JOURNAL OF VIRAL HEPATITIS
JVH

Journal of Viral Hepatitis, 2015, **22,** 461–468 doi:10.1111/jvh.12354

Antibody screening tests variably overestimate the prevalence of hepatitis C virus infection among HIV-infected adults in Ghana

S. King,[1] K. Adjei-Asante,[1,2] L. Appiah,[2] D. Adinku,[2] A. Beloukas,[1] M. Atkins,[3] S. F. Sarfo,[2] D. Chadwick,[4] R. O. Phillips[2,5] and A. M. Geretti[1] [1]*Institute of Infection and Global Health, University of Liverpool, Liverpool, UK;* [2]*Komfo Anokye Teaching Hospital University, Kumasi, Ghana;* [3]*Frimley Park Hospital NHS Foundation Trust, Frimley, Surrey, UK;* [4]*James Cook University Hospital, Middlesbrough, UK; and* [5]*Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

Received April 2014; accepted for publication August 2014

SUMMARY. HIV coinfection with HCV has been poorly studied in sub-Saharan Africa, and the reliability of available seroprevalence estimates remains uncertain. The study aim was to determine HCV RNA prevalence in HIV-infected subjects receiving care in Kumasi, Ghana, and relate the findings to HCV antibody detection. From a population of 1520 HIV-infected adults, all HBsAg-positive subjects (n = 236) and a random subset of HBsAg-negative subject (n = 172) were screened for HCV RNA using pooled plasma; positive samples were genotyped by core and NS5B sequencing. HCV antibodies were detected by three commercial screening assays and confirmed by the line immunoassay. HCV RNA was detected in 4/408 subjects (1.0%, 95% confidence interval 0.0–1.9%), comprising 3/236 (1.3%; 0.0–2.8%) HBsAg-positive and 1/172 (0.6%; 0.0–1.8%) HBsAg-negative subjects. HCV RNA-positive subjects showed reactivity in all three antibody screening assays. Among HCV RNA-negative subjects, 5/67 (7.5%), 5/67 (7.5%) and 19/67 (28.4%) showed antibody reactivity by each screening assay, respectively, including two (3.0%) with reactivity by all three assays. Only one sample (1.5%) had confirmed antibody reactivity by line immunoassay indicating past HCV infection. HCV-positive subjects (three males, two females) were aged 30–46 years, by questionnaire-based interview reported surgical procedures and blood transfusion as risk factors for infection. HCV genotypes were 2 (subtypes 2j, 2l, 2k/unassigned) and 1 (subtype unassigned). Without further testing, HCV antibody screening assays variably overestimated HCV prevalence among HIV-infected subjects in Ghana. These findings inform the interpretation of previous seroprevalence estimates based upon screening assays alone.

Keywords: Africa, antibody, genotype, RNA, serology.

INTRODUCTION

Hepatitis C virus (HCV) infection is common in Western HIV-positive cohorts, reflecting shared transmission routes, predominantly injecting drug use [1]. HIV promotes HCV replication and progression of liver disease [2], resulting in pronounced liver-related morbidity and mortality [3]. HCV testing is therefore recommended at the time of HIV diagnosis and regularly thereafter [4]. The diagnostic algorithm typically involves screening for HCV antibody (or both antibody and antigen) with chemiluminescence or enzyme immunoassays, followed by HCV RNA detection to confirm a current infection. Patients that test HCV RNA negative undergo supplementary antibody testing by blot or line immunoassay to differentiate resolved HCV infection from false antibody positivity [5].

Hepatitis C virus (HCV) infection has been poorly studied in sub-Saharan Africa. Available surveys rely mostly on antibody screening assays and few studies have tested for HCV RNA or employed supplementary antibody testing [6–9]. In 2010, a systematic review of 35 studies indicated that 7% of 9029 HIV-infected adults in sub-Saharan Africa were positive for HCV antibody, with a median seroprevalence across studies of around 5% (range 0–22%) [10]. In Ghana, a study published in the late 90s reported that 8% of 182 HIV-positive subjects tested positive for HCV antibody [11]. More recently, HCV seroprevalence was nearly 4% among 138 HIV-positive adults that in 2007 were

Abbreviations: ART, antiretroviral therapy; CO, cut-off; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; IC, internal control; OD, optical density; OD/CO, optical density/cut-off ratio; PDV, phocine distemper virus; S/CO, sample/cut-off ratio.

Correspondence: Prof Anna Maria Geretti, MD, PhD, Department of Clinical Infection, Microbiology and Immunology, Institute of Infection and Global Health, University of Liverpool, 8 West Derby Street, Liverpool L69 7BE, UK. E-mail: geretti@liverpool.ac.uk

receiving care in the capital city of Accra [12]. These estimates suggest a high disease burden that – in the absence of defined ascertainment and management strategies – poses a threat to the long-term success of ART programmes in the region.

The reliability of HCV seroprevalence estimates for sub-Saharan Africa remains uncertain. The few studies that have employed supplementary and confirmatory testing, including recent reports from Uganda and Malawi, have suggested that HCV antibody screening assays have poor specificity in sub-Saharan African populations [13–15]. To obtain more definitive evidence on the rates of HIV/HCV coinfection in Ghana, this study investigated HCV RNA prevalence in a large HIV-positive cohort receiving care in Kumasi, the second largest city of Ghana, and related the findings to HCV antibody detection by one chemiluminescence and two enzyme immunoassays, with supplementary testing by line immunoassay.

MATERIALS AND METHODS

Study population

A total of 1520 HIV-infected adults that attended the outpatient service of the Komfo Anokye Teaching Hospital in Kumasi between 2010 and 2012 underwent hepatitis B surface antigen (HBsAg) testing by the Murex enzyme immunoassay (Abbott Diagnostics, Chicago, IL, USA). All HBsAg-positive subjects (n = 236) and a random subset of HBsAg-negative subject (n = 172) had serum and plasma stored locally at −80 °C before transport on dry ice to the United Kingdom for HCV testing. Subjects with confirmed HCV infection were invited to complete a structured questionnaire about risk factors for acquiring HCV. The study conformed to the standards of the Helsinki Declaration and was approved by the Committee on Human Research Publications and Ethics at the Kwame Nkrumah University of Science and Technology of Kumasi, Ghana. Written consent was obtained from participants.

HCV RNA detection

Samples were initially screened for HCV RNA by pooling five plasma samples (200 μL each) into a single 1-mL specimen, followed by testing of individual samples from positive pools. Basematrix (SeraCare, Milford, MA, USA) was added to pools containing fewer than five specimens to maintain a total volume of 1 mL. Prior to RNA extraction, Phocine distemper virus (PDV) was added to each specimen as internal control (IC), followed by incubation at 56 °C for 30 min and 100 °C for 30 min with NucliSENS lysis buffer (2 mL) (bioMérieux, Boxtel, the Netherlands) and Proteinase K (40 μL) (Qiagen, Hilden, Germany). RNA was extracted by EasyMAG® (bioMérieux) using the Specific B protocol and 60 μL elution volume. HCV RNA was amplified by a real-time PCR assay targeting a conserved 90-bp region of the HCV 5′-UTR region [16]. The IC reaction targeted a 79-bp region of the PDV haemagglutinin gene [17]. Amplification was performed by the ABI Prism 7500 (Applied Biosystems, Foster City, CA, USA) using 20 μL of extract in a 50 μL final reaction volume containing 25 μL 2× reaction mix, 1 μL SuperScript® III/Platinum® Taq mix enzyme, and HCV (5′-GTCTAGCCATGGCGTTAGTA-3′; 5′-GTACTCACCGGTTCCGC-3′; FAM-CCCTCCCGGGAGAGCCATAGTG-TAMRA) or PDV (5′-CGGGTGCCTTTTACA AGAAC-3′; 5′-TTCTTTCCTCAACCTCGTCC-3′; JOE-ATGCAAGGGCCA ATTCTTCCAAGTT-BHQ1) forward and reverse primers (200 nM) and probes (100 nM). The thermal cycling conditions were 50 °C for 15 min and 95 °C for 2 min, followed by 45 cycles at 95 °C for 15 s and 60 °C for 32 s. The real-time PCR assay was run qualitatively. Sensitivity was determined with serial dilutions of the 4th World Health Organization (WHO) International Standard for HCV RNA (06/102, NIBSC, Potters Bar, UK) in basematrix; each dilution was tested in 40 replicates. To determine sensitivity with pooled specimens, mock pools were prepared and tested in triplicate, each comprising one HCV RNA-positive specimen and four negative specimens, maintaining 1 mL total input. The positive specimen consisted of plasma spiked with the International Standard at concentrations ranging from 500 to 10000 IU/mL.

HCV antibody detection

A total of 71 serum samples were tested for HCV antibody using the automated Architect chemiluminescence immunoassay (Abbott Diagnostics), and the enzyme immunoassays ORTHO HCV 3.0 ELISA System with Enhanced SAVe (Ortho Clinical Diagnostics, Rochester, NY, USA) and Monolisa HCV Ag–Ab ULTRA (BioRad, Hercules, CA, USA), following the manufacturer's instructions. With the Architect assay, input was 150 μL (including 130 μL dead volume) and results were calculated as ratios of the sample relative light units (RLUs)/cut-off value (S/CO); samples with a S/CO ratio ≥1 were considered reactive. With the Ortho and Monolisa assays, input was 10 and 50 μL, respectively, and samples with optical density (OD)/CO ratio ≥1 were considered reactive. According to the manufacturers' instructions, the CO of the Ortho was determined by the mean of three negative controls plus 0.600, whilst the CO of the Monolisa was determined by the mean OD of the positive control divided by four. Samples that tested reactive by Architect but negative for HCV RNA were also tested using the line immunoassay INNO-LIA HCV Score (Fujirebio Europe, Ghent, Belgium), which detects antibodies against antigens derived from core, E2, NS3, NS4A, NS4B and NS5A. Positive results were those showing reactivity of ± or higher with ≥2 antigens; indeterminate

results were those showing reactivity of 1+ or higher to one antigen or reactivity of ± or higher with NS3 alone.

Sequencing of HCV core and NS5B regions

Following nucleic acid extraction with the Qiagen Viral RNA Mini Kit (Qiagen), a 380 bp region of HCV NS5B gene was amplified and sequenced as previously described [18]. In addition, a 403 bp region of HCV core was amplified by nested PCR and sequenced using previously described primers [19]. For the latter, extracted RNA was reverse transcribed by the one-step RT-PCR kit (Qiagen) using RNasin ribonuclease inhibitor (Promega, Madison, WI, USA) under the following thermal cycling conditions: 50 °C for 30 min, 95 °C for 15 min, and then 35 cycles at 94 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min and 72 °C for 10 min. The PCR product underwent nested amplification using the HotStarTaq DNA polymerase kit (Qiagen) under the following conditions: 95 °C for 15 min, and then 35 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min and 72 °C for 10 min. PCR products were purified using QIAquick PCR purification kit (Qiagen) and sequenced using BigDye Terminator Cycle Sequencing Kit v3.1 on the ABI Prism 3730 Genetic Analyser (Applied Biosystems®). The GenBank accession numbers of nucleotide sequences analysed in the study are KJ642627 to KJ642630 and KJ642635 to KJ642638.

Analysis

HCV RNA prevalence in HBsAg-positive *vs* HBsAg-negative patients was compared by Fisher's exact test. Median anti-HCV antibody reactivity in HCV RNA-positive *vs* HCV RNA-negative subjects was compared by the Mann–Whitney–Wilcoxon test. Statistical analysis was performed using SPSS version 21 (IBM Corporation, Armonk, NY, USA). Phylogenetic analyses were performed using the PHYLIP software package (v3.69) (http://evolution.genetics.washington.edu/phylip.html). Sequences were aligned using the CLUSTAL W programme (http://www.clustal.org/clustal2/) with reference sequences from the HCV Los Alamos Database (genotypes 1–7) and analysed phylogenetically applying 1000 bootstrap replicates with Kimura two-parameter and neighbour-joining setting. Bootstrap values ≥80% were considered significant. Sequences used in the phylogenetic analyses are provided in a supplementary file.

RESULTS

Sensitivity of HCV RNA screening

The lower limit of detection of the real-time PCR assay for HCV RNA detection was first determined with serial dilutions of the 4th WHO International Standard for HCV RNA, testing 40 replicates per dilution. The HCV RNA detection rate (1 mL input) was 32/40 (80%) at 100 IU/mL and 40/40 (100%) at 110 IU/mL. It was therefore assumed that a lower limit of detection of 550 IU/mL would be obtained by pooling five specimens of 200 μL each, which was considered acceptable for screening an untreated population. To validate this assumption, HCV-negative plasma was spiked with the International Standard at a concentration of 500, 550, 1000, 5000 or 10000 IU/mL, and each spiked sample was combined with four negative plasma specimens to make up mock pools of 1 mL each. In triplicate testing, HCV RNA was detected in all pools containing a positive sample with HCV RNA concentration ≥550 IU/mL and in 2 of 3 pools containing the 500 IU/mL sample.

Prevalence of HCV RNA in the Kumasi HIV cohort

From 1520 HIV-infected adults undergoing HBsAg testing, 236 consecutive HBsAg-positive subjects and 172 randomly selected HBsAg-negative subjects were tested for HCV RNA. Overall, 270/408 (66.2%) subjects were females and the median (interquartile range, IQR) age was 40 (35–47) years. HCV RNA was detected in 4/82 pools, each pool comprising five plasma samples. Testing of the individual samples of positive pools detected one positive sample in each pool, yielding a HCV RNA prevalence of 1.0% (95% confidence interval 0.0–1.9%). HCV RNA prevalence was 3/236 (1.3%; 0.0–2.8%) and 1/172 (0.6%; 0.0–1.8%) in HBsAg positive *vs* HBsAg negative, respectively ($P = 0.64$).

Prevalence of HCV antibody in the Kumasi HIV cohort

Four HCV RNA-positive and 67 HCV RNA-negative subjects that had sufficient sample volume stored underwent HCV antibody screening using the Architect automated chemiluminescence immunoassay and the Ortho and Monolisa enzyme immunoassays. The four HCV RNA-positive subjects tested antibody reactive in all three assays. With the 67 HCV RNA-negative subjects, 5 (7.5%), 5 (7.5%) and 19 (28.4%) samples tested antibody reactive by Architect, Ortho and Monolisa, respectively. Overall, 4/67 (6.0%) samples were antibody reactive in ≥2 assays including 2/67 (3.0%) that were reactive in all three assays. Screening assay concordance with HCV RNA-negative samples was 49/67 (73.1%) for Architect *vs* Monolisa, 61/67 (91.0%) for Architect *vs* Ortho and 49/67 (73.1%) for Ortho *vs* Monolisa. In HCV RNA-positive *vs* HCV RNA-negative subjects, median (IQR) antibody reactivity was 12.1 (8.4–12.6) *vs* 3.3 (1.0–4.7) by Architect ($P = 0.016$), 5.1 (4.9–5.2) *vs* 1.5 (1.1–3.6) by Ortho ($P = 0.016$) and 6.3 (6.0–6.4) *vs* 2.0 (1.3–3.1) by Monolisa ($P < 0.0001$).

Five samples that were HCV RNA negative and Architect reactive underwent supplementary testing by the INNO-LIA line immunoassay. Of these, three tested negative, one

indeterminate (anti-C2 reactivity alone) and one positive (reactivity with C1, C2 and NS3). The prevalence of confirmed HCV antibody reactivity in HCV RNA-negative subjects was therefore 1/67 (1.5%). The INNOLIA indeterminate sample was reactive by Architect only (S/CO 3.3), whereas the INNOLIA positive sample was reactive by all three assays, with reactivity levels of 5.2, 4.5 and 5.6 by Architect, Ortho and Monolisa, respectively. Using HCV RNA-positive samples ($n = 4$) and samples testing Architect reactive/INNO-LIA positive or indeterminate ($n = 2$) as a reference of confirmed or possible HCV infection, assay sensitivity and specificity were 100% and 95.4% for Architect, 83.3% and 93.8% for Ortho, and 83.3% and 72.3% for Monolisa (Table 1).

HCV genotypes

The four HCV RNA-positive samples underwent sequencing of the HCV core and NS5B genes. Three strains clustered with genotype 2 reference sequences whilst one strain clustered with genotype 1 reference sequences with concordant results between core (Fig. 1) and NS5B sequences (not shown). Further analysis of the genotype 2 strains identified one subtype 2 l strain (bootstrap values of 100% and 99.7% for core and NS5B, respectively); the core sequence also clustered closely with a strain previously detected among Kumasi blood donors [20], with a sequence homology of 91.6% (bootstrap value of 100%). The other two genotype 2 strains had less defined subtype assignment with poor bootstrap support: one strain clustered most closely with subtype 2j (bootstrap values of 9.6% and 23.3% for core and NS5B respectively); with the second strain, the core sequence clustered most closely with subtype 2k (bootstrap value of 9.3%), whereas the NS5B sequence cluster most closely with an unassigned Ghanaian strain (bootstrap value of 18.4%) (data not shown).

The genotype 1 strain was distinct from genotype 1 sequences from elsewhere in the world and did not cluster with any previously defined subtype with either the core or NS5B sequence (Fig. 2). There were two related African genotype 1 sequences in the Los Alamos database, one from Nigeria (core) and one from Equatorial Guinea (NS5B), with sequence homologies of 92% and 86.1%, respectively (bootstrap values of 42.7% and 38.3%).

HCV-positive subjects

The five HCV-positive subjects, four with a current infection and one with a confirmed past infection, comprised three males and two females aged 30–46 years (Table 2). Using a structured questionnaire, the reported risk factors for HCV infection were surgical procedures and blood transfusion and these had occurred ≥12 months prior to HCV testing; no patient reported any history of jaundice, intravenous or illicit drug use, needle stick injury, therapeutic or cosmetic cuts, or body tattooing.

DISCUSSION

Our study, the first to employ HCV RNA testing to determine HCV prevalence in HIV-positive patients in Ghana, found that about 1% of subjects carried HCV RNA. The findings are in agreement with the reported HCV RNA prevalence of 0.6–0.9% among blood donors in Ghana [20,21]. The infections were due to HCV genotypes 1 and 2, but there was a high molecular diversity, which is similar to findings from Ghanaian blood donors [20] and consistent with the proposed long-term endemicity of HCV genotypes 1 and 2 in West Africa [22]. The genotype 1 strain in particular may represent a variant that originated in Cameroon and spread to West and Central Africa [23,24], although full genome sequencing is required to provide a more detailed molecular characterization.

A further 1.5% of HIV-positive subjects showed convincing evidence of a past, resolved HCV infection, as indicated by consistent HCV antibody reactivity in three screening assays and confirmation by the supplementary line immunoassay. This result is again consistent with data from Kumasi blood donors [20]. Taken together, our findings do not suggest a marked excess risk of HCV infection in HIV-positive subjects in the region. The conclusion is in line with the observation that the reported risk factors for HCV infection were healthcare-related, with at least one of the

Table 1 Hepatitis C virus (HCV) antibody testing in 71 subjects from the Kumasi HIV cohort*

	Reactive	Non Reactive	True positive	False negative	True negative	False positive	Sensitivity (%)	Specificity (%)
Architect	9	62	6	0	62	3	100	95.4
Ortho	9	62	5	1	61	4	83.3	93.8
Monolisa	23	48	5	1	47	18	83.3	72.3

*HCV infection was indicated by either HCV RNA detection ($n = 4$; all samples were also anti-HCV reactive) or a negative HCV RNA result with Architect anti-HCV reactivity and either a positive (confirmed HCV infection) or indeterminate (possible HCV infection) line immunoassay result ($n = 2$).

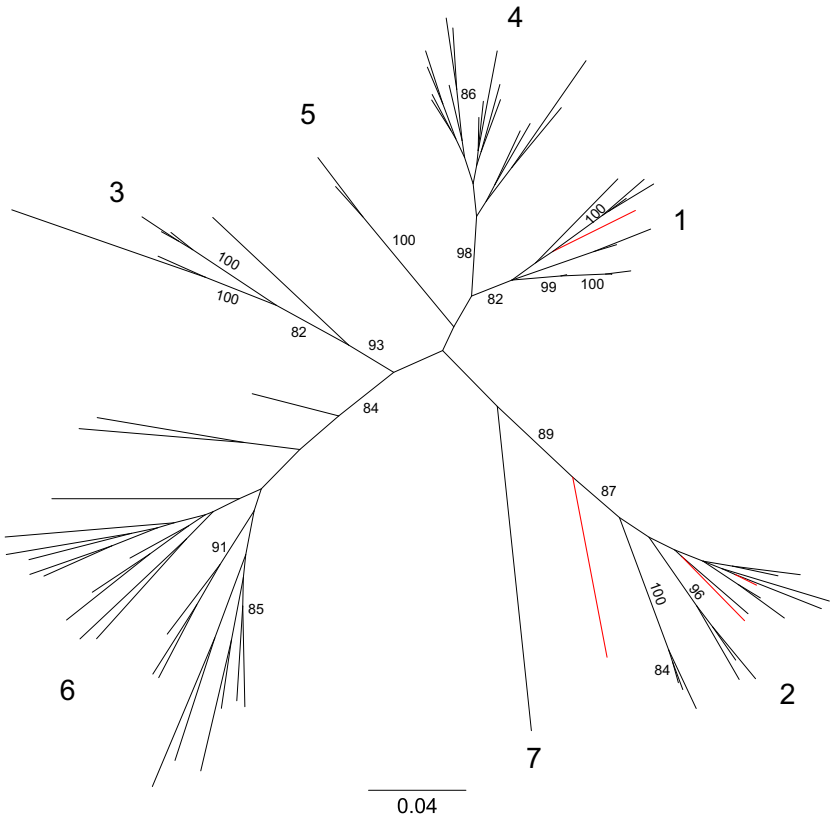

Fig. 1 Phylogenetic relationship between Hepatitis C virus (HCV) core sequences. Ghanaian sequences (in red) were aligned to reference sequences from the Los Alamos database. The seven major HCV genotypes (1–7) are indicated along the branches. Bootstrap values ≥80% are shown.

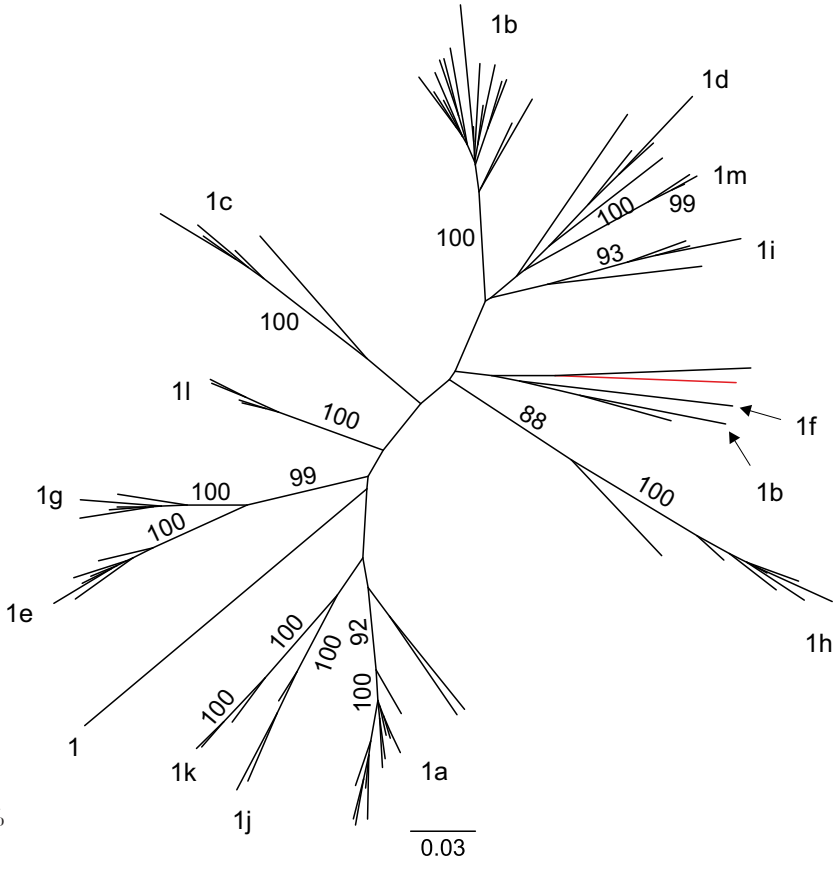

Fig. 2 Phylogenetic relationship between Hepatitis C virus (HCV) genotype 1 NS5B sequences. The genotype 1 Kumasi sample (in red) was aligned against genotype 1 sequences from throughout the world obtained from the Los Alamos database. HCV genotype 1 subtypes are indicated along the branches. Bootstrap values ≥80% are shown.

Table 2 Subjects from the Kumasi HIV cohort that tested HCV RNA positive and HCV antibody positive or indeterminate

						HCV			
ID	Gender	Age	HBsAg	CD4	ALT	Antibody	RNA	Genotype	Subtype
GH01	F	46	Negative	ND	ND	+	+	1	Unassigned
GH02	F	32	Positive	110	168	+	+	2	j/unassigned
GH03	M	43	Positive	441	50	+	+	2	l
GH04	M	41	Positive	385	44	+	+	2	k/unassigned
GH10	M	30	Positive	237	36	+	–	NA	NA
GH11	F	39	Positive	182	18	Indeterminate*	–	NA	NA

ND, Not done; NA, Not applicable. *Anti-HCV reactivity by Architect with indeterminate reactivity by line immunoassay (anti-C2 reactivity alone).

strains showing clustering with a strain previously detected in Kumasi blood donors [20]. It should be noted, however, that HCV prevalence was nearly double among HBsAg-positive subjects relative to those who tested HBsAg negative, although the difference did not reach statistical significance due to small numbers. There are an estimated 240 000 patients infected with HIV in Ghana [25], of whom 16.7% are coinfected with HBV [26]. Thus, our data indicate that Ghana harbours an estimated 521 subjects with triple HIV/HBV/HCV coinfection and 1440 subjects with HIV/HCV coinfection. Larger studies are required to confirm an association between HBV and HCV coinfection in this population.

After extensive validation, our approach proved to be effective for HCV RNA screening, reducing cost and labour, and confirming the previous observation that pooled testing is a realistic strategy for identifying HCV carriers in low-prevalence settings [27,28]. Pooling samples reduces assay sensitivity, which could result in the misdiagnosis of patients with a low HCV RNA load. However, previous studies indicated that among 20 Kumasi blood donors infected with HCV genotype 1 or 2, HCV RNA levels were median 2.5×10^5 IU/mL and ranged from 3.3×10^3 to 9.0×10^6 IU/mL [20], which is well above the lower limit of detection (550 IU/mL) of our assay.

Hepatitis C virus (HCV) antibody screening with three widely used commercial immunoassays [15,29–32] showed variable performance among subjects that tested HCV RNA negative. HCV antibody prevalence varied by screening assay, ranging from 7.5% to 28.4%, but declining to 3% when considering concordant reactivity with all three assays and to 1.5% when considering confirmed reactivity by the line immunoassay. In agreement with previous observations [31,32], there was good concordance between the Architect and Ortho assays, whereas the Architect and Monolisa assays gave highly discordant results. In Western cohorts, approximately 20–30% of HCV infections resolve spontaneously. It has been proposed that West African populations may have a higher rate of spontaneous clearance [20], possibly reflecting a high HLA-B57 frequency [33]. Our data, however, cast doubt over the reliability of available HCV prevalence estimates for Ghana [11,12,34,35] and elsewhere in Africa [10] that are based upon serological screening tests alone. Results of three previous studies from Uganda and Malawi are in agreement with our findings [13–15]. Among 500 HIV-positive subjects in Uganda, anti-HCV prevalence by the Ortho assay was 7.5% and similar to that found in the Kumasi HIV cohort. However, none of the anti-HCV reactive samples had detectable HCV RNA [15]. Among 380 hospitalized patients in Uganda, nearly 9% showed anti-HCV reactivity by a chemiluminescence assay, but only a small subset of these were confirmed by either a positive HCV RNA test (29%) or a positive blot immunoassay (7%) [13]. Among 2041 HIV-positive pregnant women in Malawi, HCV antibody prevalence by a chemiluminescence immunoassay was 5.3%, but none of the women tested HCV RNA positive and only 0.1% tested positive by blot immunoassay [14]. The mechanisms underlying the poor specificity of HCV antibody screening assays in Africa are not known. Persistent infections that trigger production of auto-antibodies (e.g. with *Schistosoma mansoni*) may cause cross-reactivity [36]. Consistent with this hypothesis, a positive Schistosoma ELISA was recently shown to be an independent predictor of Ortho-based anti-HCV reactivity among Ugandans [15]. Interestingly, the Ugandan study showed also that HIV-infected individuals were significantly less likely to have an Ortho HCV antibody reactive result than HIV-negative subjects [15].

There are limitations to this study. Sample availability allowed HCV antibody testing in a subset of HCV RNA-negative subjects. As the number of subjects with HCV infection was small, there was limited scope for detailed analyses of factors associated with HCV infection, indicating that large studies must be designed to define the epidemiological and clinical characteristics of HIV/HCV coinfection in Ghana. Nonetheless, our data provide novel

information with implications for both public health and clinical care. In the absence of additional testing, and when applying the recommended interpretative cut-offs, commercial HCV antibody screening assays variably overestimated the burden of HCV infection in HIV-infected adults in Ghana. As assay reactivity was significantly higher in samples that tested HCV RNA positive, applying higher interpretative cut-offs (i.e. 5 with the Architect and 4 with the Ortho) could reduce the number of samples requiring expensive supplementary and confirmatory testing. A possible testing algorithm suitable for resource-limited settings could employ a manual screening enzyme immunoassay with acceptable specificity such as the Ortho, followed by HCV RNA testing of samples with reactivity above the revised cut-off value. Enzyme immunoassays are easily accessible and widely used in sub-Saharan Africa, requiring little specialized infrastructure. HCV RNA testing continues to have limited availability, although the recommended introduction of routine HIV-1 RNA monitoring may in the future expand access to molecular assays in the region. In Ghana, HCV RNA testing is currently available in large research facilities and private laboratories. As an alternative, HCV antigen testing by immunoassays could be used to confirm a current infection [37]. The validity of these strategies should be investigated further.

ACKNOWLEDGEMENTS

This study was funded by the Leverhulme-Royal Society Africa Award. The authors have declared that no conflict of interests exists regarding the manuscript.

REFERENCES

1 Kim AY, Onofrey S, Church DR. An epidemiologic update on hepatitis C infection in persons living with or at risk of HIV infection. *J Infect Dis* 2013; 207(Suppl. 1): S1–S6.
2 Lin W, Weinberg EM, Chung RT. Pathogenesis of accelerated fibrosis in HIV/HCV co-infection. *J Infect Dis* 2013; 207(Suppl. 1): S13–S18.
3 Hernandez MD, Sherman KE. HIV/hepatitis C coinfection natural history and disease progression. *Curr Opin HIV AIDS* 2011; 6: 478–482.
4 Wilkins E, Nelson M, Agarwal K *et al.* British HIV Association guidelines for the management of hepatitis viruses in adults infected with HIV 2013. *HIV Med* 2013; 14(Suppl. 4): 1–71.
5 CDC. Testing for HCV infection: an update of guidance for clinicians and laboratorians. *MMWR Morb Mortal Wkly Rep* 2013; 62: 362–365.
6 Agwale SM, Tanimoto L, Womack C *et al.* Prevalence of HCV coinfection in HIV-infected individuals in Nigeria and characterization of HCV genotypes. *J Clin Virol* 2004; 31 (Suppl. 1): S3–S6.
7 Laurent C, Bourgeois A, Mpoudi-Ngolé E *et al.* High rates of active hepatitis B and C co-infections in HIV-1 infected Cameroonian adults initiating antiretroviral therapy. *HIV Med* 2010; 11: 85–89.
8 Madhava V, Burgess C, Drucker E. Epidemiology of chronic hepatitis C virus infection in sub-Saharan Africa. *Lancet Infect Dis* 2002; 2: 293–302.
9 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.
10 Barth RE, Huijgen Q, Taljaard J, Hoepelman AIM. Hepatitis B/C and HIV in sub-Saharan Africa: an association between highly prevalent infectious diseases. A systematic review and meta-analysis. *Int J Infect Dis* 2010; 14: e1024–e1031.
11 Brandful JA, Apeagyei FA, Ampofo WK *et al.* Relationship between immunoclinical status and prevalence of viral sexually transmitted diseases among human immunodeficiency virus-1 seropositive patients in Ghana. *Viral Immunol* 1999; 12: 131–137.
12 Sagoe KW, Agyei AA, Ziga F *et al.* Prevalence and impact of hepatitis B and C virus co-infections in antiretroviral treatment naïve patients with HIV infection at a major treatment center in Ghana. *J Med Virol* 2012; 84: 6–10.
13 Seremba E, Ocama P, Opio CK *et al.* Poor performance of hepatitis C antibody tests in hospital patients in Uganda. *J Med Virol* 2010; 82: 1371–1378.
14 Chasela CS, Wall P, Drobeniuc J *et al.* Prevalence of hepatitis C virus infection among human immunodeficiency virus-1-infected pregnant women in Malawi: the BAN study. *J Clin Virol* 2012; 54: 318–320.
15 Mullis CE, Laeyendecker O, Reynolds SJ *et al.* High frequency of false-positive hepatitis C virus enzyme-linked immunosorbent assay in Rakai, Uganda. *Clin Infect Dis* 2013; 57: 1747–1750.
16 Daniel HD, Grant PR, Garson JA, Tedder RS, Chandy GM, Abraham P. Quantitation of hepatitis C virus using an in-house real-time reverse transcriptase polymerase chain reaction in plasma samples. *Diagn Microbiol Infect Dis* 2008; 61: 415–420.
17 Van Doornum GJJ, Schutten M, Voermans J, Guldemeester GJJ, Niesters HGM. Development and implementation of real-time nucleic acid amplification for the detection of enterovirus infections in comparison to rapid culture of various clinical specimens. *J Med Virol* 2007; 79: 1868–1876.
18 Sandres-Sauné K, Deny P, Pasquier C, Thibaut V, Duverlie G, Izopet J. Determining hepatitis C genotype by analyzing the sequence of the NS5b region. *J Virol Methods* 2003; 109: 187–193.
19 Mellor J, Holmes EC, Jarvis LM, Yap PL, Simmonds P. Investigation of the pattern of hepatitis C virus sequence diversity in different geographical regions: implications for

virus classification. *J Gen Virol* 1995; 76: 2493–2507.
20 Candotti D, Temple J, Sarkodie F, Allain JP. Frequent recovery and broad genotype 2 diversity characterize hepatitis C virus infection in Ghana, West Africa. *J Virol* 2003; 77: 7914–7923.
21 Ampofo W, Nii-Trebi N, Ansah J *et al.* Prevalence of blood-borne infectious diseases in blood donors in Ghana. *J Clin Microbiol* 2002; 40: 3523–3525.
22 Jeannel D, Fretz C, Traore Y *et al.* Evidence for high genetic diversity and long-term endemicity of hepatitis C virus genotypes 1 and 2 in West Africa. *J Med Virol* 1998; 55: 92–97.
23 Bracho MA, Carrillo-Cruz FY, Ortega E, Moya A, González-Candelas F. A new subtype of hepatitis C virus genotype 1: complete genome and phylogenetic relationships of an Equatorial Guinea isolate. *J Gen Virol* 2006; 87: 1697–1702.
24 Ndjomou J, Pybus OG, Matz B. Phylogenetic analysis of hepatitis C virus isolates indicates a unique pattern of endemic infection in Cameroon. *J Gen Virol* 2003; 84: 2333–2341.
25 UNAIDS. http://www.unaids.org/en/regionscountries/countries/ghana/ (accessed 24 June 2014).
26 Geretti AM, Patel M, Sarfo FS *et al.* Detection of highly prevalent hepatitis B virus coinfection among HIV-seropositive persons in Ghana. *J Clin Microbiol* 2010; 48: 3223–3230.
27 Brant LJ, Ramsay ME, Balogun MA *et al.* Diagnosis of acute hepatitis C virus infection and estimated incidence in low- and high-risk English populations. *J Viral Hepat* 2008; 15: 871–877.
28 Keys JR, Leone PA, Eron JJ, Alexander K, Brinson M, Swanstrom R. Large scale screening of human sera for HCV RNA and GBV-C RNA. *J Med Virol* 2014; 86: 473–477.
29 Kim S, Kim JH, Yoon S, Park YH, Kim HS. Clinical performance evaluation of four automated chemiluminescence immunoassays for hepatitis C virus antibody detection. *J Clin Microbiol* 2008; 46: 3919–3923.
30 Wu FB, Ouyan HQ, Tang XY, Zhou ZX. Double-antigen sandwich time-resolved immunofluorometric assay for the detection of anti-hepatitis C virus total antibodies with improved specificity and sensitivity. *J Med Microbiol* 2008; 57: 947–953.
31 Maasoumy B, Bremer B, Raupach R *et al.* How to interpret borderline HCV antibody test results: a comparative study investigating four different anti-HCV assays. *Viral Immunol* 2014; 27: 7–13.
32 Berger A, Rabenau H, Allwinn R, Doerr HW. Evaluation of the new ARCHITECT anti-HCV screening test under routine laboratory conditions. *J Clin Virol* 2008; 43: 158–161.
33 Chuang WC, Sarkodie F, Brown CJ *et al.* Protective effect of HLA-B57 on HCV genotype 2 infection in a West African population. *J Med Virol* 2007; 79: 724–733.
34 Kubio C, Tierney G, Quaye T *et al.* Blood transfusion practice in a rural hospital in Northern Ghana, Damongo, West Gonja District. *Transfusion* 2012; 52: 2161–2166.
35 Nkrumah B, Owusu M, Averu P. Hepatitis B and C viral infections among blood donors. A retrospective study from a rural community of Ghana. *BMC Res Notes* 2001; 4: 529–532.
36 Agha S, El-Mashad N, El-Malky M *et al.* Prevalence of low positive anti-HCV antibodies in blood donors: *Schistosoma mansoni* co-infection and possible role of autoantibodies. *Microbiol Immunol* 2006; 50: 447–452.
37 Mederacke I, Potthoff A, Meyer-Olson D *et al.* HCV core antigen testing in HIV- and HBV-coinfected patients, and in HCV-infected patients on hemodialysis. *J Clin Virol* 2012; 53: 110–115.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1: GenBank accession numbers of reference sequences used in the phylogenetic analysis of the core region. The phylogenetic tree can be seen in Fig. 1.

Table S2: GenBank accession numbers of sequences used in the phylogenetic analysis of the NS5B region of the genotype 1 sequence GH01. The phylogenetic tree can be seen in Fig. 2.