Hepatitis C Prevalence and Validation of a Clinical Prediction Score for Targeted Screening among People Living with HIV in Ghana

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Abstract
WHO recommends hepatitis C (HCV) screening for all people living with HIV (PLHIV). Yet, HCV coinfection was shown to be rare in some Sub-Saharan HIV cohorts, and targeted testing was suggested more efficient for such settings. We studied HCV prevalence among Ghanaian PLHIV, and assessed the external validity of a score to guide targeted testing. This score was initially derived from a Cambodian HIV cohort, and uses as predictors: age, household member/partner with liver disease, diabetes, generalized pruritus, AST, platelets, and AST-to-platelet ratio index. We enrolled 4,023 PLHIV, most from Greater Accra and Central regions, 28.4% were male, median age was 47 years, and high-risk behavior was reported to be rare. HCV seroprevalence was 0.57%, and HCV-RNA was detectable in 0.5%. Sequencing revealed genotype 1(b) and 2(q/r) infections. The discriminatory performance of the score was suboptimal in the Ghanaian setting. The area under the curve was 0.69 (95% CI 0.59-0.79). HCV coinfection prevalence was very low in this Ghanaian PLHIV cohort with reported low-risk of onward transmission. To avoid the cost of screening all PLHIV in similar cohorts in resource-constrained settings, further research to develop better tools/scores to guide targeted HCV testing is needed.

Keywords
hepatitis C prevalence, HIV, HCV/HIV coinfection, clinical prediction score, targeted screening, Ghana

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Background
Worldwide, hepatitis C virus (HCV) infection is among the leading causes of chronic liver disease. An estimated 71 million people are chronically infected with hepatitis C.1 This results currently in about 400,000 deaths each year, primarily due to liver cancer and cirrhosis caused by untreated infections.2 Hepatitis C is found worldwide, but prevalence is highly unevenly distributed within and between different countries, depending on whether the epidemic is mainly driven by population subgroups with high-risk behavior, or by more widespread exposure (such as iatrogenic routes or poor infection control).3 Low- and middle-income countries (LMIC) are estimated to account for about 75% of people living with HCV.4 Until a few years ago, hepatitis C treatment was centered around the use of pegylated interferon and ribavirin, and was considered too complex and poorly successful for wide-scale use. However, with the advent of direct-acting antivirals, hepatitis C treatment has been revolutionized. Its high cure rates

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What Do We Already Know about this Topic?

In a systematic review of 2016 the HCV/HIV coinfection prevalence in Ghana was estimated at 2.8% (95% CI 0.4-6%). Resources were insufficient to offer HCV testing as part of the standard package of care.

How Does Your Research Contribute to the Field?

We found a very low HCV prevalence (0.5% viremic) in this large, primarily heterosexual infected HIV cohort. The low number of HCV/HIV coinfections did not allow to firmly conclude on the external validity of an earlier developed score for targeted screening.

What Are Your Research’s Implications toward Theory, Practice, or Policy?

Depending on local resources and epidemiology, offering targeted HCV screening to people living with HIV with low onward risk of HCV transmission might be a better (more efficient) choice. However, further research is needed to develop (and validate) better performing risk-and symptom-based scores for targeted HCV screening.

Methods

Study Design and Setting

We conducted a single-center, cross-sectional study among adult PLHIV in care at the HIV clinic of the Fevers Unit of Korle Bu Teaching Hospital in Accra (Ghana). The hospital is the largest in Ghana with a bed capacity of 2,000 and a daily outpatient attendance of 1,500. The Fevers Unit houses the adult HIV clinic that serves as a national referral hub for PLHIV. The clinic does not target any specific subgroup of PLHIV. Key populations are expected to be a minority among those attending. Most did not yet know their HCV status at the time we started our study. Poor availability of hepatitis C treatment was the main reason for not offering HCV diagnostic testing as part of the routine care package. Availability of hepatitis C treatment is now slowly improving as direct-acting antivirals are becoming available, but still beyond reach for most patients.

Study Population

All adult PLHIV of the Fevers Unit cohort who reported for a routine follow-up consultation, and who were 1) on ART or yet to be initiated on ART, and 2) in regular HIV care follow-up, i.e. minimum of 2 HIV consultations in the last 6 months, and 3) never exposed to hepatitis C treatment, were offered participation in the study. They were enrolled in a consecutive...
manner, and without any other further selection until the sample size was reached. Enrolments took place between November 2018 and August 2019.

**Background Information on the Clinical Prediction Score Derived from the Cambodian Dataset**

Researchers from the Institute of Tropical Medicine Antwerp (also authors of this paper) and Sihanouk Hospital Center of Hope in Cambodia developed a CPS for targeted HCV screening based on a dataset of a large Cambodian HIV cohort. They used the Spiegelhalter-Knill-Jones method to develop the predictive model. The score included older age (>50 years), having a household member/partner with liver disease, presence of diabetes, having generalized pruritus, high AST-to-platelet ratio index (APRI, ≥0.45), and low platelet count (<200 × 10⁹ cells/L) as risk-increasing predictors; and low aspartate aminotransferase (AST, <30 IU/L) and low APRI (<0.45) as risk-lowering predictors. In the Cambodian derivation cohort, the score would have enabled identifying 85% of the coinfected while reducing the need for testing by 70%, and a score ≥0 fitted best as threshold-to-screen, with a negative predictive value of 99.2%.

**Sample Size and Power**

The primary objective of the study was to determine the HCV prevalence and to estimate the area under the ROC curve (AUROC) of the predictor score developed in Cambodia when it would be applied in the Ghanaian setting. The score had an AUROC of 0.84 in the Cambodian population. A sample size of 4,000 participants was calculated initially to estimate the AUROC to within 7.5% (half-width of the 95% confidence interval), assuming a prevalence of current HCV coinfecion (i.e. HCV RNA detectable) of 1.0% in Ghana. The paper of King et al was the only published study to provide an estimate on current HCV infection in PLHIV in Ghana at the time of preparing the study protocol.

**Subject-Related Procedures**

After providing informed consent, enrolled PLHIV were—in addition to the usual procedures of an HIV follow-up consult—asked about 1) current socio-demographics (formal/informal profession, area of residence), 2) risk factors/behavior for HCV (injecting drug use, commercial sex work, sexual orientation, past blood transfusions, surgery, tattoos, partner/household member with liver disease), 3) clinical symptoms possibly related to liver disease (generalized pruritus, fatigue, myalgia/arthralgia, anorexia/weight loss), and 4) comorbid conditions (alcohol use, diabetes mellitus, tuberculosis, hepatitis B, hypertension). For alcohol use, we asked questions on the average number of days per week alcohol was consumed, and the type and number of drinks.

The data collection for the study was complemented by specific findings of the physical examination (clinical signs of hepatic decompensation) and retrieval of routinely collected data from the medical file such as date of enrolment in the HIV cohort, date of start on ART, baseline CD4, last HIV viral load, hepatitis B surface antigen (HBsAg) result, and current ART regimen.

This part of the study procedures covered also the systematic data collection on 4 of the predictors used in the Cambodian CPS (age, diabetes mellitus, partner/household member with liver diseases, generalized pruritus). All data were collected without prior knowledge on the HCV status of the patient.

At the end of the consultation, after hepatitis C pre-test counselling was done blood was drawn for HCV diagnostic testing (HCV antibody test, followed by HCV viral load testing if the HCV antibody test showed a positive or borderline result), liver function tests (ALT, AST) and full blood count. The above laboratory results were used to calculate the commonly used compound surrogates of the degree of liver fibrosis: AST-to-platelet ratio index (APRI) and Fibrosis-4 (FIB-4). Cut-offs of >1.5 for APRI, and >3.25 for FIB-4 are commonly used as being indicative of advanced fibrosis. They are calculated as follows:

\[
\text{APRI} = \frac{\text{AST(} IU/L \text{)}}{\text{platelet count}(10^9/L)} × 100
\]

\[
\text{FIB-4} = \frac{\text{age(} \text{years}) × \text{AST}(IU/L)}{\text{platelet count}(10^9/L) × \sqrt{\text{ALT}(IU/L)}}
\]

**Laboratory Procedures**

HCV antibody, biochemistry and full blood count analyses were done by a team of experienced laboratory technicians trained in Good Clinical Laboratory Practices (GCLP) at the Central Laboratory of Korle Bu Teaching Hospital. HCV viral load testing was performed at the University Hospital of Antwerp, Belgium. All analyses were performed according to the manufacturer’s instructions.

- **HCV antibody screening**

  The SD Bioline HCV test (Standard Diagnostics, Republic of Korea) was used to detect anti-HCV antibodies. It is an *in vitro* immunochromatographic rapid assay for the qualitative detection of antibodies specific to HCV. The test is listed on the WHO pre-qualification list for in-vitro diagnostics. Serum samples were used in this study.

- **Biochemistry**

  Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined using the VITROS ALT Slide method on the VITROS 5600 Integrated System (Ortho Clinical Diagnostics, Rochester, NY, USA). Full blood count was performed on the SYSMEX Xi 2100 hematology automated analyzer (Sysmex Europe GmbH, Germany).
• HCV viral load (quantitative PCR) and genotyping

The quantification of HCV RNA was determined using the COBAS TaqMan HCV Quantitative Test, v2.0 on the COBAS TaqMan 48 Analyzer from Roche, with lower limit of detection of 15 IU/mL. This method is approved by the Food and Drug Authority (USA). Genotyping was performed by NS5B sequencing at the Belgian National HCV Reference Centre (a consortium of the Institute of Public Health and Université Catholique de Louvain).

External quality control was performed on a subset of samples in the laboratories of the Institute of Tropical Medicine Antwerp and the University Hospital of Antwerp, Belgium. HCV antibody re-testing was done for 5% of the negative and 100% of the positive samples.

Data Collection

Routine patient files and laboratory result print-outs served as source data for the study. Standard templates were used for each of those. These source data and the study data, obtained during the consultation immediately following enrolment, were used to complete a study-specific paper case report form (CRF). On daily basis, data were transferred from the paper CRF into a password-protected Epidata database (v4.4.2.1).

Data Analysis

Statistical analyses were performed using Stata 15.1 (StataCorp LP, College Station, TX, USA). Descriptive statistics, using medians and interquartile ranges (IQRs) for continuous variables and percentages for categorical variables, were used to summarize the baseline characteristics of the study participants, by HCV coinfection status. The information on alcohol use was resumed for each participant in average grams of alcohol consumed per week. An average alcohol use of more than 140g/week was considered as moderate to heavy alcohol intake.24

The HCV seroprevalence (i.e. proportion with an HCV-antibody positive result among all enrolled), and prevalence of current hepatitis C coinfection (i.e. proportion with a detectable HCV viral load among all enrolled) were determined and reported with Wilson 95% confidence intervals.

The Cambodian CPS,20,21 containing clinical and laboratory factors with a specific weight (age over 50 years: +1, diabetes mellitus: +1, APRI <0.45: −1, APRI ≥0.45: +1, AST <30 IU/L: −1, platelets <200 × 10^9 cells/L: +1, household/partner with liver disease: +1, generalized pruritus: +1), was applied in the Ghanaian cohort based on the data collected in the cross-sectional study. Summing the weighted predictor scores of the individual’s risk factors yielded the total predictor score for each study participant. Presence of current HCV infection (=HCV RNA detectable) was defined as the outcome.

The overall performance of the CPS was assessed by calculating the AUROC with 95% confidence intervals (CI).

Ethical Approval and Informed Consent

The study was approved in Belgium by the Institutional Review Board (IRB) of the Institute of Tropical Medicine of Antwerp (approval no. ITG 1216/18), the Ethical Committee of the Antwerp University Hospital (approval no. 18/20/249), and in Ghana by the IRB of Korle Bu Teaching Hospital (approval no. KBTH-IRB-00077/2018) and the IRB of Noguchi Memorial Institute for Medical Research (approval no. CPN 001/18-19). All participants provided written informed consent prior to enrolment in the study.

Results

Socio-Demographic and Clinical Characteristics of the Study Participants

Of the 4,023 participants enrolled, the majority resided in the Greater Accra region (82.3%, where the study was conducted and the national capital Accra is located), followed by the Central region (12.3%) and Eastern region (3.7%). Less than a third (28.4%) were male, and the median age of all enrolled was 47 years (IQR 41-55). Members of key population groups were few: only 6 participants identified as MSM; none reported past or current injecting drug use.

Most study participants (91.9%) were on first-line ART, the most used first-line regimens were tenofovir(TDF)/lamivudine(3TC)/efavirenz(EFV) (42.6%), zidovudine(AZT)/3TC/EFV (29.2%), AZT/3TC/nevirapine(NVP) (21.9%) and TDF/3TC/NVP (3.7%). As second-line ART regimens, TDF/3TC/lopinavir-ritonavir(LPVR) (52.6%), TDF/3TC/atazanavir-ritonavir(ATVr) (22.8%) were most frequently used. The median duration on ART was 7.4 years (IQR 3.7-10.8). Among those with available HIV viral load result less than 1 year before enrolment in the study (N = 2,235), 72.8% had an undetectable HIV viral load. Three-hundred nineteen participants (14.3%) had an HIV viral load above 1,000 copies/mL. Forty-two participants had an ongoing opportunistic infection at enrolment, mostly tuberculosis and/or oral/esophageal candidiasis. Of those with available HBsAg result as part of routine care, 13.6% (210/1548) had a reactive result. The median APRI of those with reactive HBsAg result was 0.36 (IQR 0.24-0.49). At enrolment, 6.8% of the study participants reported that they still consumed alcohol at least once per month, but only 5 participants consumed more than 140g/week (i.e. moderate to heavy alcohol use). Table 1 shows the details of the participant characteristics.

HCV prevalence

Twenty-three of the 4,023 participants tested positive for anti-HCV antibodies yielding a seroprevalence of 0.57% (95% CI 0.38-0.86). Of these, 20 had a detectable HCV RNA viraemia, corresponding to a prevalence of current HCV infection of 0.5% (95% CI 0.32-0.77). Five out of the 20 HCV coinfected participants had an APRI >1.5 and/or FIB4 >3.25, and thus were likely suffering from advanced liver disease. The prevalence of current HCV coinfection was similar among men
and women (0.4%) (p = 0.3). For 11 out of the 20 HCV coinfected participants genotyping results were obtained by NS5B sequencing, the genotype distribution was as follows: 1 (n = 4), 1b (n = 2), 2 q (n = 3) and 2r (n = 2).

Table 1. Characteristics of the Ghanaian HIV Cohort, by Coinfection Status.

| All enrolled N = 4023 | HIV without current HCV N = 4003 | HCV/HIV coinfected N = 20 |
|-----------------------|----------------------------------|---------------------------|
| Male sex (%)          | 1141 (28.4)                      | 1133 (28.3)               | 8 (40.0) |
| Age (years): median (IQR) | 47 (41-55)                      | 47 (41-55)               | 47.5 (37.5-55) |
| Past/current injecting drug use (%) | 0 (0.0)                      | 0 (0.0)                 | 0 (0.0) |
| Past/current commercial sex work (%) | 0 (0.0)                      | 0 (0.0)                 | 0 (0.0) |
| Men having sex with men (%) | 6 (0.5)                      | 6 (0.5)                 | 0 (0.0) |
| HIV care              |                                  |                          |            |
| Duration HIV care (years): median (IQR) | 8.2 (4.4-11.8)              | 7.3 (3.7-10.8)              | 10.0 (5.3-13.2) |
| CD4 nadir: median (IQR) | 205 (82-347)                    | 205 (82-347)             | 198 (113-362) |
| On ART (%)            | 401 (99.9)                      | 399 (99.9)               | 19 (100.0) |
| First line            | 368 (91.9)                      | 3669 (91.9)              | 17 (89.5) |
| Second line           | 325 (8.1)                       | 323 (8.1)                | 2 (10.5) |
| Duration on ART (years): median (IQR) | 7.4 (3.7-10.8)                 | 7.3 (3.7-10.8)            | 10.0 (5.3-13.1) |
| Laboratory results    |                                  |                          |            |
| HIV VL undetectable (%) | 1629 (72.8)                    | 1625 (72.9)              | 4 (44.4) |
| HCV VL (x 10^3 IU/mL): median (IQR) | NA                           | NA                      | 127 (4-1491) |
| CD4 (cells/mm^3): median (IQR) | 513 (338-712)                  | 513 (337-712)            | 596 (401-706) |
| ALT (IU/L): median (IQR) | 17 (12-25)                     | 17 (12-24)               | 41 (20-67.5) |
| AST (IU/L): median (IQR) | 24 (19-31)                     | 24 (19-31)               | 42 (26-66) |
| Hemoglobin (g/dL): median (IQR) | 12 (11-13)                    | 12 (11-13)               | 12 (11-13) |
| Platelets (x10^11 cells/L: median (IQR) | 233 (194-278)                 | 233 (194-278)            | 232 (162-289) |
| APRI: median (IQR)    | 0.17 (0.85-1.64)               | 1.17 (0.85-1.64)         | 1.26 (0.78-2.21) |
| Symptoms/signs        |                                  |                          |            |
| Fatigue/weakness (%)  | 143 (3.6)                      | 143 (3.6)                | 0 (0.0) |
| Diffuse pruritus (%)  | 144 (3.6)                      | 143 (3.6)                | 1 (5.0) |
| Myalgia/arthritis (%) | 113 (2.8)                      | 113 (2.8)                | 0 (0.0) |
| Anorexia/weight loss (%) | 175 (4.4)                     | 175 (4.4)                | 0 (0.0) |
| Abdominal pain/nausea (%) | 90 (2.2)                     | 90 (2.3)                 | 0 (0.0) |
| Sensory neuropathy (%) | 466 (11.6)                    | 466 (11.7)               | 0 (0.0) |
| Current comorbidities/conditions |                                  |                          |            |
| HBsAg positivity (%) | 210 (13.6)                      | 210 (13.6)               | 0 (0.0) |
| Diabetes mellitus (%) | 126 (3.1)                      | 125 (3.2)                | 1 (5.3) |
| Arterial hypertension (%) | 913 (23.0)                     | 908 (23.0)               | 5 (25.0) |
| Alcohol use >140 g/week (%) | 5 (0.13)                     | 5 (0.13)                 | 0 (0.0) |
| Tuberculosis (%)      | 13 (0.3)                       | 13 (0.3)                 | 0 (0.0) |
| Past comorbidities/conditions |                                  |                          |            |
| Alcohol >140g/week (before enrolment HIV care, %) | 63 (1.6)                  | 63 (1.6)                 | 0 (0.0) |

Abbreviations: ART (antiretroviral treatment), VL (viral load), ALT (alanine aminotransferase), AST (aspartate aminotransferase), APRI (AST-to-platelet ratio index), FIB-4 (fibrosis-4), HBs Ag (hepatitis B surface antigen).

Missing values were less than 5% except for 1) HIV viral load results within 1 year of study enrolment, and 2) hepatitis B surface antigen.

1 For HIV viral load, 1775 participants without HCV coinfection, and 11 with HCV coinfection have missing results.

2 For HBsAg, 2462 participants without HCV coinfection, and 14 with HCV coinfection have missing results.

Discriminatory Performance of the Cambodian Clinical Prediction Score in the Ghanaian HIV Cohort

The distribution of the individual predictors included in the Cambodian CPS is presented in Table 2.

When applying the CPS, the total individual scores in the Ghanaian HIV cohort range from −2 to +4 (see Table 3). Only 36% of the PLHIV in the Ghanaian cohort had a total score ≥ 0 (i.e. at the cut-off that best fitted as threshold-to-screen in Cambodia), including 13 of the 20 HCV/HIV coinfected people. The AUROC of the CPS applied in the Ghanaian cohort was 0.69 (95% CI 0.59-0.79) (Figure 1) compared to 0.84 (95% CI 0.80-0.89) in the Cambodian derivation cohort.

Discussion

In our study among PLHIV, predominantly from the Greater Accra and Central regions in Ghana, the HCV seroprevalence and prevalence of current HCV coinfection was 0.57% and 0.5% respectively. These estimates range the lower end of the confidence interval of the 2.8% (95% CI 0.4-6.0) pooled
estimate for HCV/HIV coinfection prevalence obtained by a systematic review of Ghanaian data published up to end 2015. When this systematic review was compiled, only 3 studies had reported data about PLHIV, of which one described coinfection data for less than 50 PLHIV as the study was not designed for that purpose. The other 2 reported quite diverging results on larger sample sizes. King et al reported, similar to the estimate we found, a prevalence of current HCV coinfection of 1.0% (95% CI 0.0-1.9) among PLHIV from the Ashanti region. In the other study, from Sagoe et al, the seroprevalence was higher (3.6%) and measured among ART-naïve PLHIV in 2007 at the Korle Bu Fevers Unit in Accra. Possible explanations, for the difference in prevalence found in 2007 and our study (2018-2019) in the same HIV clinic, might be the different types/generations of antibody tests used (later generations being more specific), or excess mortality among HCV/HIV coinfe...
Our findings suggest moreover also that there is no excess risk of HCV infection in PLHIV in this region compared to the HIV uninfected population. Seroprevalence among blood donors in and around Accra was reported to be 0.9% \(^5\)\(^{33}\) Hence, screening all PLHIV for HCV infection (as recommended by the WHO)\(^9\) in the Ghananian setting may not be cost-effective, and targeted screening of a subgroup of PLHIV with higher risk or suggestive symptoms could be considered as alternative strategy, in case resources are insufficient to offer testing for all PLHIV. However, such targeted screening strategy can only work if low-cost, easy-to-apply tools are available to guide identification of those at greater risk of HCV coinfection.

We intended to assess the discriminatory performance of a score for targeted HCV screening that was developed based on data from a Cambodian HIV cohort where overt high-risk groups for HCV, such as PWID or MSM, were also rare.\(^{20}\) As the number of participants with current HCV infection was however very low (n = 20), we were not able to obtain a robust estimate of the score performance in this external cohort. The possibility for further exploration of other factors associated with HCV infection in the Ghananian setting was limited due to this low number of participants with the outcome event, and the lack of complete data on other diseases that may frequently cause chronic liver disease, such as hepatitis B. The AUC of the Cambodian score applied in the Ghanaian cohort was 0.69, so potentially of some use, but difficult to interpret because of the wide confidence interval. Applying the score cut-off (≥ 0) as had been proposed for Cambodia, 36% would have needed screening and 13 out of 20 coinfections would have been found, if the score would have been applied once. Our study did not provide data to evaluate whether the missed cases would have been picked up timely if the score would be applied on yearly basis. In contrast to the findings in Cambodia, we did not see a birth-cohort (older age) effect in the Ghanaian cohort. In absence of a well-performing validated score, clinical alertness focused on signs/symptoms compatible with (chronic) liver disease (such as increased ALT or AST, increased APRI, clinical symptoms compatible with chronic liver disease) may be the best available guide for targeted screening in such settings.

**Conclusion**

Hepatitis C coinfection prevalence was very low among this cohort of Ghanaians from the Greater Accra and Central regions in whom HIV was largely heterosexually-acquired. The performance of a clinical prediction score for targeted HCV testing, originally derived from data of a Cambodian HIV cohort, was suboptimal in the Ghanaian setting. In order to identify PLHIV at risk, tools that are tailored to local context and disease epidemiology are needed for targeted HCV testing.

**Authors’ Note**

KT, ADW, JB and LL conceptualized the study. KT, ADW, LO, VC, SA, AA-N, JOC oversaw data collection. ADW analyzed the data, KT, ADW, LO, VC, SA, AA-N, JOC and LL drafted the manuscript. KT, ADW, LO, JB and LL provided critical input in the development and revision of the manuscript. All authors read and approved the final manuscript. The datasets used and analyzed in this study are available from the corresponding author upon request. Personal identifiers were excluded from the data set before analyses were performed. We therefore did not report any individual identifier. The study was approved by the Institutional Review Board of the Institute of Tropical Medicine in Antwerp, the Ethical Committee (EC) of the Antwerp University Hospital, the Institutional Review Board of Korle Bu Teaching Hospital, and the Noguchi Memorial Institute for Medical Research Institutional Review Board (NMIMR-IRB). Only patients who provided written informed consent for study participation were enrolled.

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**Declaration of Conflicting Interests**

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