (IQR)	7.0 (3.00)	7.0 (3.00)	0.4245 [a]
CTP class, N (%)			
A	499 (34.3)	40 (30.3)	0.3435 [b]
B	610 (41.9)	59 (44.7)	
C	288 (19.8)	33 (25.0)	
Maximum diameter of largest lesion (cm), median (IQR)	4.8 (5.00)	5.5 (5.25)	0.0493 [a]
Extrahepatic disease, N (%)	604 (41.5)	37 (28.0)	0.0035 [b]
Portal vein thrombosis, N (%)	536 (36.8)	47 (35.6)	0.3940 [b]
Multinodular disease, N (%)	738 (46.5)	67 (50.8)	0.6947 [b]
On antiretroviral treatment, N (%)	-	85 (64.4)	-
Duration of antiretroviral treatment (years), median (IQR)		8.3 (8.59)	
Antiretroviral regimen			
PI	-	35 (26.5)	-
NNRTI	-	25 (18.9)	-
NRTI	-	16 (12.1)	-
INI	-	5 (3.8)	-
NNRTI+INI	-	1 (0.8)	-
INI+PI	-	1 (0.8)	-
Risk factor for HIV, N (%)			
Intravenous drug use	-	66 (50.0)	-
Heterosexual contact	-	14 (10.6)	-
Homosexual contact	-	10 (7.6)	-
Blood products	-	3 (2.3)	-
Unknown	-	25 (18.9)	-
CD4+ cell count (cells/mm^3), median (IQR)	-	256.0 (284.00)	-
Undetectable HIV viral load, N (%)	-	68 (51.5)	-
Undetectable HBV DNA, N (%)	-	6 (4.5)	-
Undetectable HCV RNA, N (%)	-	8 (6.1)	-
HCV genotype			
1	-	45 (34.1)	-
2	-	3 (2.3)	-
3	-	5 (3.8)	-

[a] Mann-Whitney test. [b] Pearson's chi-squared test.

IRQ, interquartile range. AFP, alpha-fetoprotein. ALT, Alanine aminotransferase. AST, Aspartarte aminotransferase. PT, prothrombin time. BCLC, Barcelona Clinic Liver Cancer. CTP, Child-Turcotte-Pugh. PI, protease inhibitor. NNRTI, non-nucleoside reverse transcriptase inhibitor. NRTI, nucleoside reverse transcriptase inhibitor. INI, Integrase inhibitor.
Please note that the sum of percentages may be lower than 100% owing to missing data.

Table 2. Effects of HIV+ status and common prognostic factors on overall survival in multiply-imputed datasets

A. In the full sample of HCC patients (N=1,588)

Predictor	Univariable models		Multivariable model	
	Hazard ratio (95%CI)	**P-value**	**Hazard ratio (95%CI)**	**P-value**
HIV+ status	1.49 (1.22 to 1.82)	<0.0001	1.24 (1.02 to 1.52)	0.0333
Male gender	1.62 (1.36 to 1.94)	<0.0001	1.34 (1.12 to 1.61)	0.0016
BCLC stage C/D vs 0/A/B	3.06 (2.57 to 3.65)	<0.0001	1.76 (1.44 to 2.13)	<0.0001
CTP class				
B vs A	1.69 (1.45 to 1.97)	<0.0001	1.76 (1.50 to 2.06)	<0.0001
C vs A	2.44 (2.04 to 2.93)	<0.0001	2.42 (1.98 to 2.95)	<0.0001
Log AFP level (ng/dl)	1.18 (1.16 to 1.21)	<0.0001	1.15 (1.12 to 1.17)	<0.0001
Continent: Americas/Asia vs Europe	2.59 (2.24 to 3.00)	<0.0001	1.92 (1.63 to 2.25)	<0.0001

B. In HIV+ HCC patients (N=132)

Predictor	Univariable models		Multivariable model	
	Hazard ratio (95%CI)	**P-value**	**Hazard ratio (95%CI)**	**P-value**
BCLC stage C/D vs 0/A/B	1.58 (1.01 to 2.46)	0.0441	1.38 (0.82 to 2.31)	0.2226
CTP class				
B vs A	1.67 (0.86 to 3.26)	0.1331	1.43 (0.72 to 2.86)	0.3048
C vs A	3.45 (1.72 to 6.92)	0.0005	2.78 (1.31 to 5.91)	0.0079
Log AFP level (ng/dl)	1.20 (1.11 to 1.31)	<0.0001	1.18 (1.09 to 1.28)	<0.0001
CD4+ cell count (x 100)	0.93 (0.83 to 1.04)	0.1914	0.96 (0.85 to 1.08)	0.4689
Log HIV viral load (copes/ml)	1.02 (0.96 to 1.08)	0.6126	0.99 (0.92 to 1.06)	0.7124
Continent: Americas/Asia vs Europe	1.09 (0.70 to 1.69)	0.7154	0.90 (0.55 to 1.46)	0.6666

BCLC, Barcelona Clinic Liver Cancer. CTP, Child-Turcotte-Pugh. AFP, alpha-fetoprotein.

Figure 1.

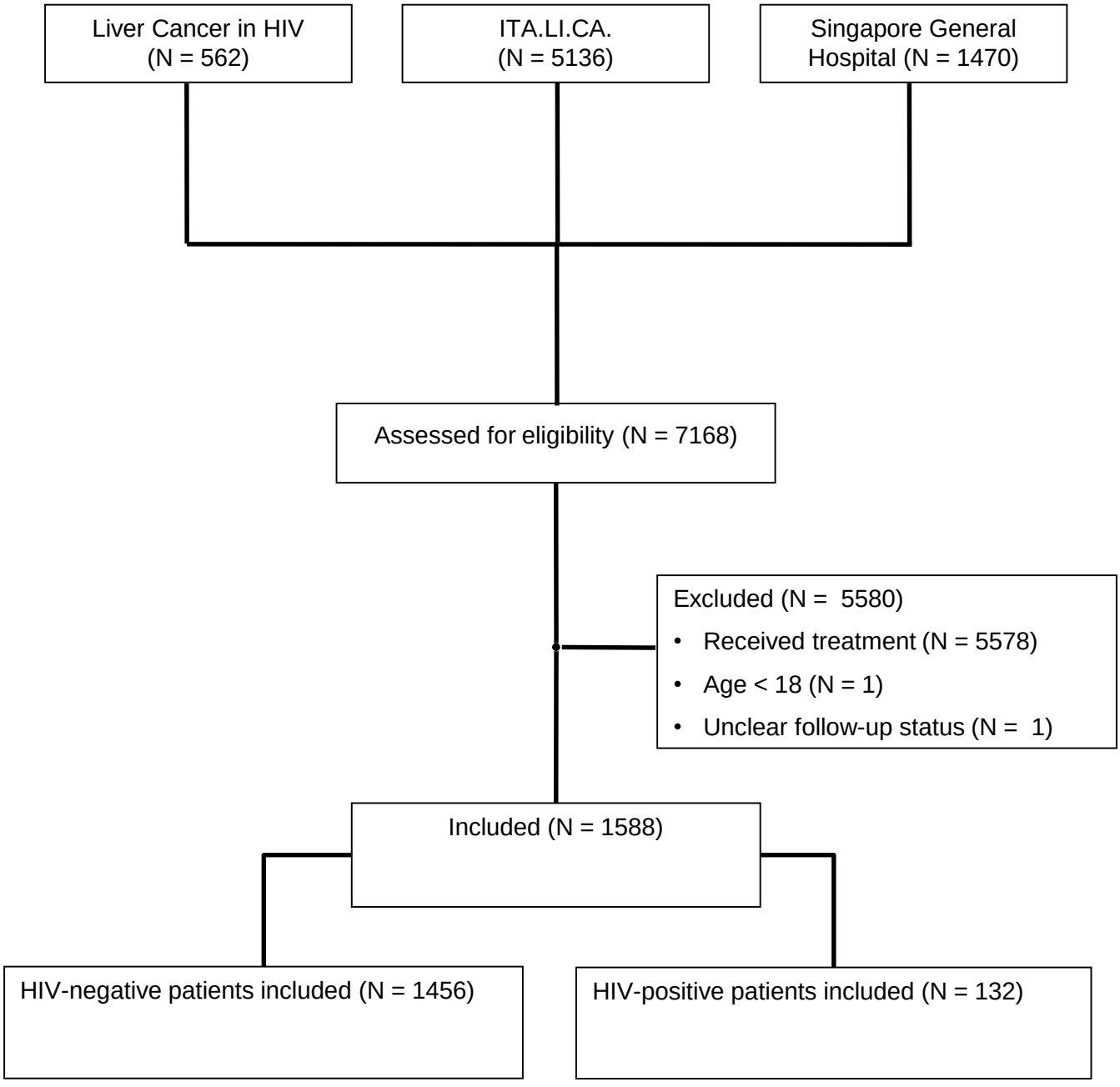
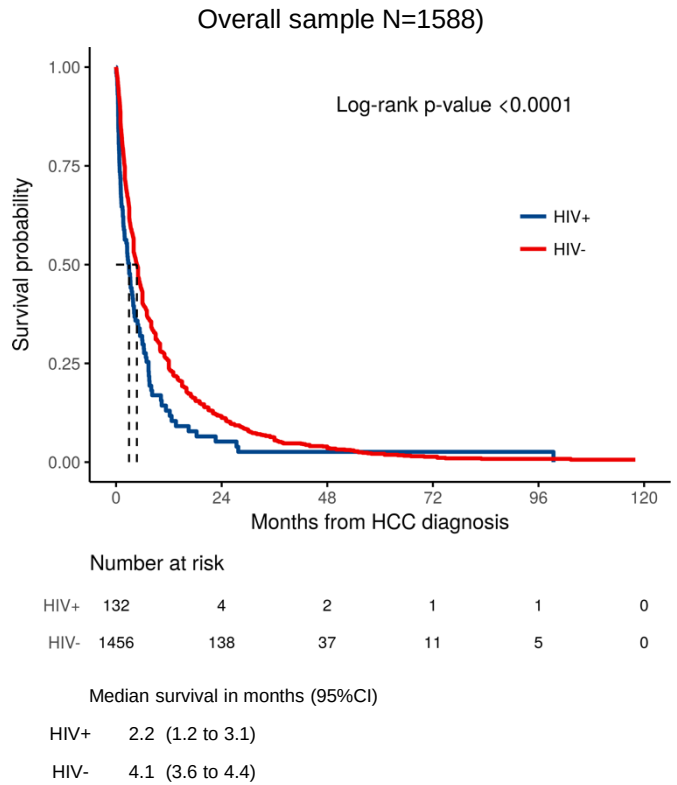

Liver Cancer in HIV (N = 562)
ITA.LI.CA. (N = 5136)
Singapore General Hospital (N = 1470)
Assessed for eligibility (N = 7168)
Excluded (N = 5580)
• Received treatment (N = 5578)
• Age < 18 (N = 1)
• Unclear follow-up status (N = 1)
Included (N = 1588)
HIV-negative patients included (N = 1456)
HIV-positive patients included (N = 132)

Figure 2.


Overall sample N=1588)
Log-rank p-value <0.0001
HIV+
HIV-
Survival probability
1.00
0.75
0.50
0.25
0.00
0
24
48
72
96
120
Months from HCC diagnosis
Number at risk
HIV+ 132 4 2 1 1 0
HIV- 1456 138 37 11 5 0
Median survival in months (95%CI)
HIV+ 2.2 (1.2 to 3.1)
HIV- 4.1 (3.6 to 4.4)

The influence of HIV infection in the natural history of hepatocellular carcinoma in the era of combined anti-retroviral therapy.

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Supplementary Methods

Multiple imputation procedures

Due to the considerable proportion of missing data for some variables in the analysis datasets (up to ~50% for the full dataset of 1588 patients and up to ~ 90% for the subset of 132 HIV+ patients with HIV-related phenotypes), we used a multiple imputation with chained equations (MICE) approach assuming that the data in were missing at random (MAR).

We imputed each dataset separately to allow for imputation of HIV-related prognostic factor. For both the full dataset of 1588 patients and the subset of 132 HIV+ patients, we included in the imputation models (i) all predictors of missingness (log-likelihood p <0.10) for any of the variables included in the initial analysis models specified in the following paragraph “Backward selection procedures”, (ii) the event indicator (subject alive/death), and (iii) the Nelson-Aalen estimator of baseline hazard(1). From each original dataset, we generated 100 imputed datasets to provide stable estimates. Diagnostics plots of trace lines revealed satisfactory convergence of imputation models. Plots of imputed values vs the observed values suggested that imputed values were plausible.

Backward selection procedures

We applied a backward selection algorithm on the imputed datasets following the “Rubin rule” approach(2). For each step, a Cox regression model was fitted in the imputed datasets and parameter estimates were averaged as per Rubin rule. The backward selection algorithm stopped when all pooled estimates from the multiply-imputed datasets had a p-value lower than 0.05.

In the full sample (N=1588) of all untreated HCC patients, the initial multivariable Cox model comprised age, gender, BCLC stage, CTP class, log-transformed AFP, year of HCC diagnosis, a binary variable representing continent of study centre (Americas/Asia vs Europe), and HIV status. After three steps,

backward selection identified the following prognostic factors associated with OS in the full dataset: HIV, gender, BCLC stage, CTP class, log-transformed AFP, and continent.

In the subset (N=132) of HIV+ untreated HCC patients, the initial model comprised factors associated to OS in HIV+ patients(3, 4) in addition to common prognostic factors for HCC: age, BCLC stage, CTP class, log-transformed AFP, CD4+ concentration, year of HCC diagnosis, continent, CD4+ concentration, log-transformed HIV viral load, presence of antiretroviral treatment. This initial model could not include gender as only 2/132 patients were female. After eight steps, two parameters were associated to OS at the 5% level of significance in the subset of HIV+ patients: CTP class and log-transformed AFP. We re-added common prognostic factor for HCC and HIV as well as continent owing to its significance in the full-sample analysis, so that the final model for HIV+ patients included: CTP class, BCLC stage, log-transformed AFP, HIV viral load, CD4+ concentration, and continent.

Supplementary tables and figures

Supplementary Table 1. Complete-case analyses: effects of HIV+ status and selected prognostic factors on survival

A. In the full sample of HCC patients (N=1588)

Predictor	Univariable models			Multivariable model		
	Hazard ratio (95%CI)	P-value	N	Hazard ratio (95%CI)	P-value	N
HIV+ status	1.49 (1.22 to 1.82)	<0.0001	1,588	1.23 (1.00 to 1.51)	0.0515	1,463
Male gender	1.62 (1.35 to 1.93)	<0.0001	1,586	1.29 (1.07 to 1.56)	0.0071	
BCLC stage C/D vs 0/A/B	3.09 (2.59 to 3.69)	<0.0001	1,567	1.85 (1.52 to 2.26)	<0.0001	
CTP class						
B vs A	1.63 (1.40 to 1.90)	<0.0001	1,529	1.76 (1.51 to 2.05)	<0.0001	
C vs A	2.35 (1.97 to 2.82)	<0.0001		2.42 (1.99 to 2.95)	<0.0001	
Log AFP	1.19 (1.16 to 1.21)	<0.0001	1,538	1.15 (1.12 to 1.17)	<0.0001	
Continent: Americas/Asia vs Europe	2.59 (2.24 to 3.00)	<0.0001	1,588	2.01 (1.71 to 2.38)	<0.0001	

A. In HIV+ HCC patients (N=132)

Predictor	Univariable models			Multivariable model		
	Hazard ratio (95%CI)	P-value	N	Hazard ratio (95%CI)	P-value	N
BCLC stage C/D vs 0/A/B	1.58 (1.01 to 2.46)	0.0441	132	1.56 (0.90 to 2.69)	0.1141	118
CTP class						
B vs A	1.67 (0.86 to 3.26)	0.1331	132	1.49 (0.73 to 3.04)	0.2739	
C vs A	3.45 (1.72 to 6.92)	0.0005		2.40 (1.07 to 5.39)	0.0344	
Log AFP	1.21 (1.12 to 1.32)	<0.0001	125	1.20 (1.10 to 1.30)	<0.0001	
CD4 count (x 100)	0.92 (0.83 to 1.02)	0.1329	129	0.96 (0.85 to 1.08)	0.4755	
Log viral load	1.02 (0.96 to 1.08)	0.5963	125	0.98 (0.91 to 1.05)	0.5401	
Continent: Americas/Asia vs Europe	1.09 (0.70 to 1.69)	0.7154	132	0.69 (0.42 to 1.13)	0.1359	

BCLC, Barcelona Clinic Liver Cancer. CTP, Child-Turcotte-Pugh. AFP, alpha-fetoprotein.

Supplementary Table 2. Distribution of demographic and prognostic characteristics by continent

Characteristics		Americas/Asia (N=958)	Europe (N=630)	P-value
Age (years), median (IQR)		62.8 (17.20)	67.0 (14.00)	<0.0001 [a]
Male gender, N (%)		844 (88.1)	474 (75.2)	<0.0001 [b]
Survival (months), median (IQR)		2.8 (4.99)	7.0 (13.00)	<0.0001 [a]
HIV+ status, N (%)		97 (10.1)	35 (5.6)	0.0017 [b]
Viral aetiology of liver disease, N (%)				
	HBV	544 (56.8)	154 (24.4)	<0.0001 [b]
	HCV	254 (26.5)	389 (61.7)	<0.0001 [b]
Alcohol aetiology of liver disease, N (%)		189 (19.7)	226 (35.9)	<0.0001 [b]
BCLC stage, N (%)				
	0	5 (0.5)	12 (1.9)	<0.0001 [b]
	A	72 (7.5)	103 (16.3)	
	B	66 (6.9)	141 (22.4)	
	C	610 (63.7)	151 (24.0)	
	D	184 (19.2)	223 (35.4)	
CTP score, median (IQR)		7.0 (3.00)	7.0 (3.00)	0.0495 [a]
CTP class, N (%)				
	A	295 (30.8)	244 (38.7)	0.0014 [b]
	B	428 (44.7)	241 (38.3)	
	C	176 (18.4)	145 (23.0)	
AFP (ug/L), median (IQR)		1361.8 (15019.30)	51.0 (840.00)	<0.0001 [a]
Year of HCC diagnosis, median (IQR)		1996.0 (9.00)	2000.0 (10.00)	<0.0001 [a]
Cirrhosis, N (%)		230 (24.0)	616 (97.8)	<0.0001 [b]
Extrahepatic disease, N (%)		594 (62.0)	47 (7.5)	<0.0001 [b]
Portal vein thrombosis, N (%)		414 (43.2)	169 (26.8)	<0.0001 [b]

[a] Mann-Whitney test. [b] Pearson's chi-squared test.

IRQ, interquartile range. AFP, alpha-fetoprotein. BCLC, Barcelona Clinic Liver Cancer. CTP, Child-Turcotte-Pugh.

Please note that the sum of percentages may be lower than 100% owing to missing data.

Supplementary References.

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