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High Frequency of Advanced Hepatic Disease among HIV/HCV Co-Infected Patients in Cambodia: The HEPACAM Study (ANRS 12267)

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Abstract

Background: Little is known about HIV/Hepatitis C Virus (HCV) co-infection in resource-limited countries, although chronic HCV infection is one of the most relevant comorbidities in HIV population. The aim of this study was to determine the severity of liver disease in a cohort of HIV/HCV co-infected patients followed in Calmette Hospital, Phnom Penh, Cambodia, and to analyse the impact of HCV infection on antiretroviral therapy efficacy and hepatotoxicity.

Methods: HIV patients with positive HCV antibodies were enrolled in this cross-sectional study. HIV mono-infected patients formed the control group. Transverse evaluation of co-infected patients was performed collecting clinical, biological, virological and ultrasonographic data. HIV course, response to antiretroviral therapy and frequency of hepatocytolysis were compared in both groups.

Results: Among 50 HIV patients known with HCV antibodies, 31 (62%) had positive plasma HCV RNA and were included (58% men, median age 44 years). HCV genotype 1 was the most prevalent (68%), followed by genotype 6 (25%). Twelve patients (39%) met clinical, biological and/or ultrasonographic criteria for cirrhosis. FibroTest stage was 3-4 in 16 patients (52%). HIV/HCV co-infected patients demonstrated similar immune restoration and virological response to antiretroviral therapy as the 160 HIV mono-infected patients. Co-infected patients were more likely to have alanine aminotransferase elevation at baseline and to develop grade 2 or 3 hepatocytolysis in the two years after antiretroviral therapy initiation, specifically when nevirapine was used during the first six months of treatment.

Conclusions: HIV/HCV co-infected patients are at increased risk for acquiring severe hepatic fibrosis. HCV co-infection does not affect response to ART. Efavirenz should be preferred to nevirapine in co-infected patients due to hepatotoxicity. Further research is required to target access to appropriate management of HIV/HCV co-infections in resource-limited countries.

Keywords: HIV; HCV co-infection; Fibrotest; Cirrhosis; Resource-limited country; Nevirapine

Abbreviations: HIV: Human Immunodeficiency Virus; HCV: Hepatitis C Virus; ART: Antiretroviral Therapy; ALT: Alanine Aminotransferase; HAART: Highly Active Antiretroviral Therapy; ART: Antiretroviral Therapy; AIDS: Acquired Immune Deficiency Syndrome; PLWHA: People Living with HIV/AIDS; ARV: Antiretroviral; EFV: Efavirenz; NVP: Nevirapine; HBV: Hepatitis B Virus; HbsAg: HBs Antigen; OR: Odds Ratios; SVR: Sustained Virological Response

Introduction

Since the advent of highly active antiretroviral therapy (HAART), both mortality and incidence of new-Acquired Immune Deficiency Syndrome (AIDS) defining events have dramatically declined among people living with HIV/AIDS (PLWHA) in developed countries [1,2]. Accordingly, the main underlying causes of morbidity and mortality in this population are more frequently associated to non HIV-related conditions such as neoplasia, cardiovascular diseases and chronic viral hepatitis [3,4]. In France, hepatitis C virus (HCV) was found to be the third cause of death among PLWHA between 2000 and 2005 [5], and in the Data collection on Adverse events of anti-HIV Drugs (D:A:D study), liver disease was the first cause of non-AIDS related death [4]. Compared to HIV sero-negative patients, HCV course among PLWHA is more severe: progression to cirrhosis occurs faster [6], and global survival is shortened [7]. Moreover, liver toxicity of ART is a source of growing concern and seems to be magnified by HCV co-infection [8-10]. Data are more controversial regarding the potential impact of HCV co-infection on HIV disease progression and immune reconstitution with ART [11].

In developing countries, stimulated by the "3 by 5" and Universal Access initiatives from the World Health Organization (WHO) and UNAIDS (United Nations Programme on HIV/AIDS), a growing number of PLWHA gained access to ART: by the end of 2008, 4 million

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had started the treatment. In 13.4 million inhabitants Cambodia, the number of PLWHA on first-line antiretroviral (ARV) regimen has significantly increased in the last 6 years, reaching more than 40,000 at the end of 2010, which covers 80% of those who needed the treatment based on the WHO 2010 recommendations (Cambodian Ministry of Health's annual report). ART access programs have proven the feasibility and efficacy of first-line ARV regimen in resource-limited settings similarly to those reported in developed countries [12,13]. In Cambodia, similar positive outcomes was reported after two and three years of first-line ARV regimen combining retrovir (AZT) or stavudine (d4T), epivir (3TC), and nevirapine (NVP) or efavirenz (EFV) [14-18]. As AIDS-related morbidity and mortality are declining in Cambodia, the burden of HIV/chronic viral hepatitis co-infection will likely increase in the near future. However, little is known about viral hepatitis in Cambodia. The prevalence in the overall Cambodian population ranges from 8 to 9% for hepatitis B and 6 to 14% for hepatitis C according to Cambodian unpublished and published surveys [19-21]. Compared with neighbouring countries, the prevalence of hepatitis B is similar but the prevalence of hepatitis C appears to be greater [22,23]. The high frequency of therapeutic injections practices before the 1990s in Cambodia could explain this difference [24], whereas intravenous drug abuse seems marginal. The prevalence of HIV/HCV co-infection is not precisely known in the different Cambodian HIV care centres, as HCV antibodies are not routinely screened in the country, but it was estimated to be around 10% in ESTHER/Calmette cohort, Phnom Penh, Cambodia, in a recent survey. Accurate assessment of the extent of liver disease among HIV/HCV co-infected patients in Cambodia, as in most developing countries, is lacking.

The study aimed at evaluating the ratio of active hepatitis among positive HCV antibodies patients and to describe the severity of hepatic disease, specifically the stage of fibrosis, among HIV/HCV co-infected patients. Secondary objectives were to identify major HCV genotypes circulating in this population and to analyse response to ART and hepatic toxicity of drugs among HIV/HCV co-infected patients compared to a HIV mono-infected control group.

Patients and Methods

Study population

A cross sectional study was conducted from March to June 2009 among patients issued from the "Ensemble pour une Solidarité Thérapeutique Hospitalière En Réseau" (ESTHER) cohort followed at Calmette Hospital in Phnom Penh, Cambodia. The French ESTHER program was implemented in this hospital in February 2003 in collaboration with the Cambodian Ministry of Health. ART initiation began in July 2003 according to WHO recommendations and National Guidelines. The initial first-line regimen was d4T/3TC/EFV. To avoid d4T toxicity and because of EFV supply difficulties, the AZT/3TC/NVP combination was progressively introduced and has become the initial combination since July 2004. Patients are clinically followed every month. CD4 counts are evaluated every 6 months but viral load monitoring is not routinely available. Nurses provide adherence support via a patient education program. In 2006, viral load assessment of the ESTHER Cohort was <250 copies in 69% of all patients and in 83% of naive patients in intent to treat analysis at 36 months [18]. At the time of the study, around 1400 PLWHA were followed-up in ESTHER/Calmette cohort and 1200 were treated with ART. In 2007, 507 patients, among the 1000 patients followed at this time, have been screened for HCV and Hepatitis B Virus (HBV) antibodies thanks to a financial support of ESTHER program (enzyme immunoassay, EIA 3.0, Abbott Laboratories). Screening was made gradually among patients seen in consultation and without selection criteria. HCV antibodies were positive for 52 patients (10.5%) and HBs Antigen (HbsAg) for 63 (12%) patients (personal unpublished data).

For inclusion in our study, HCV status was retrieved from the ESTHER/Calmette cohort database. Qualitative measurement of plasma HCV RNA was performed in all patients with positive HCV antibodies. Patients were eligible if they had positive HCV antibodies and detectable plasma HCV RNA. Patients were not eligible if plasma HCV RNA was negative, if they were pre-treated or treated with anti-HCV therapy, or if they were enrolled in a prospective study. The selected Control group consisted of HIV infected patients (ratio 3/1) with negative HCV antibodies followed at the Hospital's outpatient clinic during the study period and randomly chosen. HCV antibodies were controlled after inclusion among all control patients (enzyme immunoassay, EIA 3.0, Abbott Laboratories). HBV status was not restricted but was adjusted in analysis.

Assessment of liver disease among HIV-HCV co-infected patients

Transverse evaluation of HIV/HCV co-infected patients was performed by collecting clinical, biological, virological and ultrasonographic data during follow-up visits, from March to June 2009. Information about socio-demographic background, lifestyle (including alcohol intake [25], tobacco and illicit drug use), previous hepatic history and detailed physical examination (including vital signs, body mass index, hepatomegaly, splenomegaly, jaundice, ascites, collateral venous circulation, spider angiomata, leukonychia, palmar erythema, encephalopathy) were collected on a standardised case report form. In addition to the routine blood tests and HBV/HCV serology, prothrombin time, serum albumin level, alpha-fetoprotein (Calmette Hospital), and FibroTest-ActiTest (Clinical Laboratory, Institut Pasteur in Cambodia) were obtained. The results from FibroTest-ActiTest were analysed on automated system Integra 400 (Roche Diagnostics) following manufacturer's protocol (Bio predictive, Paris, France). An abdominal ultrasound was performed, within two days following inclusion, to look for hepatomegaly, steatosis, and signs of liver cirrhosis, ascites, portal hypertension and focal hepatic lesions.

Virological analysis

Qualitative measurement of HCV RNA and HCV genotyping was performed in the HIV/Hepatitis Laboratory, Pasteur Institute in Cambodia, as described below. HCV RNA was extracted from 140 µL of plasma using QIAamp Viral RNA mini kit (Qiagen, Hilden, Germany). Extracted RNA was immediately used for qualitative RNA viral load and viral genotyping. Qualitative HCV RNA viral load was assed using reverse transcription (RT) and nested-PCR amplification of 5'NC region. The cut-off value of detection was 100 copies per ml.

HCV Genotyping was performed by Reverse transcription and nested-PCR amplification of NS5B region as previously described [26,27]. The nested-PCR amplified fragments were sent to the Macrogen Company (Macrogen Inc., Seoul, Republic of Korea). Chromatograms sent back electronically to the Pasteur Institute were interpreted using Ceq2000 (Beckman Coulter) software. Virus subtypes were determined by sequence analysis of amplified fragments using CLUSTAL X 1.81 software and the subtype sequences reference sets from the Genbank database.

Comparison with HIV mono-infected control group

In order to compare HIV/HCV co-infected patients with HIV mono-infected patients, socio-demographic background and lifestyle data were collected. HIV disease progression, clinical, immunological (Cyflow, Partec, Germany) and virological (real-time PCR, ANRS, France) response to ART and hepatotoxicity (e.g. ALT at screening and 6, 12 and 24 months after ART initiation) were retrospectively analysed using the outpatient database, and compared between HIV/HCV co-infected and HIV mono-infected patients. Normal values of ALT were defined as 19U/L for women and 31U/L for men. Hepatocytolysis grade 2 was defined as ALT >2.5x normal values and grade 3 as ALT >5 times normal values.

Ethical aspects

The study received approval from the Cambodian National Ethics Committee for Health Research (NECHR). A signed informed consent form was required from each patient before inclusion in the study. Data collection was anonymous and stored safely in a computer.

Statistical analysis

Comparison between HIV/HCV co-infected and HIV mono-infected patients was made using Chi-square test or Fischer test for qualitative variables, and Student test or Kruskal-Wallis test for quantitative variables. Crude association between cases and controls was determined by estimating the Odds ratios (OR) and their 95% CI. This was done by bivariate logistic regression, adjusted for age (in x-year classes), residence and HBV status. OR significance was assessed by the likelihood test. Adjusted ORs were reported. Statistical analysis was performed using STATA 2010 (Texas, USA).

Results

Virological results

Between March and June 2009, 52 HIV infected patients with HCV positive antibodies were enrolled in the study. Two patients were excluded: one because of ongoing treatment for hepatitis C and one because of previous inclusion in a prospective trial study. Qualitative PCR for HCV was positive for 31 patients (62%). HCV genotyping could be done for 28 patients: genotype 1 was the most common (68%), followed by genotype 6 (25%) and genotype 2 (7%). Amplification failed for three patients.

Characteristics of hepatic disease among HIV/HCV co-infected patients

HCV infection was diagnosed around a median of 29 months. No diagnosis of cirrhosis was made before enrolment in our study. Based on physical examination, two patients had clinical signs suggestive of cirrhosis (jaundice without other explanation for one patient and collateral venous circulation for the other). Serum liver function tests were consistent with cirrhosis in eight patients (prothrombin time ≤ 70%: five patients; bilirubinemia >35 mg/l: three patients), whereas albuminemia and α-fetoprotein were normal in all patients. Abdominal ultrasound revealed signs of chronic hepatopathy in five patients (dysmorphic liver: two patients; widened portal vein diameter: three patients; splenomegaly: three patients). No patients had ascites, portal thrombosis or suspicious focal liver lesion. Twelve patients (39%) presented at least one criterion for possible cirrhosis (Table 1).

Non-invasive assessment of hepatitis activity and liver fibrosis (FibroTest-ActiTest) is shown in Table 1. The severity of liver inflammation was graded 2-3 in 12 patients (39%). Advanced hepatic fibrosis (i.e. FibroTest stage 3-4) was found in 16 patients (52%) and significantly correlated with the presence of, at least, one criterion for cirrhosis (p=0.043). FibroTest revealed stage 0-1 for ten patients (32%) and stage 2 for five patients (16%). Overall, 68% of patients presented a fibrosis score ≥ 2.

Comparison of HIV/HCV co-infected patients with HIV mono-infected control subjects

Demographic, behavioural and general medical data are reported in Table 2. Median age was 44 years, and 58% of the population was male. More than half of the patients came from Phnom Penh or its suburb of Kandal. The majority of patients were married or widowed and had children. Seven patients (23%) were heavy alcohol users and all patients denied any intravenous drug use history. HBsAg was detected in one patient, whereas half of the study population had cured hepatitis B (data not showed).

HIV/HCV co-infected and HIV mono-infected patients were compared according to general characteristics (Table 2), duration of HIV infection, clinical, immunological and virological response to ART

Median HCV duration in months (IQR)	29 (25-51)
Criteria of cirrhosis	
≥ 1 clinical criterion*, N (%)	2 (7)
≥ 1 biological criterion**, N (%)	8 (26)
≥ 1 echographic criterion***, N (%)	5 (16)
≥ 1 criterion among clinical, biological and echographic, N (%)	12 (39)
2 types of criterion, N (%)	3 (10)
3 types of criterion, N (%)	0
ActiTest-FibroTest results	
A: 0/1, N (%)	19 (61)
2/3, N (%)	12 (39)
F: 0/1, N (%)	10 (32)
2, N (%)	5 (16)
3/4, N (%)	16 (52)

IQR: Interquartile Ratio

*Ascites, collateral venous circulation, splenomegaly, jaundice and/or encephalopathy without other explanation

**Prothrombin time ≤ 70% and/or bilirubinemia>35 mg/l without other explanation

***Dysmorphic liver, increased diameter of portal vein, ascites, portal thrombosis and/or suspect liver focal lesion without other explanation

Table 1: Characteristics of hepatic disease among PLWHA with active hepatitis C (N=31).

	HIV/HCV co-infected patients (N=31)	HIV mono-infected patients (N=160)	p value
Median age in years (IQR)	44 (38-51)	39 (35-45)	0.038
<30, N (%)	3 (10)	17 (11)	0.016
31-40, N (%)	9 (29)	82 (51)	
41-50, N (%)	11 (35)	48 (30)	
>50, N (%)	8 (26)	13 (8)	
Gender, male, N (%)	18 (58)	85 (53)	0.61
Residence, Phnom Penh, N (%)	16 (52)	122 (76)	0.005
Marital status, married, N (%)	23 (74)	113 (71)	0.61
Duration of HIV infection in years (IQR)	7 (4-9,5)	6 (5,5-7)	0.79
Heterosexual HIV transmission, N (%)	31 (100)	160 (100)	
Injected drug use, N (%)	0 (0)	0 (0)	
HBsAg positive, N (%)	1 (3)	25 (16)	0.047
Alcohol daily consumption, N (%)	7 (23)	31 (19)	0.68

IQR: Interquartile Ratio

Table 2: General data among HIV/HCV co-infected patients and comparison to HIV mono-infected patients.

(Table 3), and frequency of hepatocytolysis before and after initiation of ART (Table 4).

Comparison of demographic data revealed no statistically significant differences in terms of gender, marital and familial status, but co-infected subjects were older (median age 44 versus 39 years, p= 0.038), and were more likely to reside outside of Phnom Penh or Kandal provinces (48% vs. 24%, p=0.005). Remarkably, HBV co-infection was more frequent in control subjects (16% vs. 3%, p=0.047).

Duration of HIV infection (i.e. time since diagnosis), frequency of ART pre-treatment before inclusion in the cohort, incidence of AIDS, baseline CD4 cell count, ART proportion and ART duration were similar in both groups. Clinical, immunological and virological therapeutic responses were also equivalent. At baseline and at the time of the study, ART regimens were comparable in the two populations (nevirapine, efavirenz, or protease inhibitors-based regimens).

Co-infected patients were more likely to have grade 2 ALT elevation at baseline (30% vs. 14%, p=0,017) and to develop grade 2 or more hepatocytolysis six months after ART initiation in case of nevirapine use (46% vs. 11%, p=0.08). In all cases, the likelihood to experience at least one episode of hepatic cytolysis grade 2 or 3 during the first two years of treatment was significantly higher in HIV/HCV co-infected patients (42% vs. 13%, p=0.004 for grade 2 and 29% vs. 8%, p=0.007 for grade 3).

Discussion

Among the 50 HIV infected patients with HCV positive antibodies, 62% had active HCV defined by positive plasma HCV PCR. This result

	HIV/HCV co-infected patients (N=31)	HIV mono-infected patients (N=160)	Crude OR	p value
Duration of HIV infection in months				
<60, N (%)	13 (46)	78 (53)	1	
60-90, N (%)	8 (29)	52 (35)	0.85 (0.31-2.31)	0.75
>90, N (%)	7 (25)	18 (12)	2.39 (0,75-7.62)	0.14
ART Pre-treated, N (%)	16 (53)	53 (34)	1.94 (0.82-4.62)	0.13
CDC stage C, N (%)	12 (40)	49 (32)	1.41 (0.63-3.18)	0.39
Median baseline CD4 cell count, cells/mm^3 (IQR)	171 (32-295)	113 (44-258)		0.58
<50, N (%)	10 (32)	46 (29)	1	
50-200, N (%)	9 (29)	64 (40)	0.63 (0.22-1.79)	0.39
>200, N (%)	12 (39)	50 (31)	1.17 (0.44-3.13)	0.75
ART treated at the time of the study (%)	28 (90)	150 (94)	0.40 (0.09-1.68)	0.21
NVP-based regimen	15 (54)	94 (64)	0.65 (0.27-1.56)	0.33
EFV-based regimen	7 (25)	26 (18)	1.61 (0.58-4.47)	0.36
PI-based regimen	6 (21)	25 (17)	1.28 (0.43-3.84)	0.66
ART duration in months				
<36, N (%)	9 (32)	32 (21)	1	
36-60, N (%)	9 (32)	37 (25)	0.75 (0.24-2.31)	0.62
>60, N (%)	10 (36)	81 (54)	0.34 (0.11-1.01)	0.053
Opportunistic infection upon ART, N (%)	6 (29)	24 (20)	1.88 (0.61-5.81)	0.27
Median latest CD4 cell count, cells/mm^3 (IQR)	372 (283- 472)	324 (227-422)		0.94
<200, N (%)	7 (22)	26 (16)	1	
200-350, N (%)	8 (26)	67 (42)	0.61 (0.18-2.03)	0.42
>350, N (%)	16 (52)	67 (42)	1.16 (0.38-3.58)	0.79
Median CD4 cell count gain under ART, cells / mm^3 (IQR)	160 (0-280)	183 (78-280)		0.51
Latest $\log_{10}$ HIV RNA>250 copies/ml, N (%)	3 (11)	17 (12)	0.86 (0.20-3.53)	0.83
WHO immunological failure*, N (%)	9 (29)	28 (17,5)	1.85 (0.73-4.70)	0.20
WHO clinical and/or immunological failure*, N (%)	14 (52)	49 (37)	2.00 (0.83-4.83)	0.12

IQR: Interquartile ratio; CDC: Center for Disease Control and Prevention; ART: Antiretroviral Therapy; EFV: Efavirenz; NVP: Nevirapine; PI: Protease Inhibitors; WHO: World Health Organization

*WHO definition of treatment failure: immunological failure = CD4 decrease to pre-therapy baseline or below, 50% fall from the on-treatment peak value, or persistent CD4 levels <100 cell/mm^3; clinical failure = new or recurrent WHO stage 4 or certain stage 3 conditions

Table 3: Comparison of HIV/HCV co-infected and HIV mono-infected patients adjusted for age, residence and HBV status, HIV course and ART response.

	HIV/HCV co-infected patients (N=31)	HIV mono-infected patients (N=160)	Crude OR	p value
Cytolysis grade 2* at baseline, N (%)	9 (30)	22 (14)	3.46 (1.25-9.60)	0.017
Cytolysis grade 2, 6 months after ART initiation, N (%)	6 (23)	14 (9)	2.94 (0.93-9.33)	0.067
NVP-based regimen	6 (46), N=13	6 (11), N=56	4.19 (0.84-20.80)	0.08
EFV- based regimen	0, N=13	8 (9), N=86		0.31
≥ 1 cytolysis grade 2 during the first two years of ART, N (%)	10 (42), N=24	18 (13), N=138	4.71 (1.66-13.36)	0.004
≥ 1 cytolysis grade 3** during the first two years of ART, N (%)	7 (29), N=24	10 (8), N=134	5.52 (1.59-19.16)	0.007

*ALT value>2.5x normal value

**ALT value>5x normal value

Table 4: Comparison of HIV/HCV co-infected and HIV mono-infected patients adjusted for age, residence and HBV status, cytolysis at baseline and on ART.

is lower than those reported in developed countries, which vary from 70 to 80%. Presence of false positive antibodies with EIA (Ortho HCV 3.0) [28] could explain this difference. Genotyping revealed genotype 1 predominance, followed by genotype 6. This pattern is similar to the distribution amongst Vietnamese blood donors [29] but differs from Thailand, where genotype 3 is predominant [30]. The latter discrepancy could be explained by the absence of intravenous drug users in our cohort.

Half of the 31 patients with active HCV infection had advanced hepatic fibrosis according to FibroTest results, most of them meeting at least one criterion of cirrhosis, although none were previously diagnosed with such disease. This finding is consistent with those reported in the French ANRS HC02 (Ribavic) study, where liver biopsies suggested bridging fibrosis or cirrhosis in 40% of patients [31]. Ideally, hepatic fibrosis should be evaluated by liver biopsy. However, the associated costs, risks and need for a trained examining pathologist render the procedure impractical in a resource-limited context. The FibroTest measurement of HCV biomarkers has been proven to have a prognostic value similar to that of liver biopsy, especially for severe fibrosis, even if several conditions, such as infection, inflammation or hemolysis, can invalidate its results [32-34]. Moreover, HIV infection does not seem to affect the performance of non-invasive markers of fibrosis [35], and in our study, the finding of severe fibrosis was significantly associated with the presence of at least one criterion for cirrhosis. Thus, implementing liver fibrosis blood tests and elastometry should be a valuable diagnostic contribution [32,34] in resource-limited settings may be essential.

This study is the first assessing virological data and liver disease evaluation among HIV/HCV co-infected patients in Cambodia. Some limitations deserve to be cited: patients seen in Calmette Hospital, a tertiary care center in the main town of Cambodia, may not be representative of those in the general community. Moreover, our small population size consisted only of patients whose HCV status was available, actually half of the entire cohort. Therefore, the estimated prevalence of HIV/HCV co-infection in our cohort is still unclear, as in the Cambodian population, and seroprevalence assays must be promoted further in the future. Although the seroprevalence survey performed in our cohort in 2007 was supposed to screen half of the patients without particular criteria, some additional patients may have been tested for HBV and HCV status because of liver dysfunction, which could have biased the study population.

Comparison between HIV/HCV co-infected patients and HIV mono-infected patients demographics in our study population indicated that co-infected patients were older and more likely to live outside Phnom Penh. The high frequency of unsafe therapeutic injections in Cambodia before the year 2000 [24] was suspected to be the cause of HCV epidemic infection, and could explain the disparities in age and geographic origin in this study. For that reason, potential confounding effects of those incomparable variables were adjusted in analysis. Further studies are needed to estimate the past and actual routes of HCV transmission in general population and PLWHA in Cambodia.

Amongst ART treated patients, HIV disease progression and clinical, immunological and virological responses were not different between the two populations. These results contradict findings from developed countries. Several studies suggest that active HCV infection affects immune restoration, even after years of exposure to ART [36,37]. Others studies indicate that mortality and increase in CD4 T-cell count do not differ during ART administration [38]. Confounding factors such as drug or alcohol use might be associated with poorer outcomes in co-infected patients in developed countries, but in this study no patients declared being intravenous drug users, and daily alcohol consumption was similar in HIV/HCV co-infected patients compared with HIV mono-infected patients.

After ART initiation, hepatocytolysis grade 2 and 3 occurred more frequently in HIV/HCV co-infected patients. However, the difference between the two groups was statistically significant mainly for nevirapine-containing regimens. Similarly, several studies in developed countries have revealed that chronic HCV infection is associated with an increased risk of drug-induced hepatotoxicity, especially with the use of nevirapine [9,39-41]. Nevirapine should probably be avoided in co-infected patients, and efavirenz should be the drug of choice whenever possible. However, a recent study in Cameroon suggests that this adverse event did not impact negatively on the effectiveness of treatment [42]. Moreover, ART slows down the progression of hepatic fibrosis and improves survival in co-infected patients [43], and hepatotoxicity of ART is reduced after successful treatment of HCV [44]. For these reasons, the control of HIV disease must be the ultimate priority in the management of co-infected patients.

Among the 31 co-infected patients, 21 (68%) presented indications for HVC treatment according to current French recommendations (Fibrosis score ≥ 2). None of these subjects demonstrated criteria for decompensated cirrhosis, suggesting that all patients with severe fibrosis were potentially treatable. HCV treatment in resource-limited settings is profoundly limited due to the prohibitive cost of the drugs and diagnostic tools, the need for cold-chain and the difficulties to manage the side effects. However, in the absence of treatment, these patients rapidly develop cirrhosis-related complications and are at increased risk of mortality from HCV infection. In Southeast Asia, increasing evidences support that HCV infected patients are more likely to achieve sustained virological response (SVR) than Caucasians with corresponding treatment regimen [45], probably because of the high proportion of genotype 6 [29,30] and of particular genetic polymorphisms of IL28B in East Asian population, which are both associated with high therapeutic success rate [46,47].

In conclusion, this study demonstrates the urgent need to develop HCV screening, as well as to increase access to virological assay and fibrosis assessment for HCV-infected individuals in developing countries, specifically among PLWHA, since liver disease progresses faster in these patients. Early therapeutic intervention should be implemented in affected individuals to prevent progression into decompensated cirrhosis and death. We expect that our preliminary data will lead to further investigations, in order to determine the feasibility and effectiveness of treatments of chronic HCV among PLWHA, in Cambodia and other resource-limited countries, and will help to improve health policies about management of HIV/HCV co-infection in these settings.

Authors Contributions

NL contributed to the study design, participant enrolment and follow-up and manuscript preparation. SL contributed to study design. IFN contributed to study design and data analysis. SL contributed to data analysis. JN and SK contributed to virological analysis. BG contributed to study design and FibroTest performance. AD, PK, JFD and VO contributed to study design. OS contributed to the study design, data analysis and manuscript preparation. All authors read and approved the final manuscript.

International Conflicts of Interest

JFD declared being board membership for BM, MSD and Gilead.

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