# . Deriving the optimal limit of detection for an HCV point-of-care test for viraemic infection: Analysis of a global dataset

Authors	Freiman, JM; Wang, J; Easterbrook, PJ; Horsburgh, CR; Marinucci, F; White, LF; Kamkamidze, G; Krajden, M; Loarec, A; Njouom, R; Nguyen, KV; Shiha, G; Soliman, R; Solomon, SS; Tsertsvadze, T; Denkinger, CM; Linas, B
Publisher	Elsevier
Journal	Journal of Hepatology
Rights	With thanks to Elsevier.
Download date	03/10/2021 17:03:34
Link to Item	http://hdl.handle.net/10144/619469

Deriving the optimal limit of detection for an HCV point-of-care test for viraemic infection: Analysis of a global dataset

Graphical abstract
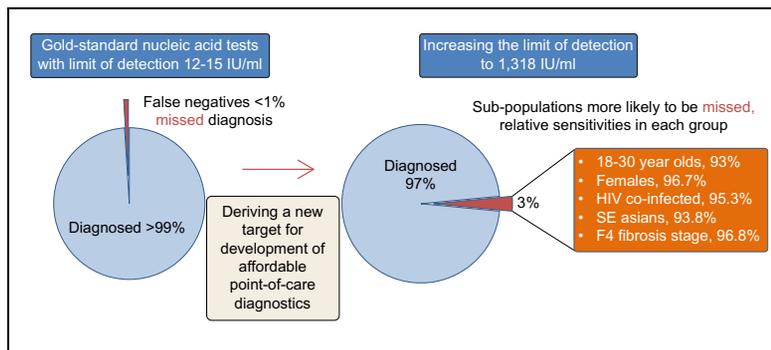
Highlights

- >97% of those with chronic hepatitis C virus have viraemia >1,318 IU/ml.

- Low-level viraemia among 66,640 individuals did not vary significantly by genotype.

- The sensitivity of HCV diagnostic tests was maintained even when increasing the detection limit by 100×.

Authors

J. Morgan Freiman, Jianing Wang, Philippa J. Easterbrook, ..., Tengiz Tsertsvadze, Claudia M. Denkinger, Benjamin Linas

Correspondence

j.morgan.freiman@bmc.org
(J.M. Freiman) Benjamin.linas@bmc.org
(B. Linas)

Lay summary

We created and analysed a dataset from 12 countries with 66,640 participants with chronic hepatitis C virus infection. We determined that about 97% of those with viraemic infection had 1,300 IU/ml or more of circulating virus at the time of diagnosis. While current diagnostic tests can detect as little as 12 IU/ml of virus, our findings suggest that increasing the level of detection closer to 1,300 IU/ml would maintain good test accuracy and will likely enable development of more affordable portable tests for use in low- and middle-income countries.

Deriving the optimal limit of detection for an HCV point-of-care test for viraemic infection: Analysis of a global dataset

J. Morgan Freiman[1,*], Jianing Wang[1], Philippa J. Easterbrook[5], C. Robert Horsburgh[2,3,4], Francesco Marinucci[6], Laura F. White[2], George Kamkamidze[7], Mel Krajden[8], Anne Loarec[9], Richard Njouom[10], Kihn V. Nguyen[11], Gamal Shiha[12,13], Reham Soliman[13], Sunil S. Solomon[14,15], Tengiz Tsertsvadze[7], Claudia M. Denkinger[6,†], Benjamin Linas[1,3,*,†]

[1]Boston Medical Center, Section of Infectious Diseases, Boston University School of Public Health, Boston, USA; [2]Department of Biostatistics, Section of Infectious Diseases, Boston University School of Public Health, Boston, USA; [3]Department of Epidemiology, Section of Infectious Diseases, Boston University School of Public Health, Boston, USA; [4]Global Health, Section of Infectious Diseases, Boston University School of Public Health, Boston, USA; [5]Global Hepatitis Programme, World Health Organization, Geneva, Switzerland; [6]FIND, Geneva, Switzerland; [7]Georgia Hepatitis C Elimination Program, Tbilisi, Georgia; [8]British Columbia Centre for Disease Control, Vancouver, Canada; [9]Epicentre, Medecins Sans Frontières, Paris, France; [10]Centre Pasteur of Cameroon, Yaoundé, Cameroon; [11]National Hospital of Tropical Diseases, Hanoi, Viet Nam; [12]Department of Internal Medicine, University of Mansoura, Egypt; [13]Egyptian Liver Research Institute and Hospital, Mansoura, Egypt; [14]YR Gaitonde Centre for AIDS Research and Education, Chennai, India; [15]Johns Hopkins University School of Medicine, Baltimore, USA

Background & Aims: Affordable point-of-care tests for hepatitis C (HCV) viraemia are needed to improve access to treatment in low- and middle-income countries. Our aims were to determine the target limit of detection (LOD) necessary to diagnose the majority of people with HCV eligible for treatment, and identify characteristics associated with low-level viraemia (LLV) (defined as the lowest 3% of the distribution of HCV RNA) to understand those at risk of being misdiagnosed.

Methods: We established a multi-country cross-sectional dataset of first available quantitative HCV RNA measurements linked to demographic and clinical data. We excluded individuals on HCV treatment. We analysed the distribution of HCV RNA and determined critical thresholds for detection of HCV viraemia. We then performed logistic regression to evaluate factors associated with LLV, and derived relative sensitivities for significant covariates.

Results: The dataset included 66,640 individuals with HCV viraemia from across the world. The LOD for the 95th and 99th percentiles were 3,311 IU/ml and 214 IU/ml. The LOD for the 97th percentile was 1,318 IU/ml (95% CI 1,298.4–1,322.3). Factors associated with LLV, defined as HCV RNA <1,318 IU/ml, were younger age 18–30 vs. 51–64 years (odds ratios [OR] 2.56; 95% CI 2.19–2.99), female vs. male sex (OR 1.32; 95% CI 1.18–1.49), and advanced fibrosis stage F4 vs. F0-1 (OR 1.44; 95% CI 1.21–1.69). Only the younger age group had a decreased relative sensitivity below 95%, at 93.3%.

Conclusions: In this global dataset, a test with an LOD of 1,318 IU/ml would identify 97% of viraemic HCV infections among almost all populations. This LOD will help guide manufacturers in the development of affordable point-of-care diagnostics to expand HCV testing and linkage to care in low- and middle-income countries.

Lay summary: We created and analysed a dataset from 12 countries with 66,640 participants with chronic hepatitis C virus infection. We determined that about 97% of those with viraemic infection had 1,300 IU/ml or more of circulating virus at the time of diagnosis. While current diagnostic tests can detect as little as 12 IU/ml of virus, our findings suggest that increasing the level of detection closer to 1,300 IU/ml would maintain good test accuracy and will likely enable development of more affordable portable tests for use in low- and middle-income countries.

Introduction

Globally, viral hepatitis is responsible for 1.34 million deaths[1,2] and more than 50 million of the estimated 70 million cases of chronic hepatitis C virus (HCV) occur in low and middle-income countries (LMICs).[3] The World Health Organization (WHO) defined goals towards the elimination of viral hepatitis as a public health threat, with a 90% reduction in new infections, and a 65% reduction in mortality by 2030.[1,4] Achievement of these targets requires scale-up of access to affordable testing and treatment alongside interventions for HCV prevention (harm reduction and safe blood donation and injections).[5] Progress in treatment scale-up is encouraging with more than 3 million treated with direct-acting antivirals since 2015, however, testing coverage and diagnosis rates are still less than 10% in LMICs.[6]

Approximately 15–45% of people infected with HCV will spontaneously clear the virus[7,8] and therefore confirmation of HCV viraemia is necessary to identify those needing treatment. The standard diagnostic algorithm recommended by the WHO includes an initial HCV antibody test followed by confirmatory testing for viraemia with either a nucleic acid test (NAT) for HCV RNA or core antigen (HCVcAg) where RNA tests are not available.[9–11] High proportions of those with positive antibody fail to have confirmatory testing and are never linked to treatment.[12,13] Further, available tests for viraemia are expensive, and require advanced laboratory facilities, electricity, water, and refrigerated reagents. Few LMICs have testing policies or the requisite laboratory infrastructure in place[13–15]

Innovations in testing technology, and research to inform optimal implementation strategies for HCV in LMICs are needed.[9,16,15] A rapid, affordable, easy-to-use test for confirmation of HCV viraemia at the point-of-care (POC) that can be deployed on a large scale has the potential to improve outcomes across the diagnosis and care continuum, particularly in high HCV prevalence settings.[16–18]

Presently, there are no data to determine a limit of detection (LOD) for WHO prequalification criteria for a POC HCV viraemia test. Thus, POC tests are held to the same standards as laboratory-based NATs. The laboratory-based Abbott RealTime HCV viral load test, for example, is able to detect and measure HCV RNA down to 12 international units per milliliter (IU/ml) with >99% sensitivity; similarly, the Roche COBAS®TaqMan® HCV Test reports an LOD of 15 IU/ml.[19] The laboratory-based Abbott ARCHITECT HCVcAg test has an LOD corresponding to 3,000 IU/ml with 93.4% sensitivity.[20] Requiring POC assays to achieve the same prequalification criteria as laboratory assays may limit the ability to expand HCV testing and treatment in LMICs. The POC Genedrive® HCV assay, however, acquired European *in vitro* diagnostics approval this year with an LOD of 2,362 IU/ml,[21] but is not yet WHO prequalified. Additionally, Cepheid Xpert® HCV Viral Load finger-stick assay detects as little as 40 IU/ml and can utilise consolidated near-patient Xpert platforms or the POC Omni version.[22]

A consensus target product profile in 2017 outlined price targets and operational characteristics for a near-patient HCV viraemia test[18] including but not limited to: a minimal LOD of 1,000–3,000 IU/ml, minimal test sensitivity of 95%, test cost <$15 though ideally <$5, and instrument cost <$20,000 but ideally <$2,000. Currently, available platforms struggle to meet these price targets. Data are needed to estimate the clinical sensitivity of the potential minimum LOD recommendations. Integrating NAT data outlined above with the goals from the target product profile, we hypothesise that a POC assay with an LOD of 1,000 IU/ml (3 log IU/ml) would have >97% clinical sensitivity for confirming HCV viraemia. A single-step POC test would allow for accessible, low-cost testing of viraemia without loss to follow-up in LMICs, despite having a lower analytical sensitivity.[18,23,24] Our objective is to determine the requisite LOD for an affordable POC assay to diagnose the majority of people with chronic HCV, and to identify characteristics of those with low-level viraemia (LLV) who might be missed by a less sensitive test.

Patients and methods
Study design
We assembled a cross-sectional dataset of the first available HCV RNA measurement for HCV antibody positive persons with viraemia from high, moderate, and low HCV prevalence settings in 12 countries (Cambodia, Cameroon, Canada, Egypt, Georgia, India, Indonesia, Malaysia, Mozambique, Pakistan, Thailand, and Vietnam) with representation of the 6 major HCV genotypes, a broad range of liver fibrosis stages, and varying prevalence of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) co-infection. To test our hypothesis, we analyzed the distribution of HCV RNA at the time of diagnosis, and performed bivariate and multivariable analyses to identify demographic and clinical characteristics associated with LLV, defined as those in the lowest 3% of the distribution of HCV viral load (*i.e.*, those missed by a test with 97% clinical sensitivity).

We performed a cross-sectional analysis of initial HCV viral load data and linked demographic (age, sex, country of testing) and clinical (HIV and HBV co-infection, HCV genotype, fibrosis stage) data collected between January 1, 2007 and June 1, 2017. We included males and females of all ages with detectable quantitative HCV RNA. We excluded participants with missing age or sex demographics and those on HCV treatment. We grouped countries by WHO regions: African, Americas, Eastern Mediterranean, European, South East Asia, and Western Pacific.

Data sources
We identified potential patient cohorts for inclusion from 2 main sources: the WHO global hepatitis programme contacts database that includes implementing partners and international HCV researchers, and a PubMed literature search using the search terms "HCV RNA quantification" and "cohort study" to identify additional cohorts of people with HCV infection. Criteria for potential inclusion in this analysis were available HCV RNA quantification linked to comprehensive demographic and clinical data among populations outside of the United States. We contacted study authors and established a working group with all respondents who agreed to share data. Fig. S1 shows a flow-chart of contributing sites and countries, and Table 1 summarises characteristics of the source data, including: HCV epidemiology of the country or region of origin (prevalence, population affected, World Bank country classification), patient inclusion and exclusion criteria if from a research cohort, and reason for HCV testing.

We determined the main source of patient samples and basis for HCV testing from study protocols or direct communication with collaborators. Reasons for testing were categorised as: i) targeted among specific high-risk populations (people who inject drugs, birth cohorts, healthcare workers), or ii) clinically indicated (*i.e.* testing of those with clinical signs or symptoms or laboratory features suggestive of hepatitis), or iii) routine as part of large-scale screening programmes (*i.e.* antenatal clinics, blood donors, seroprevalence surveys). We included data from 1 large reference laboratory in a high-income setting (Canada) as a comparison to LMICs.

Data concatenation
We predefined a protocol for data concatenation. We outlined categories and associated dummy variables for all categorical variables (age, sex, country, WHO region, HCV genotype, fibrosis stage). HCV viral load measurements at each site were reported in IU/ml. Sample specifics (serum or plasma) and platform used for quantification were collected where available. We determined fibrosis stage either from transient elastography (Fibroscan®) results reported in Metavir stage, or calculated

Table 1. Data source characteristics.

Site, location, region	Dates of collection	Country HCV epidemiology,[3,39] economy[40]	Sample size, population	Testing purpose*	Inclusion/exclusion criteria and cohort notes
Centre Pasteur, Yaoundé, Cameroon, African	2010–2016	Viraemic prevalence: 0.7% Pop. Infected: baby boomers, iatrogenic Genotype Distribution: G1 44.8%, G2 24.3%, G4 30.7% Economy: Lower-middle	4,861, Specialty clinics	Clinical	
British Columbia Center for Disease Control Hepatitis C Testers Cohort (BC-HTC), Vancouver, Canada, Americas	Jan. 2007–Dec. 2016	Viraemic prevalence: 0.6% Pop. Infected: IDU, ex-IDU, iatrogenic, unknown. Incident infections are occurring in PWID, males 2× more likely than females Genotype Distribution: G1 50.3%, G2 15.4%, G3 22.3%, G4 2.3% Economy: High	27,448, General	Reference laboratory	BC-HTC includes data for >95% of all individuals tested for HCV in the province of British Columbia. Data is collected and merged from the province public health laboratory[41]
Egyptian Liver Research Institute and Hospital, Mansoura, Egypt, Eastern Mediterranean		Viraemic prevalence: 6.3–14.7% Pop. Infected: general, iatrogenic Genotype Distribution: G1 3.8%, G3 0.8%, G4 93.1% Economy: Lower-Middle	1,063, General		
Georgia HCV Elimination Program, Tbilisi, Georgia, European	April 2015–May 2017	Viraemic prevalence: 4.2–7.7% Pop. Infected: IDU, iatrogenic transmission, 50% of prison population Genotype Distribution: G1 61%, G2 11.0%, G3 27% Economy: Lower-Middle	29,568, General	Mixed: targeted, routine, clinical	The Georgia HCV Elimination Program is a partnership between the Georgia Ministry of Health, the US Centers for Disease Control, and Gilead Sciences[42–44]
Médecins Sans Frontières (MSF), 1. Cambodia, Western Pacific 2. Mozambique, African 3. Pakistan, Eastern Mediterranean	Sept.–Dec 2016	Cambodia Viraemic prevalence: 2.3% Pop Infected: IDU, MSM, iatrogenic Genotype Distribution: G1 24.0%, G3 20.0%, G6 56.0% Economy: lower-middle Mozambique Viraemic prevalence: no data Genotype Distribution: no data Economy: low Pakistan Viraemic prevalence: 3.8–6.7% Pop. Infected: IDU, iatrogenic Genotype Distribution: G1 10.9%, G2 3.8%, G3 79%, G41.6%, G5 0.1%, G6 0.1%, Mixed 8.3% Economy: lower-middle	Cambodia: 1737, general Mozambique: 13, HIV infected Pakistan: 1293, general	Targeted	Cambodia & Mozambique: Observational cohorts. Inclusion: ≥18 years old, detectable HCV RNA, able to provide written informed consent Pakistan: Retrospective analysis of operational data. Inclusion: ≥18 years old, detectable HCV RNA
TREAT Asia, Kirby Institute Jakarta, Indonesia; Kuala Lumpur, Malaysia; Bangkok, Thailand; Hanoi, Vietnam South East Asia	Dec 2013-Jan 2015	Viraemic prevalence: Region = 0.7–1.2% Pop. Infected: IDU, MSM, iatrogenic Genotype Distribution: G1 35.2%, G2 11.1%, G3 19.9%, G4 0.9%, G5 0.4%, G6 30.8%, Mixed 1.7% Economy: Indonesia – Lower-Middle Malaysia – Upper-Middle Thailand – Upper-Middle Vietnam – Lower-Middle	413, HIV infected	Targeted	Inclusion: HIV-infected patients under care at participating sites. Detectable HCV antibody within 6 months of enrollment. Exclusion: <18 years old, CD4 count <200, Child-Pugh score >A, ascites, encephalopathy, bleeding esophageal varices, liver cancer, pregnant, breastfeeding or the male partner of a pregnant female[45]

Table 1 (*continued*)

Site, location, region	Dates of collection	Country HCV epidemiology,[3,39] economy[40]	Sample size, population	Testing purpose[*]	Inclusion/exclusion criteria and cohort notes
Y.R. Gaitonde Centre for AIDS Research and Education, Chennai, India, South East Asia	2010–2016	Viraemic prevalence: 0.5% Pop. Infected: IDU, iatrogenic Genotype Distribution: G1 24.0%, G3 54.4%, G4 5.8%, G5 0.2%, Mixed 15.6% Economy: Lower-middle	5,476, IDU, MSM, HIV infected	Mixed: targeted and routine	Four study cohorts: 1. Respondent-driven sampling strategy used for recruitment. Inclusion: >18 years old, self-report of IDU in prior 2 years, provide informed consent, valid referral coupon.[46] 2. Inclusion: >18 years old, provide written informed consent, report IDU in prior 6 months.[47] 3. CDOT[48] 4. Inclusion: >18 years old, provide informed consent, self-reported history of IDU in prior 5 years, no intention of migrating for 2 years during study period[49]

Including site, country, World Health Organization region, dates of sample collection, country HCV epidemiology and World Bank economy classification, sample size, sample population, testing purpose[*] (targeted, clinical, routine, reference laboratory, mixed), and enrolment inclusion and exclusion criteria (for research cohorts).

Pop = Population; IDU = injection drug user(s); G1 = genotype 1; G2 = genotype 2; G3 = genotype 3; G4 = genotype 4; G5 = genotype 5; G6 = genotype 6; US = United States; HIV = human immunodeficiency virus; AIDS = acquired immunodeficiency syndrome.

[*]1) Targeted among specific high-risk populations (injection drug users, birth cohorts, healthcare workers), 2) clinical (testing of those with clinical signs or symptoms or laboratory features suggestive of hepatitis), 3) and routine from large-scale screening (antenatal clinics, blood donors, seroprevalence surveys).

from Fibrosis-4 score[25,26] as these scores correlate well with Fibroscan.[27–29] For those with a Fibrosis-4 score <1.45, we assigned Metavir stage F0-F1. For scores between 1.45 and 3.25, we assigned stage F2-F3, and scores above 3.25 we assigned to stage F4.

Statistical analysis

We employed descriptive statistics to derive the HCV viral load distribution in $\log_{10}$ IU/ml for initial HCV RNA among all patients in the combined dataset. From this distribution, we identified the LOD levels of HCV RNA in IU/ml corresponding to the 95th, 97th, and 99th percentiles, *i.e.* the level of HCV RNA below which the infection would be missed. To estimate the 95% CI for each LOD, we performed bootstrap and Markov chain Monte Carlo method[30] to randomly simulate a population of 10,000 patients from the total dataset. We then calculated the LOD at the 95th, 97th, and 99th percentiles from this sample population, and repeated the procedure 10,000 times to obtain an LOD range for each percentile. We then calculated the 95% CIs from these sample distributions.

We defined LLV as HCV RNA in the lowest 3% of the distribution, below the LOD corresponding to the 97th percentile determined above. We then calculated summary statistics for covariates of interest and tested associations between each covariate and the odds of having LLV. We used a step-wise approach to construct a multivariable logistic regression model of the odds of LLV. Covariates remained in the multiple logistic regression model when their *p* value was ≤0.05 and we found no substantial multicollinearity. We tested for effect modification with predefined stratified analyses: i) HIV subset analysis, ii) HBV subset analysis, iii) fibrosis stage with transient elastography data only. We also assessed the effect of varying the fibrosis classification thresholds of the Fibrosis-4 score. First, we shifted the cut-offs toward F4: scores <1.20, we assigned stage F0-F1, 1.20 to 3.0 stage F2-F3, and scores >3.0 stage F4. We then shifted the cut-offs away from F4: scores <1.60 stage F0-F1, 1.60 to 3.45 stage F2-F3, and scores >3.45 stage F4.

Next, we performed data imputation for the missing exposures of interest (HIV co-infection, HBV co-infection, HCV genotype, fibrosis stage) to create a dataset for sensitivity analyses. We employed parametric regression imputation with a prediction model to impute the missing values for fibrosis stage.[31] We utilised prevalence data specific to each country for HCV genotype, HIV and HBV co-infection, and imputed missing data for these variables within each country cohort. For example, we used the genotype distributions described in each country as the probabilities of having each genotype, we then employed Markov chain Monte Carlo techniques to stochastically assign a genotype to each individual. We used the same method adapting country and sex specific HIV and HBV prevalence data.

We then used the imputed dataset to test associations between each covariate and the odds of having LLV with bivariate and multivariable logistic regression, and compared the results with the non-imputed total population dataset. Finally, we quantitatively compared the performance of the LOD from the total population dataset among the covariates with significant associations with LLV in the imputed dataset by deriving the relative percentiles from HCV RNA distributions for subsets from each significant covariate. We used R version 1.0.136 to perform all statistical analyses.

Results

Dataset characteristics

The dataset included 66,640 individuals with HCV viraemia from Cambodia (2.6%), Canada (40.9%), Cameroon (0.4%), Egypt (1.6%), Georgia (44.4%), India (8.1%), Indonesia (0.2%), Malaysia (0.05%), Mozambique (0.02%), Pakistan (1.3%), Thailand (0.2%), and Vietnam (0.1%) (Fig. S1). Table 1 contains data source characteristics and summarises country-level HCV prevalence data and genotype distribution.

Characteristics for the total population cohort (TPC) are presented in Table 2. Females comprised 24.4% (16,320) of participants with a median age of 48 years. Among those also tested for HIV (54.3%) and HBV (50.7%), 10.9% (3,945) were HIV co-infected, and 21.4% (7,221) were HBV co-infected. We identified the HCV genotype distribution as follows: 40.9% (27,245) genotype 1, 13.9% (9,287) genotype 2, 22.7% (15,157) genotype 3, 3.0% (2,030) genotype 4, <1% (13) genotype 5, 1.3% (889) genotype 6, <1% (170) with mixed genotype, and 17.8% (11,849) with

Table 2. Characteristics of 66,640 participants in a combined cross-sectional dataset.

Variable	Total cohort N (Col%)	Low-level viraemia[*] n (Row%)	OR (95% CI)	aOR[1] (95% CI)
Age				
<18	75 (0.1)	4 (5.3)	2.39 (0.73–5.79)	1.73 (0.52–4.22)
18–30	5,883 (8.8)	396 (6.7)	**3.06 (2.68–3.49)**	**2.56 (2.19–2.99)**
31–50	31,724 (47.6)	962 (3.0)	**1.33 (1.19–1.48)**	**1.30 (1.16–1.45)**
51–64	24,173 (36.3)	556 (2.3)	Ref	Ref
≥65	4,785 (7.2)	84 (1.8)	**0.76 (0.60–0.95)**	**0.75 (0.59–0.94)**
Sex				
Female	16,320 (24.4)	526 (3.2)	1.1 (1.00–1.22)	**1.32 (1.18–1.49)**
Male	50,320 (75.5)	1,476 (2.9)	Ref	Ref
Country				
Cambodia	1,730 (2.6)	34 (1.9)	**0.66 (0.46–0.92)**	
Cameroon	293 (0.4)	3 (1.0)	**0.34 (0.08–0.89)**	
Canada	27,277 (40.9)	805 (3.0)	Ref	
Egypt	1,063 (1.6)	5 (0.5)	**0.16 (0.06–0.34)**	
Georgia	29,569 (44.4)	780 (2.6)	**0.89 (0.81–0.98)**	
India	5,430 (8.1)	360 (6.6)	**2.33 (2.05–2.65)**	
Indonesia	141 (0.2)	2 (1.4)	0.47 (0.08–1.49)	
Malaysia	34 (0.05)	1 (2.9)	1.51 (0.08–7.35)	
Mozambique	13 (0.02)	1 (7.7)	4.16 (0.23–22.02)	
Pakistan	854 (1.3)	11 (1.3)	**0.43 (0.22–0.74)**	
Thailand	142 (0.2)	1 (0.7)	0.23 (0.01–1.04)	
Vietnam	94 (0.1)	1 (1.1)	0.35 (0.02–1.59)	
WHO Region				
African	306 (0.5)	3 (1.0)	**0.33 (0.08–0.85)**	**0.21 (0.05–0.65)**
Americas	27,277 (40.9)	805 (3.1)	Ref	Ref
E. Mediterranean	1,917 (2.9)	16 (0.8)	**0.28 (0.16–0.44)**	**0.05 (0.02–0.09)**
European	29,569 (44.4)	780 (2.6)	**0.89 (0.81–0.98)**	**0.13 (0.07–0.27)**
S.E. Asia	5,841 (8.8)	364 (6.2)	**2.19 (1.92–2.48)**	**0.66 (0.54–0.81)**
W. Pacific	1,730 (2.6)	34 (1.9)	**0.66 (0.46–0.92)**	**0.21 (0.12–0.36)**
HIV				
Co-infected	3,945 (5.9)	191 (4.8)	**1.57 (1.34–1.84)**	1.01 (0.83–1.21)
Negative	32,253 (48.4)	1,012 (3.1)	Ref	Ref
Missing data	30,442 (45.7)	799 (2.6)	NA	NA
HBV				
Co-infected	7,221 (10.8)	214 (3.0)	1.08 (0.92–1.25)	1.14 (0.97–1.34)
Negative	26,579 (39.9)	734 (2.8)	Ref	Ref
Missing data	32,840 (49.3)	1,054 (3.2)	NA	NA
HCV Genotype				
Genotype 1	27,245 (40.9)	623 (2.3)	Ref	Ref
Genotype 2	9,287 (13.9)	261 (2.8)	**1.24 (1.07–1.43)**	**1.25 (1.07–1.45)**
Genotype 3	15,157 (22.7)	405 (2.7)	**1.17 (1.03–1.33)**	**1.17 (1.03–1.34)**
Genotype 4	2,030 (3.0)	28 (1.4)	**0.59 (0.39–0.86)**	1.14 (0.75–1.67)
Genotype 5	13 (0.02)	1 (7.7)	3.55 (0.19–18.08)	4.22 (0.05–17.15)
Genotype 6	889 (1.3)	12 (1.3)	**0.58 (0.31–0.99)**	0.61 (0.31–1.12)
Mixed	170 (0.3)	4 (2.4)	1.03 (0.32–2.44)	0.97 (0.29–2.3)
Missing	11,849 (17.8)	669 (5.6)	NA	NA
Fibrosis stage				
F0-F1	12,460 (18.7)	313 (2.5)	Ref	Ref
F2-F3	11,923 (17.8)	242 (2.0)	**0.80 (0.68–0.95)**	0.89 (0.76–1.07)
F4	8,366 (12.5)	239 (2.9)	1.14 (0.96–1.35)	**1.43 (1.19–1.70)**
Missing	33,891 (50.9)	1,115 (3.4)	NA	NA

Data from patients with chronic HCV in Cambodia, Canada, Cameroon, Egypt, Georgia, India, Indonesia, Malaysia, Mozambique, Pakistan, Thailand, and Vietnam. The association of covariates with low-level viraemia, HCV RNA <1,318 IU/ml, is indicated by the bivariate ORs and aORs with 95% CI – statistically significant OR are in bold font. Col%, column percent; Row%, row percent; Ref, reference group; OR, odds ratio; aOR, adjusted OR; NA, not applicable; WHO, World Health Organization, E., eastern; S.E., South East; W., western; HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus.

[*]Low-level viraemia = HCV RNA <1,318 IU/ml.

[1]aOR derived from a multivariable model adjusting for age, sex, country, HCV genotype, and fibrosis stage.

missing genotype data. We categorised 12,880 (38.1%) individuals as Metavir fibrosis stage F0-F1, 12,242 (36.3%) as F2-F3, and 8,644 (25.6%) as F4 among the 33,766 individuals with available fibrosis staging data (Fibroscan or Fibrosis-4 score).

HCV viral load distribution & limit of detection analyses

The HCV RNA ($\log_{10}$ IU/ml) frequency distribution is depicted in Fig. 1. We derived the LOD for the 95th, 97th and 99th percentiles as: 3,311 IU/ml (95% CI 3,256.3–3,368.0), 1,318 IU/ml (95% CI 1,298.4–1,322.3), and 214 IU/ml (95% CI 207.1–218.6) respectively. We further visualised the HCV RNA distribution to compare the mean HCV RNA for each covariate with violin plots (Fig. 2). Violin plots depict a box plot where a circle denotes the median and the interquartile range is shown by a box in solid black. Overlaid on this box plot is a kernel density plot indicating more data where the plot is thicker and less where it narrows. We then derived violin plots for each country to illustrate the viral load distribution by site as a surrogate approach to control for variation in quantification platforms and sampling techniques (Fig. S2). The mean RNA lies between 5 and 6 log IU/ml in all cohorts.

Identification of subgroups with low-level viraemia

We derived the odds of association with LLV for each covariate with the following groups selected as a reference because each contained the largest volume from the reference laboratory dataset in Canada: 51–64 years of age, male sex, Canada, Americas, genotype 1. Bivariate analyses indicated increased odds of LLV <1,318 IU/ml for those aged 18–30 and 31–50 years, partic-

Fig. 1. Frequency distribution of HCV RNA ($\log_{10}$ IU/ml) among participants in a combined cross-sectional dataset. Data from patients with chronic HCV in Cambodia, Canada, Cameroon, Egypt, Georgia, India, Indonesia, Malaysia, Mozambique, Pakistan, Thailand, and Vietnam. The analytic level of detection for: a) centralized NAT Xpert® HCV Viral Load (40 IU/ml) and Abbott RealTime HCV Viral load (12 IU/ml), b) point-of-care NAT Genedrive® HCV ID Kit (2,362 IU/ml) and c) Abbott ARCHITECT HCV core antigen (HCVcAg) test (in IU/ml; 3,000 IU/ml approximately equivalent to 3 fmol/L) are marked in comparison to the LOD derived for the 99th, 97th, and 95th percentiles in the dataset. *Marks the lower LOD for tests performed in India that were detected but not quantified. HCV, hepatitis C virus; LOD, limit of detection; NAT, nucleic acid test. (This figure appears in colour on the web.)

Fig. 2. Violin plot of the HCV RNA distribution ($\log_{10}$ IU/ml) for each covariate in the total population cohort. In each violin plot, a circle denotes the median and the interquartile range is shown by box in solid black. Overlaid on this box plot is a kernel density plot indicating more data where the plot is thicker and less where it narrows. The dashed horizontal marker indicates the 1,318 IU/ml level of detection derived from the HCV RNA frequency distribution. HCV, hepatitis C virus. (This figure appears in colour on the web.)

ipants from India, the South East Asian region, and those with HIV co-infection or genotype 2 and 3 infection (Table 2). The OR was highest between those aged 18–30 years and the reference group aged 51–64 years, at 3.06 (95% CI 2.68–3.49). India had an OR of 2.33 (95% CI 2.05–2.65) compared to Canada. South East Asia had an OR of 2.19 (95% CI 1.92–2.48) compared to the Americas. HIV co-infected persons had an OR for LLV of 1.57 (95% CI 1.34–1.84) compared to those without HIV. Lastly, genotype 2 had an OR of 1.24 (95% CI 1.07–1.43) and genotype 3 OR 1.17 (95% CI 1.03–1.33) compared to genotype 1.

In a multivariable model controlling for age, sex, WHO Region, HIV and HBV co-infections, genotype, and fibrosis stage, the 18–30-year age group, female sex, genotypes 2 and 3, and fibrosis stage F4 remained associated with increased odds for LLV (Table 2). Persons 18–30 years of age had an adjusted OR (aOR) of 2.56 (95% 2.19–2.99) compared to the 51–64-year-old age group. For female sex, the aOR was 1.32 (95% CI 1.18–1.49) compared to males. The aORs for genotype 2 and 3 compared to genotype 1 were 1.24 and 1.17, respectively. Lastly, for advanced fibrosis stage F4, the aOR increased to 1.44 (95% CI 1.21–1.69) compared to stage F0-1. We did not detect significant interactions between: i) sex and fibrosis stage, ii) genotype and country, iii) genotype and sex, iv) genotype and age group.

Stratified and sensitivity analyses

Stratified analyses for HIV and HBV co-infection did not suggest an effect measure modification for either covariate (Tables S2 and S3). A subset analysis of those with Fibroscan results did not differ from that of the total dataset population (Table S4). Similarly, sensitivity analyses varying the cut-off thresholds for fibrosis stage classification by Fibrosis-4 scores did not impact the associations found for fibrosis stage and LLV (data not shown).

Characteristics for the cohort after missing data imputation for HIV, HBV, genotype, and fibrosis stage are presented in Table S5. Now 6.3% (4,169) are classified as HIV-coinfected (compared to 5.9% in the TPC and 10.9% among those tested,
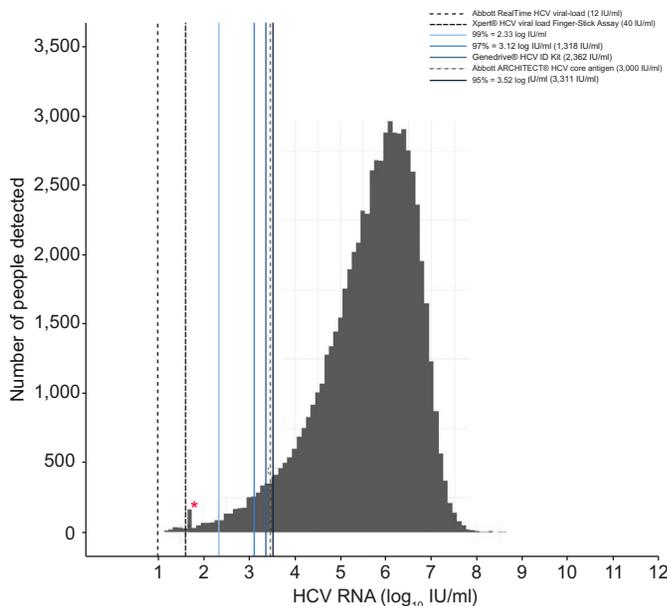
Table 2), and 12.2% (8,161) as HBV co-infected (10.8% in TPC, 21.4% among those tested). The genotype distribution was similar to the TPC. Fibrosis stage categories were also similar to those with data in the TPC. In the imputation regression analyses, significant associations with LLV remained after multivariable adjustment for: groups aged 18–30 (aOR 2.44) and 31–50 (aOR 1.29) years, female sex (aOR 1.31), participants from South East Asia (aOR 1.59), and fibrosis stage F4 (aOR 1.14) (Table S5). The increased odds among HIV co-infection, and HCV genotypes 2 and 3 in the TPC attenuated in the imputed dataset.

Relative percentiles for significant covariates

While an HCV RNA of 1,318 IU/ml correlated to the 97th percentile in the total population dataset, the relative percentiles corresponding to the 1,318 IU/ml LOD for significant covariates were: 93% for age group 18–30, 96.7% for females, 93.8% for participants from South East Asia, 95.3% for HIV co-infected, and 96.8% for fibrosis stage F4 (Fig. 3A). Though genotypes 2 and 3 had increased odds for LLV, the relative percentiles remained >97%. All percentiles were similar in the imputed dataset sensitivity analyses (Fig. 3B).

Discussion

This dataset of 66,640 individuals from 12 countries representative of 6 global regions represents the largest and most comprehensive dataset assembled to address the issue of clinical sensitivity of different LODs for detection of HCV viraemia. Our data confirm that with an LOD of 1,318 IU/ml (3.12 log IU/ml), 97% of viraemic HCV infections would be identified. These data further support the recent European Association for the Study of the Liver recommendation for an LOD of 1,000 IU/ml among diagnostic nucleic acid assays for use in LMICs.[32] While several covariates were associated with increased odds for LLV below 1,318 IU/ml, we report a relative sensitivity below 95% only for the 18–30-year age group (93.6% sensitivity) and among participants in South East Asia (93.8% sensitivity); genotypes 2 and 3 were associated with LLV but the relative sensitivity remained >97%. The prevalence of LLV among the 18–30-year age group in this study may reflect fluctuating viraemia that occurs with early HCV infection.[33,34] Of note, many of the participants from sites in South East Asia were active injection drug users and may also have had early HCV infection. We have insufficient data in the TPC to conduct a subset analysis of people who inject drugs.

Our findings differ from previous studies that evaluated HCV viral load quantification as many were designed to evaluate predictors of high-level viraemia; however, the degree of LLV we describe is similar to an evaluation of 2,472 people with genotype 1 infection.[35] Another study of 148 people with HCV infection found lower levels of circulating HCV RNA among those with decompensated cirrhosis.[36] We captured a trend toward LLV, but this was not sufficient to alter the relative sensitivity of the LOD in this sub-population. From the available data, we could not identify those who had decompensated cirrhosis within the F4 stage. Therefore, the OR may be slightly diminished by the number of participants with compensated cirrhosis in this group who still have abundant healthy hepatocytes that allow for HCV replication. Prior studies reported higher HCV RNA levels among those with HIV.[37,38] In contrast, our data suggest increased odds for LLV among those with HIV co-infection and a decrease in the sensitivity of the LOD to 95.2%. Data on
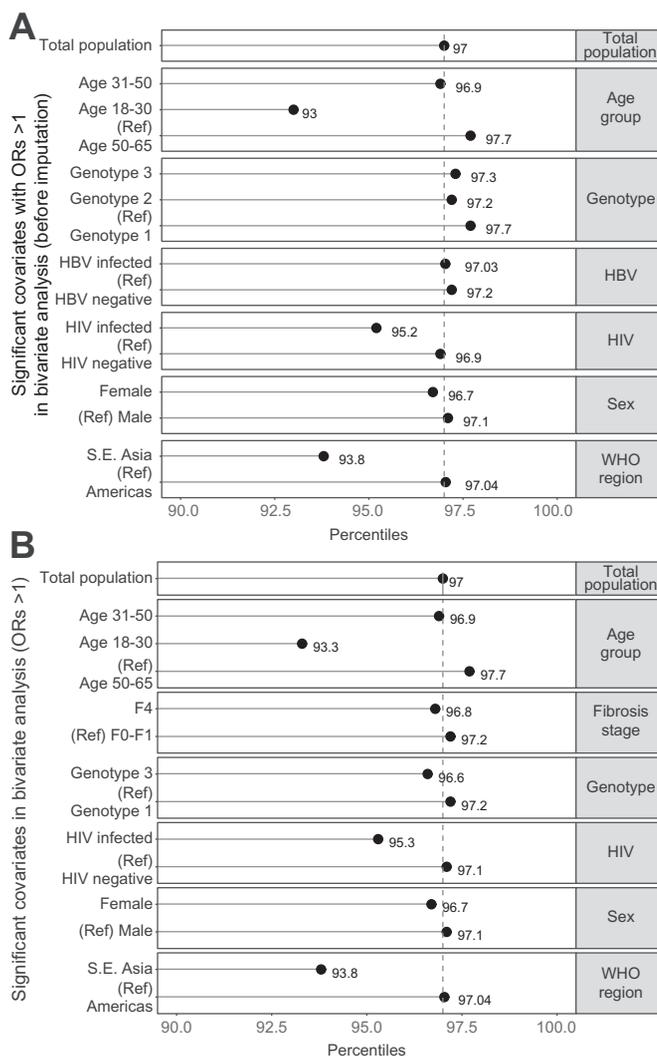
Fig. 3. Graph of relative percentiles of HCV viraemia corresponding with the limit of detection derived from the total population dataset (1,318 IU/ml). For covariates significantly associated with low-level viraemia in the (A) total population dataset and (B) imputed dataset. The reference (ref) groups are included for each category. The dashed vertical line marks the 97th percentile from the total population dataset. HCV, hepatitis C virus.

use of antiretroviral therapy and level of viral load suppression were not available from all data sources, and therefore the level of HCV viral load among those with advanced stage HIV either not receiving or failing on therapy, could not be compared to those with well-controlled HIV infection on effective antiretroviral therapy. Lastly, these studies were designed to investigate outcomes or treatment predictors and had much smaller sample sizes that limit their power to evaluate the LLV frequency.

The main strengths of this study are the large sample size powered to investigate covariates of interest and the wide representation of different geographic regions and genotypes. Regions with high HCV prevalence (Egypt, Georgia) were well represented as well as lower prevalence settings (Cameroon).

There are several limitations to this study. Identified and included patient cohorts were those with available HCV RNA linked with clinical and demographic data and therefore may not be generalisable. We conducted a secondary analysis of data from populations that were tested for HCV for a range of reasons. There were also different methods for data collection at

each site. While some data represent a broad sample of tests performed in the general population at a reference laboratory, other data were collected as part of research protocols with strict inclusion and exclusion criteria. There were missing data for several covariates (HIV and HBV co-infection, HCV genotype, fibrosis stage) in 20–52% of the participants. We performed sensitivity analyses including a comparative regression model using imputed data to better characterise bias introduced by missing data and found the introduced bias to be limited overall.

This study investigated the viral load distribution among those with chronic HCV infection from 12 countries in different geographic regions to estimate the requisite clinical sensitivity of a POC test for HCV diagnosis and inform sub-populations that may be at risk of false negative testing. Our findings suggest that a test with an LOD of 1,318 IU/ml, which is about 100 times higher (less sensitive) than the current gold-standard NATs, will likely detect 97% of viraemic HCV infections. While an increase in LOD may not impact cost and development of near-patient molecular technologies, it sets an achievable LOD for immunoassays such as those that involve HCV core antigen detection. Comparative and cost-effectiveness analyses will be needed to investigate settings that may benefit the most, and to quantify how a less sensitive test might impact the diagnosis, treatment and cure cascade in LMICs. A product specification that allows for an LOD of 1,318 IU/ml could facilitate development of an affordable non-molecular POC test that could dramatically increase rates of HCV testing and treatment initiation in LMICs, thus substantially impacting health outcomes for chronic HCV infection on a population-level.

Please refer to the accompanying ICMJE disclosure forms for further details.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2019.02.011.

References

[1] World Health Organization. Global Hepatitis Report 2017. 2017 [cited; Available from: http://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/].
[2] . Global Tuberculosis Control Report Geneva 2007.
[3] Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. J Hepatol 2014;61:S45–S57.
[4] World Health Organization. Global Health Sector Strategy on Viral Hepatitis 2016-2021: Towards Ending Hepatitis. 2016 [cited; Available from: http://www.who.int/hepatitis/strategy2016-2021/ghss-hep/en/].
[5] Hill A, Simmons B, Gotham D, Fortunak J. Rapid reductions in prices for generic sofosbuvir and daclatasvir to treat hepatitis C. J Virus Erad 2016;2:28–31.
[6] World Health Organization. Progress report on access to hepatitis C treatment:Focus on overcoming barriers in low- and middle-income countries. 2018 [cited; Available from: http://www.who.int/hepatitis/publications/hep-c-access-report-2018/en/].
[7] Jauncey M, Micallef JM, Gilmour S, Amin J, White PA, Rawlinson W, et al. Clearance of hepatitis C virus after newly acquired infection in injection drug users. J Infect Dis 2004;190:1270–1274.
[8] Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology 2013;57:1333–1342.
[9] Easterbrook PJ. Who to test and how to test for chronic hepatitis C infection – 2016 WHO testing guidance for low- and middle-income countries. J Hepatol 2016;65:S46–S66.

[10] World Health Organization. Guidelines on Hepatitis B and C Testing. 2017. [cited; Available from: http://www.who.int/hepatitis/publications/guidelines-hepatitis-c-b-testing/en/].

[11] World Health Organization. Guidelines on Hepatitis B and C Testing: Policy Brief. 2017 [cited; Available from: http://www.who.int/hepatitis/publications/hepatitis-testing-recommendation-policy/en/].

[12] McGibbon E, Bornschlegel K, Balter S. Half a diagnosis: gap in confirming infection among hepatitis C antibody-positive patients. Am J Med 2013;126:718–722.

[13] Ishizaki A, Bouscaillou J, Luhmann N, Liu S, Chua R, Walsh N, et al. Survey of programmatic experiences and challenges in delivery of hepatitis B and C testing in low- and middle-income countries. BMC Infect Dis 2017;17:696.

[14] Roberts T, Cohn J, Bonner K, Hargreaves S. Scale-up of Routine Viral Load Testing in Resource-Poor Settings: Current and Future Implementation Challenges. Clin Infect Dis 2016;62:1043–1048.

[15] FIND. Strategy for Hepatitis C, 2015-2020. https://www.finddx.org/wp-content/uploads/2016/02/HepatitisC_Strategy_WEB.pdf. Access date October 11, 2018.

[16] Peeling RW, Boeras DI, Marinucci F, Easterbrook P. The future of viral hepatitis testing: innovations in testing technologies and approaches. BMC Infect Dis 2017;17:699.

[17] Trianni A, Paneer N, Denkinger C. High-priority target product profile for hepatitis C diagnosis in decentralized settings: Report of a consensus meeting. Vienna, Austria: FIND; 2015.

[18] Ivanova Reipold E, Easterbrook P, Trianni A, Panneer N, Krakower D, Ongarello S, et al. Optimising diagnosis of viraemic hepatitis C infection: the development of a target product profile. BMC Infect Dis 2017;17:707.

[19] Maasoumy B, Vermehren J. Diagnostics in hepatitis C: The end of response-guided therapy? J Hepatol 2016;65:S67–S81.

[20] Freiman JM, Tran TM, Schumacher SG, White LF, Ongarello S, Cohn J, et al. Hepatitis C Core Antigen Testing for Diagnosis of Hepatitis C Virus Infection: A Systematic Review and meta-analysis. Ann Intern Med 2016;165:345–355.

[21] Llibre A, Shimakawa Y, Mottez E, Ainsworth S, Buivan TP, Firth R, et al. Development and clinical validation of the Genedrive point-of-care test for qualitative detection of hepatitis C virus. Gut 2018;67:2017–2024.

[22] Grebely J, Lamoury FMJ, Hajarizadeh B, Mowat Y, Marshall AD, Bajis S, et al. Evaluation of the Xpert HCV Viral Load point-of-care assay from venepuncture-collected and finger-stick capillary whole-blood samples: a cohort study. Lancet Gastroenterol Hepatol 2017.

[23] Fytili P, Tiemann C, Wang C, Schulz S, Schaffer S, Manns MP, et al. Frequency of very low HCV viremia detected by a highly sensitive HCV-RNA assay. J Clin Virol 2007;39:308–311.

[24] Peeling RW, Mabey D. Point-of-care tests for diagnosing infections in the developing world. Clin Microbiol Infect 2010;16:1062–1069.

[25] Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43:1317–1325.

[26] Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, et al. inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. Hepatology 2007;46:32–36.

[27] Bachofner JA, Valli PV, Kroger A, Bergamin I, Kunzler P, Baserga A, et al. Direct antiviral agent treatment of chronic hepatitis C results in rapid regression of transient elastography and fibrosis markers fibrosis-4 score and aspartate aminotransferase-platelet ratio index. Liver Int 2016.

[28] Sanchez-Conde M, Montes-Ramirez ML, Miralles P, Alvarez JM, Bellon JM, Ramirez M, et al. Comparison of transient elastography and liver biopsy for the assessment of liver fibrosis in HIV/hepatitis C virus-coinfected patients and correlation with noninvasive serum markers. J Viral Hepat England 2010:280–286.

[29] Xiao G, Yang J, Yan L. Comparison of diagnostic accuracy of aspartate aminotransferase to platelet ratio index and fibrosis-4 index for detecting liver fibrosis in adult patients with chronic hepatitis B virus infection: a systemic review and meta-analysis. Hepatology 2015;61:292–302.

[30] Efron B. The bootstrap and Markov chain Monte Carlo. J Biopharm Stat 2011;21:1052–1062.

[31] Schafer JL. Analysis of Incomplete Multivariate Data. USA: Chapman & Hall; 1997.

[32] Pawlotsky J-M, Negro F, Aghemo A, Berenguer M, Dalgard O, Dusheiko G, et al. EASL recommendations on treatment of hepatitis C. J Hepatol 2018;69:461–511.

[33] Glynn SA, Wright DJ, Kleinman SH, Hirschkorn D, Tu Y, Heldebrant C, et al. Dynamics of viremia in early hepatitis C virus infection. Transfusion 2005;45:994–1002.

[34] Hajarizadeh B, Grebely J, Applegate T, Matthews GV, Amin J, Petoumenos K, et al. Dynamics of HCV RNA levels during acute hepatitis C virus infection. J Med Virol 2014;86:1722–1729.

[35] Ticehurst JR, Hamzeh FM, Thomas DL. Factors affecting serum concentrations of hepatitis C virus (HCV) RNA in HCV genotype 1-infected patients with chronic hepatitis. J Clin Microbiol 2007;45:2426–2433.

[36] Duvoux C, Pawlotsky JM, Bastie A, Cherqui D, Soussy CJ, Dhumeaux D. Low HCV replication levels in end-stage hepatitis C virus-related liver disease. J Hepatol 1999;31:593–597.

[37] Matthews-Greer JM, Caldito GC, Adley SD, Willis R, Mire AC, Jamison RM, et al. Comparison of hepatitis C viral loads in patients with or without human immunodeficiency virus. Clin Diagn Lab Immunol 2001;8:690–694.

[38] Hajarizadeh B, Grady B, Page K, Kim AY, McGovern BH, Cox AL, et al. Factors associated with hepatitis C virus RNA levels in early chronic infection: the InC3 study. J Viral Hepat 2015;22:708–717.

[39] Blach S, Zeuzem S, Manns M, Altraif I, Duberg A-S, Muljono DH, et al. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. Lancet Gastroenterol Hepatol 2017;2:161–176.

[40] World Bank. World Bank List of Economies. 2017 [cited; Available from: https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups].

[41] Janjua NZ, Kuo M, Chong M, Yu A, Alvarez M, Cook D, et al. Assessing hepatitis C burden and treatment effectiveness through the British Columbia hepatitis testers cohort (BC-HTC): design and characteristics of linked and unlinked participants. PLoS ONE 2016;11 e0150176.

[42] Gvinjilia L, Nasrullah M, Sergeenko D, Tsertsvadze T, Kamkamidze G, Butsashvili M, et al. National progress toward hepatitis C elimination — Georgia, 2015–2016. MMWR 2016;65:1132–1135.

[43] Mitruka K, Tsertsvadze T, Butsashvili M, Gamkrelidze A, Sabelashvili P, Adamia E, et al. Launch of a nationwide hepatitis c elimination program-Georgia, April 2015. MMWR Morb Mortal Wkly Rep 2015;64:753–757.

[44] Nasrullah M, Sergeenko D, Gvinjilia L, Gamkrelidze A, Tsertsvadze T, Butsashvili M, et al. The role of screening and treatment in national progress toward hepatitis C elimination – Georgia, 2015–2016. MMWR Morb Mortal Wkly Rep 2017;66:773–776.

[45] Durier N, Yunihastuti E, Ruxrungtham K, Kinh NV, Kamarulzaman A, Boettiger D, et al. Chronic hepatitis C infection and liver disease in HIV-coinfected patients in Asia. J Viral Hepat 2017;24:187–196.

[46] Solomon SS, Mehta SH, Srikrishnan AK, Solomon S, McFall AM, Laeyendecker O, et al. Burden of hepatitis C virus disease and access to hepatitis C virus services in people who inject drugs in India: a cross-sectional study. Lancet Infect Dis 2015;15:36–45.

[47] Solomon SS, Srikrishnan AK, McFall AM, Kumar MS, Saravanan S, Balakrishnan P, et al. Burden of liver disease among community-based people who inject drugs (PWID) in Chennai, India. PLoS ONE 2016;11.

[48] Solomon SS, Sulkowski MS, Amrose P, Srikrishnan AK, McFall AM, Ramasamy B, et al. Directly observed therapy of sofosbuvir/ribavirin +/− peginterferon with minimal monitoring for the treatment of chronic hepatitis C in people with a history of drug use in Chennai, India (C-DOT). J Viral Hepat 2018;25:37–46.

[49] Mehta SH, Vogt SL, Srikrishnan AK, Vasudevan CK, Murugavel KG, Saravanan S, et al. Epidemiology of hepatitis C virus infection & liver disease among injection drug users (IDUs) in Chennai, India. Indian J Med Res 2010;132:706–714.