

## Hepatocellular carcinoma: Clinical-pathological features and HIV infection in Mozambican patients<sup>☆</sup>

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HCC, hepatocellular carcinoma  
HBV, hepatitis B virus  
HCV, hepatitis C virus  
HIV, human immunodeficiency virus  
AFB1, Aflatoxin B1  
AFP, Alpha-fetoprotein.

### ABSTRACT

**Background and aims:** Mozambique had been ranked among the countries with the highest global incidence of HCC with chronic hepatitis B infection and high exposure to aflatoxin-B1 (AFB1) being major risk factors. Indeed, HCC remains one of the most frequent cancer in Maputo. On the other hand, Mozambique has a high prevalence of infection with Human Immunodeficiency virus (HIV). Our study aims to describe the epidemiology, clinicopathological and serological features of patients with HCC in Maputo Central Hospital and its relationship with HIV.

**Methods:** A series of 206 patients, diagnosed with HCC via fine needle aspiration, were consecutively included in the study. Patient data was collected using a questionnaire and all patients were tested for HBV, HCV, HIV. **Results:** Median age was 49 years old and the M: F sex ratio was 2.4. A total of 114 (56.2%) of the patients were HBsAg positive. Hepatitis C antibodies were present in 8.9% of cases, and co-infection with HBV and HCV (HBsAg/anti-HCV) was observed in 4 (2.0%) cases. The remainder, 36.3%, were neither hepatitis B- nor C-related. HIV was detected in 34 cases (18.0%) cases. HIV-HBV or HIV-HCV co-infections were observed in 22 (68.8%) and 2 (6.2%) cases. Overall, positivity for HIV was associated with younger age, and especially in patients with HBsAg + /anti-HCV +.

**Conclusions:** Our data emphasize the need for a reinforcement of secondary prevention measures in Mozambique. Serological screening for HBV in people born before universal anti-hepatitis B immunization (2001), effective screening, and specific management in HIV(+) patients are urgently needed.

### Introduction

Hepatocellular carcinoma (HCC) is a leading cancer in sub-Saharan Africa and the second leading cause of death from malignancy

worldwide [1]. In sub-Saharan Africa, the major risk factors of HCC are chronic infection with hepatitis B virus (HBV) and early exposure to aflatoxin-B1 (AFB1) [2], but excessive consumption of alcohol, iron overload, or HIV infection are thought to also play a role in HCC

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incidence and presentation [3–5].

Geographical variability of incidence was noticed in the first half of 20th century by investigators working in various locations of Africa [6]. During that period, Mozambique was considered as a place where HCC incidence was very high and marked by an early presentation, even when comparing to other sub-Saharan African countries [7–9]. Later work confirmed these seminal observations by analysing the incidence of liver cancer in Mozambicans working in the South African mines and the prevalence was estimated at 98 cases/10<sup>5</sup> male habitants, placing Mozambique as the country with the highest incidence of HCC worldwide at that stage [10–12]. Since then, and for reasons that remain to be established, the frequency of HCC has decreased [13]. Despite the reduction in its frequency, data from the recently implemented Hospital-based cancer registry of Maputo Central Hospital shows that liver cancer remains the third and fourth most frequent cancers in Maputo in men (13.1%) and women (6.7%), respectively [14]. Cancer data in Mozambique are restricted to cancer's registries from Maputo and Beira cities. Recent data based on the Maputo Central Hospital registry, showed that HCC is the third most frequent cancer in men in Maputo city, responsible for 13.8% of all cancers in men and corresponding to an age-standardized incidence rates (ASIR) of 13.3 per 100 000. In women, HCC was the fifth most common (6.3% of cases, ASIR 6.6 per 100,000) [15]. In Beira city, located in the center of the country, HCC was the fourth and ninth most common cancer in men (ASIR 2.8 per 100,000) and women (ASIR 2.8 per 100,000), respectively [16].

Mozambique is considered an endemic country for HBV infection, which seems to be responsible for 80% of the HCC in sub-Saharan Africa [17]. Despite the introduction of anti-HBV vaccination in 2001, the prevalence of HBV in Mozambique still remains high [18].

In Mozambique, interest in HCC has been renewed in recent years due to the high HIV prevalence in the general population [19, 20]. HIV is considered a potential modulator of liver tumorigenesis and in presence of co-infection with HBV, could accelerate progression to cirrhosis and consequently to HCC [21]. Data from the recent HIV survey demonstrated that the HIV epidemic in Mozambique is worsening with a national prevalence of infection increasing from 11.5% in 2009 to 13.2% in 2015 [19, 20]. Hence, the impact of HIV on HCC in Mozambique might be important, despite the lack of published evidence.

Despite this, HCC still remains neglected in the country as no public health program coordinating preventive and care interventions to reduce the burden of the disease have been hitherto implemented in Mozambique. In this context, in order to update the epidemiology of HCC in the country, we conducted a survey describing the clinicopathological presentation and risk factors of HCC in patients attended Maputo Central Hospital and its relationship with HIV.

## Patients and methods

### Study design and patients

The study was performed at Maputo Central Hospital (MCH), which is the largest hospital in the country, situated in Maputo city, and is the national referral centre in Mozambique. It was a cross-sectional study, where we included all patients who consecutively attended the Gastroenterology Department of MCH for HCC during two periods, namely, from March 2011 to April 2012 ( $n = 101$ ) and then from September 2012 to October 2013 ( $n = 104$ ). A lack of availability of HBV tests accounted for the interruption in the follow up period. All enrolled participants answered a structured questionnaire focusing on demographic characteristics, alcohol and smoking habits, level of education, place of residence and place of birth. The study was approved by the Mozambican National Bioethics Committee. All patients signed an informed consent.

All patients suspected of HCC were followed using the routine protocol being implemented in the hospital. Patients were submitted to a clinical examination and laboratory blood tests, including HCV, HBV,

and HIV serologies. Patients were subsequently submitted to an abdominal ultrasound performed by a senior gastroenterologist. Following tumor localization with ultrasound, a Fine Needle Aspiration Cytology (FNAC) was done. A 23 G needle was used to aspirate material to confirm cytological features of HCC. Slides from the aspirate spread were subsequently interpreted by an experienced pathologist after staining with Hematoxylin-Eosin (95% Alcohol fixed) and May-Grunwald-Giemsa (air-dried).

### Laboratory tests

Hepatitis B and C infection were determined using an ELISA assays detecting hepatitis B surface antigens and anti-HCV antibodies, respectively, (both from Healthease, Neomedic, RSA). Additional markers, such as HBeAg and anti-HBc (HBV core), were also performed using ELISA techniques (DSI-EIA, DSI, Italy). Anti-HIV antibodies were detected following the national algorithm, based on serial testing using two immuno-chromatographic rapid tests. For this purpose, sera were first tested with Determine HIV 1–2 (Abbott Laboratories, Japan) and each seroreactive sample was confirmed using Uni-Gold HIV1-2 (Trinity Biotech, Ireland). Alpha-fetoprotein (AFP) levels were measured using ELISA Kit (DSI-EIA-AFP DSI, Italy). Levels were considered as normal when AFP < 10 ng/mL, suspect when AFP was between 10–350 ng/mL, and diagnostic when measurement was > 350 ng/mL. All patients were tested for the first time for the different serological markers described above, including HBV, HCV and HIV, at the moment of diagnosis. Therefore, they were all naïve anti-HIV or anti-HBV/HCV treatment.

### Statistical analyses

Statistical analyses were performed using a Prism 6.0 statistical package. Numerical variables were summarized by their median, mean, and range according to their types of distribution (normal or not). They were compared either by a Student's *t*-test, ANOVA or by a Mann-Whitney test as appropriate. Categorical variables were summarized as frequencies that were compared by Fischer's exact test. All tests were univariate and two-sided. Level of significance was set at  $p < 0.05$ .

## Results

### Patient demographics

Characteristics of the 206 patients are described in Table 1. A total of 145 and 61 participants were male and female, respectively (M: F gender ratio of 2.4). The median age was 49 (33–62) years old. A large majority of patients (86.7%) were living in Maputo area. Around 91% of patients were born in the three southern provinces of Mozambique: Maputo, Inhambane and Gaza with 33.2, 31.2 and 26.8% respectively. Patients tended to originate mostly from coastal districts (especially Manjacaze and Zavala) in Gaza and Inhambane provinces, with very few cases coming from inland districts (Fig. 1A and B).

### Clinic-pathological features

Frequency of HBV infection (measured by presence of HBsAg) among HCC participants was 56.1%, and only 8.9% were anti-HCV positive (Table 1). HBV/HCV co-infection was observed in 4 cases (2.0%). Frequency of anti-HIV antibodies among participants was 18.6% (34/206). HIV-HBV co-infection was observed in 22 out of 32 HIV+ tested for HBV (68.8%) cases and HIV-HCV in 2 out of 32 HIV+ patients tested for HCV (6.2%). In 36.3% (73/201) of HCC, both HBsAg and anti-HCV were negative, representing non-B non-C (nBnC) patients. Anti-HBc antibodies, a biomarker of previous contact with HBV, was present in 96.1% of patients tested for this marker (149 out of 155 tested).

A significant proportion of patients (59.6%; 121/203) reported

**Table 1**  
Demographic, clinic-biological features of the 206 patients with HCC attending Maputo Central Hospital.

Characteristics	All patients n (%) <sup>a</sup> (n = 206)	HIV infection status		P <sup>†</sup>
		HIV(-) n (%) <sup>a</sup> (n = 172)	HIV(+) n (%) <sup>a</sup> (n = 34)	
Sex (male)	145 (70.4)	124 (72.1)	21 (61.8)	0.228
Median (P25–P75) age, years	49 (33–62)	51.5 (36–64)	38.5 (29–49)	0.003
Province of birth				
Maputo	68 (33.2)	55 (32.2)	13 (38.2)	0.331
Inhambane	64 (31.2)	57 (33.3)	7 (20.6)	
Gaza	55 (26.8)	46 (26.9)	9 (26.5)	
Other	18 (8.8)	13 (7.6)	5 (14.7)	
Province of residence				
Maputo	177 (86.8)	144 (84.7)	33 (97.1)	0.190
Inhambane	13 (6.4)	13 (7.6)	0 (0.0)	
Gaza	9 (4.4)	9 (5.3)	0 (0.0)	
Other	5 (2.4)	4 (2.4)	1 (2.9)	
Education level				
None	29 (14.1)	23 (13.4)	6 (17.6)	0.751
Primary	108 (52.4)	90 (52.3)	18 (52.9)	
Secondary or University	69 (33.5)	59 (34.3)	10 (29.4)	
HBV and HCV infection markers <sup>‡</sup>				
HBsAg(+)	114 (56.2)	92 (53.8)	22 (68.8)	0.118
anti-HCV(+)	18 (9.0)	16 (9.5)	2 (6.2)	
HBsAg(+) and anti-HCV(+)	4 (2.0)	3 (1.8)	1 (3.1)	0.503
HBsAg(-) and anti-HCV(-)	73 (36.3)	64 (37.9)	9 (28.1)	0.293
Alcohol consumers <sup>§</sup>	121 (59.6)	101 (59.4)	20 (60.6)	0.898
Smokers	56 (27.2)	43 (25.0)	13 (38.2)	0.113
Multinodular tumor <sup>¶</sup>	131 (71.6)	107 (71.3)	24 (72.7)	0.872
Median (P25-P75) nodule diameter <sup>‡</sup> , mm	78 (60–105)	78 (60–102.5)	77 (60–105.5)	0.934
AFP, ng/ml <sup>‡</sup>				
< 10	44 (22.0)	36 (21.4)	8 (25.0)	0.834
10–350	71 (35.5)	61 (36.3)	10 (31.2)	
> 350	85 (42.5)	71 (42.3)	14 (43.8)	

HIV – Human Immunodeficiency Virus; HBV – Hepatitis B Virus; HCV – Hepatitis C Virus; HBsAg – HBs antigen; AFP – Alpha-fetoprotein.

<sup>a</sup> Results are presented as n(%), except if otherwise specified; percentages may not add up to 100% due to rounding's.

<sup>†</sup> P-value for the comparison between HIV+ and HIV- participants.

<sup>‡</sup> The overall number of participants does not add up to 206 due to missing data: Province of birth, n = 205; Province of residence, n = 204; HBsAg, n = 203; anti-HCV, n = 201; HBsAg and anti-HCV, n = 201 (169 HIV- and 32 HIV+); alcohol consumption, n = 203; multinodular tumor, n = 183; AFP, n = 200.

<sup>§</sup> In multinodular tumors, the diameter of the largest nodule was considered; data was available for 132 patients.

frequent consumption of alcohol and 27.1% (56/206) were smokers.

Most of the patients presented with a multinodular tumor (71.6%; 131/183), with a median diameter of the largest nodule of 78 mm. AFP levels were > 350 ng/ml in 42.5% (85/200) of patients, whereas 22.0% (44/200) displayed AFP within normal ranges (< 10 ng/mL).

#### Age trend in patients infected with hepatitis virus

Following stratification for serological markers, patients displayed significant differences of age distribution (Fig. 2). The prevalence of HBsAg was higher before 30 years old, with a declining trend to its minimum in the age group of 70 or more years old, whereas the prevalence of anti-HCV and of nBnC (HBsAg-/anti-HCV-) cases increased with age.

#### Association between HIV infection and clinic-demographic data and laboratory biomarkers

Overall, patients' HIV positive were significantly younger than those HIV negative (38.5 vs 51.5 years old, respectively;  $P = 0.003$ ) (Table 1). In HBV and/or HCV infected patients, the presentation of HCC was earlier in HIV positive than those HIV negative ( $P = 0.0197$ ) (Fig. 3). Presence of HIV is also associated with early presentation of HCC in patients HBsAg+ and/or anti-HCV(+), when compared with nBnC (HBsAg-/anti-HCV-) patients ( $P = 0.0033$ ) (Fig. 3). There were no significant differences between age presentation of HCC and HIV status in nBnC patients. HIV presence was not associated with the

province of birth or residence, education level, Hepatitis B and C virus markers, clinical presentation alcohol consumption, smoking, and AFP levels (Table 1).

#### Other etiologies

Prevalence of nBnC (HBsAg-/anti-HCV-) cases increased from 13% before 30 years old to more than 50% after 50 years old (Fig. 2). There were no differences of prevalence of HIV infection and smoking in both patients HBV and/or HCV infected or nBnC patients. Interestingly, the prevalence of alcohol consumption was significantly higher in nBnC patients than in patients positive for HBsAg or anti-HCV ( $P = 0.002$ ) (Fig. 4).

#### Discussion

In the present study, we describe clinic-pathological characteristics and frequency of hepatitis virus and HIV in Mozambican patients with HCC. Age and gender distribution of HCC in our study are similar from those reported in other HBV-endemic regions, with a relatively younger age at diagnosis and a higher male to female ratio [2, 22, 23].

The majority of HCC patients were infected with HBV (56.3%), reinforcing the critical role of HBV in the carcinogenesis of HCC. Our results are in accordance with those of the most sub-Saharan countries where HBV infection is endemic and where chronic HBV infection and HCC have a similar geographical distribution, suggesting that HBV remains the most important risk factor to develop HCC in this region [17,

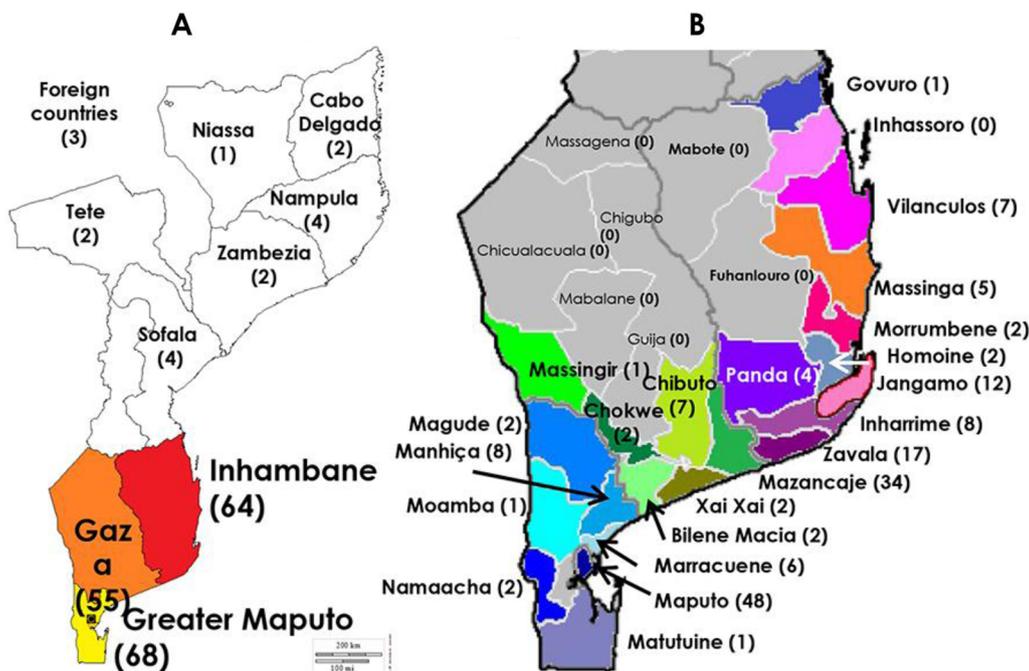


Fig. 1. Distribution of HCC cases in according to place of birth – province (A) and district (B).

23, 24]. In the past, this high prevalence was also reported by Kew and coworkers (62% and 86% of HBsAg and anti-HBc, respectively), in Mozambican miners with HCC [25]. Dazza and coworkers found similar rates of HBsAg seropositivity (66%) two decades later [26]. Regarding HCV, when compared with the only available Mozambican data obtained more than 20 years ago (6.1%) [26], the percentage of anti-HCV (+) HCC seem to have increased during the recent period, but still low (9%), as usually observed in sub-Saharan Africa [27–29].

It is well-known that HBV-HIV co-infected patients are at higher risk to be affected with a liver-related cause of death than mono-infected patients [30]. With the advent of highly antiretroviral treatment (HAART) in the last two decades, there was an improvement of survival of HIV patients due to a decrease of morbidity and mortality by HIV related diseases [31]. This has led to an increase in the incidence of diseases not directly related to HIV, such as liver disease, including HCC

[31–34]. By contrast with the situations reported from Europe or North America where HIV patients co-infected either by HCV or HBV develop an HCC earlier in lifespan, in sub Saharan Africa early death from opportunistic infection in co-infected patients has been considered so far to precede HCC development and very few studies have described the fate of co-infected patients [35–37].

To the best of our knowledge, this is the first study in Mozambique reporting the burden of HIV on HCC. Prevalence of HIV in Mozambique is 13.2%, and varies in different provinces of the country. The highest prevalence is observed in the southern regions, namely Gaza (24.4%), Maputo Province (22.9%) and Maputo city (16.9%) [20]. Almost all of the patients of our series were from the southern region, and 86.7% was living in Maputo area. Lowest prevalence of HIV (5.2%) is observed in Tete Province (located in the centre of the country).

Prevalence of HIV in our HCC series was 18%, in accordance with

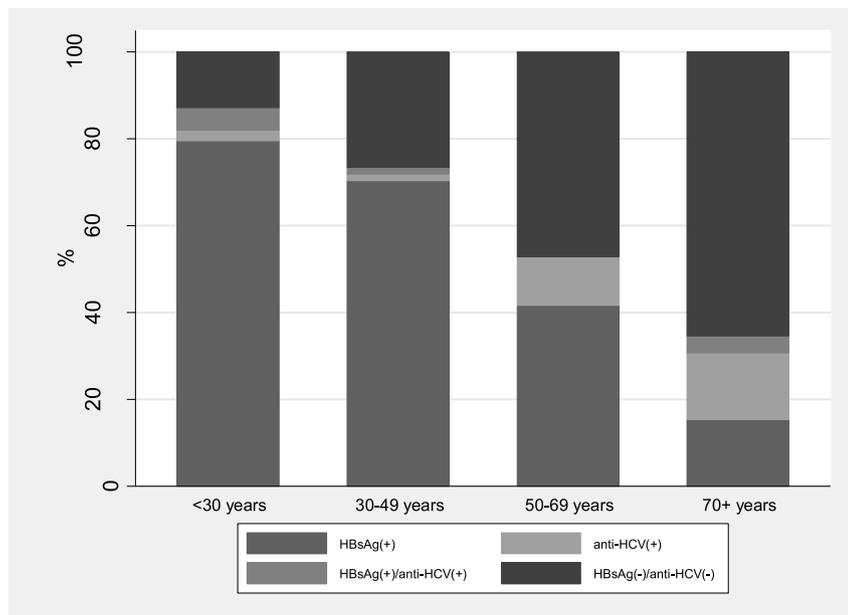
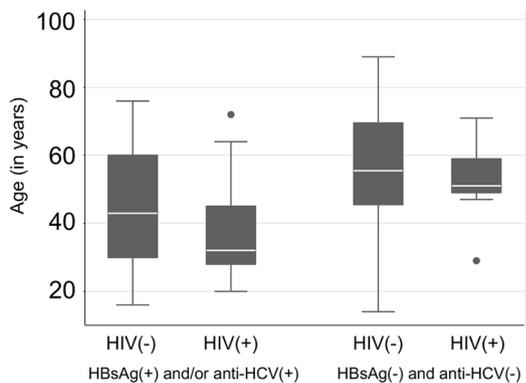


Fig. 2. Distribution of infectious risk factors according to age group.

HIV(+) vs. HIV(-) in patients HBsAg(+) and/or anti-HCV(+) –  $p = 0.0197$ ; HIV(+) vs. HIV(-) in patients HBsAg(-) and anti-HCV(-) –  $p = 0.2501$ ; HBsAg(+) and/or anti-HCV(+) vs. HBsAg(-) and anti-HCV(-) in HIV(-) –  $p < 0.001$ ; HBsAg(+) and/or anti-HCV(+) vs. HBsAg(-) and anti-HCV(-) nos HIV(+) –  $p = 0.0033$ .



HIV(+) vs. HIV(-) in patients HBsAg(+) and/or anti-HCV(+)  
 p=0.0197;  
 HIV(+) vs. HIV(-) in patients HBsAg(-) and anti-HCV(-)  
 p=0.2501; HBsAg(+) and/or anti-HCV(+)  
 vs. HBsAg(-) and anti-HCV(-) in HIV(-)  
 p<0.001;  
 HBsAg(+) and/or anti-HCV(+) vs. HBsAg(-) and anti-HCV(-)  
 nos HIV(+)  
 p=0.0033

Fig. 3. Impact of HIV co-infection on patient age.

reported in sub-Saharan Africa, like in Uganda (20%), in South Africa (19%) [23, 38] or in other countries [39]. There is reported in the literature that HIV increases the risk of HCC, reduces the age of development of HCC and is responsible for unfavourable evolution (17,30–38). We did not find significant association between the presence of HIV alone (without any hepatitis virus) and development of HCC. Our result is consistent with the other series recently reported [21, 38, 39].

However, we found that the large majority of HIV-infected patients who developed HCC were coinfecting with HBV and that Mozambican patients with HIV are subject to an earlier HCC development than HBV-monoinfected patients or patients without any overt infection with a persistent hepatitis virus. The early onset of HCC in HIV positive patients has been also reported in Uganda or South Africa [21, 38, 39]. These results suggest that HIV infection appears currently as a significant modulator of liver carcinogenesis, probably accelerating liver damage related to HBV infection or accelerating the T-cell exhaustion that participates to cancer evasion. Mak et al. (2018) demonstrated that levels of HBV DNA were significantly higher in patients with HCC co-infected with HIV and HBV than those mono-infected with HBV, thus contributing to the increase of HBV replication in those patients [38]. However, to better understand the role of HIV in the development of HCC a further true case-control study will be necessary.

Since 2011, the number of people under anti-retroviral therapy (ART) has increased threefold in Mozambique [19, 20]. The country faces an exponential increase of treated patients since the launch of the “Acceleration Plan” resulting in the rapid expansion of health ministry supported facilities offering ART. This proactive policy will hopefully reduce the burden of opportunistic infections due to HIV, but might result, on the long term, in a delayed increase of HCC cases in co-infected patients.

Regarding non-infectious etiology, the prevalence of alcohol consumption in our study was 59.6%. We did not find a significant association between alcohol and HIV status, despite the evidence reported by some studies concerning the role of alcohol as a risk factor for HIV [40]. However, alcohol consumption was significantly higher in patients negative for HBsAg and/or anti-HCV (nBnC patients), showing that in absence of HBV and/or HCV infection, alcohol is also an important risk factor for HCC in both HIV positive and negative patients [41]. In our series, 50% of patients from Inhambane province were nBnC cases, suggesting a locally important impact of non-infectious

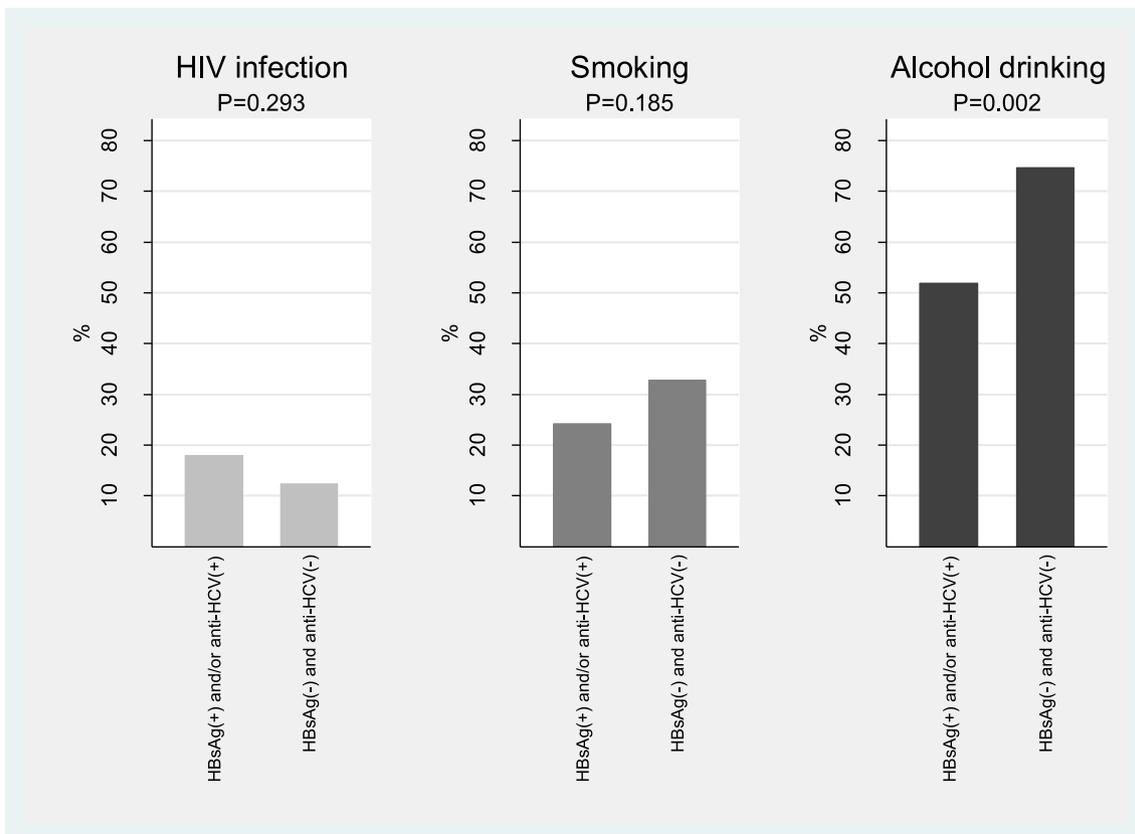


Fig. 4. Frequency of HIV, alcohol drinking and smoking in HBsAg or anti-HCV+ and nBnC patients.

etiology. A decisive achievement in the understanding of the Mozambican problem was accomplished in the 1970s through nutrient analyses that clearly demonstrated the importance of food contamination by AFB1 considered as the highest ever measured worldwide at that time [42, 43]. As underlined by van Rensburg and coworkers, high exposure to AFB1 in the context of high endemicity for chronic infection with HBV was most probably the cause of early presentation and high incidence of HCC in Mozambique. The majority of our patients were living in the south of the country, most of them living in Maputo region and most of them born in Inhambane. These regions are considered endemic for aflatoxins and Maputo city and Inhambane are regions with high prevalence of aflatoxin contamination. According with a review paper that included data from studies conducted between 1985 and 2017, the distribution of aflatoxins varies in different regions of the country. Aflatoxins B1 (AFB1) and G1 (AFG1) have been found in several Mozambican commodities, mainly groundnuts and maize [44, 45]. The highest prevalence of aflatoxins contamination was found in Nacala district (Nampula Province) in the north of the country, followed by Maputo City. Inhambane also had high aflatoxin levels in their foods [44]. The absence of cancer data, including HCC, in the north of the country does not allow us to do comparison with our current series mostly originating from the south.

We hypothesize that the significant decrease of HCC incidence in Mozambique observed in recent studies [13], rather than being linked to an improvement of HBV infection endemicity, might actually turn out to be linked with a sizeable reduction of food contamination by AFB1.

We did not have the possibility to assess AFB1 exposure through albumin or DNA adducts measurement, but an indirect measurement of AFB1 effect in these patients through the search for tissue or circulating ARG249SER mutations of *TP53* appears as an almost mandatory further step for our research group [46]. We also did not have the opportunity to check the patients for occult B infection (OBI) that is currently considered a significant cause of HCC worldwide [47, 48]. In the region, a seminal work exploring OBI in patients with HCC was conducted a few years ago by MC Kew and colleagues in neighboring South Africa [49]. The authors reported a surprisingly high rate (75%) of positivity for this type of stealth infection. In the series of Mak et al. (2018) prevalence of OBI in the HCC cases was 12% and was associated with increased HCC risk [38]. We are, thus, entitled to suspect that a proportion of our cases tagged as nBnC can be actually patients with an OBI.

## Conclusion

In conclusion, our study shows that persistent infection with HBV is still the predominant risk factor for HCC in Mozambique. HIV contributes for earlier development of HCC, mainly in patients co-infected with HBV. Studies aiming to assess precisely OBI and the current role of aflatoxin are now warranted to generate a truly comprehensive landscape of HCC in Mozambique. Along the high prevalence of HBV, Mozambique has also one of highest prevalence of HIV worldwide. Although universal immunization of newborn against HBV was introduced in 2001 in national calendar of vaccination of in Mozambique and testing for HBV infection is part of the routine monitoring of HIV-infected patients, more attention must be given to high-risk group in adults including HIV-HBV coinfections. Increasing access to HAART, together with introduction of screening program for HCC in patients co-infected with HIV-HBV may have impact on decreasing of mortality rates in HIV patients.

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