High Frequency of False-Positive Hepatitis C Virus Enzyme-Linked Immunosorbent Assay in Rakai, Uganda

Caroline E. Mullis,[1,a,b] Oliver Laeyendecker,[1,2,a] Steven J. Reynolds,[1,2] Ponsiano Ocama,[3] Jeffrey Quinn,[1] Iga Boaz,[4] Ronald H. Gray,[5] Gregory D. Kirk,[5] David L. Thomas,[1] Thomas C. Quinn,[1,2] and Lara Stabinski[2,c]

[1]Department of Medicine, School of Medicine, Johns Hopkins University, Baltimore, and [2]Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; [3]Department of Medicine, Makerere University, Kampala, and [4]Rakai Health Sciences Program, Entebbe, Uganda; and [5]Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland

The prevalence of hepatitis C virus (HCV) infection in sub-Saharan Africa remains unclear. We tested 1000 individuals from Rakai, Uganda, with the Ortho version 3.0 HCV enzyme-linked immunosorbent assay. All serologically positive samples were tested for HCV RNA. Seventy-six of the 1000 (7.6%) participants were HCV antibody positive; none were confirmed by detection of HCV RNA.

Keywords. hepatitis C virus; ELISA; Africa.

Chronic hepatitis C virus (HCV) infection is a leading cause of hepatocellular carcinoma and cirrhosis [1, 2]. Estimates of the burden of HCV infection in sub-Saharan Africa vary widely, but the overall seroprevalence is estimated at 3.0%, compared to <1%–2% in developed countries [2]. Understanding the burden of HCV in sub-Saharan Africa is of particular importance because of the high human immunodeficiency virus (HIV) prevalence and the increased rate of complications arising from HIV and HCV coinfection [1, 3]. As more HIV-infected individuals in sub-Saharan Africa gain access to highly active antiretroviral therapy (HAART) with resulting declines in AIDS-related complications, liver disease associated with hepatitis B virus (HBV) and HCV could become an increasingly important cause of morbidity and mortality [4]. Additionally, knowledge of HCV seroprevalence is critical for screening the blood supply [3, 5].

In sub-Saharan Africa, HCV prevalence has primarily been estimated by HCV antibody screening, without confirmatory virologic testing because of the high cost associated with the latter tests [1, 3, 4]. In Uganda, HCV seroprevalence estimates range from 0.0% to 14.6% [1], but the observed variation may reflect the performance of HCV antibody tests in this setting [5]. Despite high sensitivity and specificity in US populations [6], HCV antibody tests have been shown to have high false-positive misclassification in African populations, suggesting that the reported seroprevalence may be inflated [7, 8]. Infection with malaria, syphilis, or HIV, malnutrition, and various chronic diseases have been hypothesized to increase the false positivity of HCV antibody tests, although these associations remain speculative [7].

MATERIALS AND METHODS

We report analyses conducted on 500 HIV-infected individuals enrolled in HIV care with the Rakai Health Sciences Program frequency-matched by age, sex, and community to 500 HIV-uninfected participants of the Rakai Community Cohort Study [9]. Data collection included a structured interview focusing on exposures potentially associated with liver disease, collection of blood specimens, and a transient elastography (Fibroscan, Echosense, Paris, France) examination to noninvasively quantify liver fibrosis [9]. Methods for determining HIV type 1 (HIV-1) status and CD4 nadir were previously described [9]. Presence of HBV surface antigen and antibodies to schistosomiasis were determined using an ETI-EKB s Plus enzyme-linked immunosorbent assay (ELISA; Diasorin, Vercelli, Italy) and Schisto-96 soluble egg antigen ELISA (IVD Research Inc, Carlsbad, California), respectively [9]. All participants provided informed consent. Institutional review board approval for this study was received from the National Institute of Allergy and Infectious Diseases, the Johns Hopkins Medical Institutions, the Western Institutional Review Board (Olympia, Washington), the Scientific and Ethics Committee of the Uganda Virus Research Institute, and the Uganda National Council for Science and Technology.

Serum samples were tested manually using the Ortho HCV version 3.0 ELISA Test System (Ortho Clinical Diagnostics,

Received 27 June 2013; accepted 10 September 2013; electronically published 18 September 2013.

[a]C. E. M. and O. L. contributed equally to this work.

[b]Present affiliation: New York Medical College, Valhalla, New York.

[c]Present affiliation: Office of the Global AIDS Coordinator, US State Department, Washington, District of Columbia.

Correspondence: Oliver Laeyendecker, MS, MBA, PhD, LIR/NIAID/NIH, 855 N Wolfe St, Rm 538A, Baltimore, MD (olaeyen1@jhmi.edu).

Clinical Infectious Diseases 2013;57(12):1747–50

Published by Oxford University Press on behalf of the Infectious Diseases Society of America 2013. This work is written by (a) US Government employee(s) and is in the public domain in the US.

DOI: 10.1093/cid/cit602

Raritan, New Jersey). All samples were tested in a single well, with initially reactive samples being retested in duplicate before final interpretation. Any sample upon retesting that was reactive for HCV antibody in either or both wells was then considered repeatedly reactive and classified as HCV ELISA positive for this analysis. Viral RNA from repeatedly reactive samples was extracted using a QIAamp Viral RNA Mini Kit (Qiagen, Valencia, California) and quantified using an Abbott RealTime HCV Assay (Abbott Molecular Inc, Des Plaines, Illinois). Nested reverse transcription polymerase chain reaction (RT-PCR) was then performed on the CORE/E1 region of the HCV genome for qualitative analysis of samples reactive by the HCV RealTime assay [10]. To determine if the samples were free of inhibitors for the amplification of HCV virus, we performed a second experiment where we added 50 000 IU of HCV viral particles (AcroMetrix HCV Panel, AcroMetrix Corporation, Carlsbad, California) into 10 samples preextraction with strongly reactive (S/C > 5.0) HCV ELISA results. Additionally, HIV RNA loads using the Abbott RealTime HIV-1 assay were generated for 10 infected subjects who were HCV ELISA positive.

Analyses examined associations of HCV-positive ELISA with risk factors for HCV or liver disease, including age, sex, current alcohol use, heavy liquor use (≥1.25 L/week), lifetime occupational fishing, and current herb use, and with clinical parameters including HBV surface antigen, *Schistosoma* antibody, and liver stiffness measured by transient elastography. Univariate and multivariate logistic regression was used to estimate the odds ratios and 95% confidence intervals of HCV ELISA positivity. Multivariate logistic regression analysis was performed including age, sex, and covariates with a *P* value of <.1 in univariate analyses. All statistical analysis was performed using Stata software, version 11.0.

RESULTS

Among the 500 HIV-infected and 500 HIV-uninfected samples tested, 76 (7.6%) were classified as HCV ELISA positive

Table 1. Factors Associated With Positive Hepatitis C Virus Enzyme-Linked Immunosorbent Assay

Factor	% HCV ELISA Positive (No.)	OR (95% CI)	*P* Value	Adjusted OR (95% CI)	*P* Value
Age, y					
<30	18.42 (14/191)	1		1	
30–34	13.16 (10/220)	0.60 (.26–1.39)	.234	0.61 (.26–1.42)	.255
35–39	18.42 (14/192)	0.99 (.46–2.15)	.989	0.99 (.46–2.17)	.989
40–44	23.68 (18/167)	1.53 (.73–3.17)	.257	1.57 (.75–3.30)	.235
45–49	17.11 (13/152)	1.18 (.54–2.60)	.676	1.20 (.54–2.66)	.653
≥50	9.21 (7/78)	1.25 (.48–3.22)	.649	1.39 (.53–3.67)	.505
Sex					
Female	6.87 (46/670)	1		1	
Male	9.09 (30/330)	1.36 (.84–2.19)	.213	1.23 (.754–2.02)	.404
HIV status					
Negative	9.00 (45/500)	1		1	
Positive	6.20 (31/500)	0.67 (.42–1.08)	.097	0.61 (.37–1.00)	.049
Schistosoma Ab					
Negative	6.58 (58/882)	1		1	
Positive	15.38 (18/117)	2.58 (1.46–4.56)	.001	2.80 (1.57–5.01)	.001
Liver stiffness					
<7.3 kPa	7.41 (5/675)	1		. . .	
7.3–9.3 kPa	8.67 (13/150)	1.18 (.93–2.24)	.600	. . .	. . .
≥9.3 kPa	7.43 (13/175)	1.00 (.53–1.89)	.992	. . .	. . .
Current herb use					
No	6.52 (3/46)	1		. . .	
Yes	7.65 (73/954)	0.84 (.26–2.78)	.778	. . .	. . .
Current alcohol use					
No	7.54 (61/809)	1		. . .	
Yes	7.85 (15/191)	1.05 (.58–1.88)	.883	. . .	. . .

Abbreviations: Ab, antibody, CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; HCV, hepatitis C virus; HIV, human immunodeficiency virus; kPa, kilopascal; OR, odds ratio.

(median signal to cutoff ratio = 2.83; interquartile range [IQR], 1.70–6.57). None of the 76 participants had a history of treatment for HCV infection. Two of the 76 HCV ELISA–positive samples were reactive for HCV RNA using the Abbott RealTime HCV Assay but had low levels (<30 IU/mL). Those 2 samples were not positive by a highly sensitive in-house nested RT-PCR. Furthermore, all 10 samples spiked with a known quantity of HCV amplified the correct viral load, demonstrating that no inhibition to viral amplification existed in the samples. Samples from HIV-positive subjects not on HAART had HIV loads comparable to historical data (median viral load, 4.4 $\log_{10}$ copies/mL; IQR, 3.7–4.6).

In univariate analysis, HCV seropositivity was not associated with liver fibrosis. Age, sex, HIV status, and current herb or alcohol use were also not significantly associated with HCV seropositivity (Table 1). Having a positive HCV ELISA result was significantly associated with a positive *Schistosoma* antibody ELISA ($P = .001$). No individuals with a false-positive HCV ELISA were classified as having chronic HBV infection (HBV antigen positive), being a lifetime occupational fisher, or being a heavy liquor user (≥1.25 L/week). In multivariable analysis, HIV-infected individuals were significantly less likely to have an HCV ELISA–positive result ($P = .049$), whereas individuals with a positive *Schistosoma* ELISA were more likely to be HCV seropositive ($P = .001$). Of 76 samples with a positive HCV ELISA, 18 samples (23.7%) were positive for *Schistosoma* antibody.

CONCLUSIONS

No HCV third-generation ELISA–positive samples were confirmed by dual HCV RNA assays in this rural population in Rakai, Uganda. The absence of detectable viremia strongly suggests a low prevalence of ongoing chronic HCV infection. As approximately 30% of HCV infections spontaneously resolve and clear HCV RNA but not antibody, it is possible that some of the observed ELISA-positive, HCV RNA–negative samples reflect cleared HCV infections. However, the absence of any ELISA-positive, RNA-positive samples would suggest that the majority represent false-positive tests. As all Ortho ELISA plates met the manufacturer's quality control acceptance criteria and the RT-PCR controls were also valid, it is unlikely that the observed findings were a result of a defective kit. Additionally, the HCV spiking experiment and the presence of HIV RNA demonstrated that no inhibitors were present in samples highly reactive by the HCV enzyme immunoassay. These results are strikingly similar to those from a recent study from Malawi, where none of the 110 samples that were serologically reactive for HCV using the Ortho Vitros anti-HCV chemiluminescent immunoassay were HCV RNA positive with a Cobas Amplicor HCV Test version 2.0 [8]. We did find a strong association between a positive HCV ELISA result and a positive *Schistosoma* ELISA, which may reflect a cross-reaction of autoimmune markers associated with *Schistosoma mansoni* infection [11]. Most importantly, our data demonstrate no association of HCV seroreactivity with the degree of liver fibrosis measured by transient elastography.

These findings have public health implications. The high HCV seroreactivity combined with the rarity of detectable HCV RNA suggests that screening blood donations in this population with an ELISA test may result in the inappropriate disposal of a substantial proportion of blood products. The high frequency of misclassification observed when using the Ortho version 3.0 ELISA suggests that prevalence estimates based on ELISA results alone may be inflated in similar sub-Saharan African populations; confirmation with nucleic acid testing should be emphasized.

Notes

Acknowledgments. The authors acknowledge the contributions of the participants and the members of the Rakai Health Sciences Program.

Financial support. This work was supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH). Additional support was provided by the HIV Prevention Trials Network sponsored by the NIAID, the Eunice Kennedy Shriver National Institute of Child Health and Human Development, the National Institute on Drug Abuse, the National Institute of Mental Health, and the Office of AIDS Research, of the NIH, Department of Health and Human Services (UM1-AI068613 and R37 DA 13806).

Author contributions. Conceived and designed the experiments: C. E. M., O. L., L. S. Organized data collection: S. J. R., P. O., I. B., L. S. Performed the experiments: C. E. M., O. L., J. Q., L. S. Analyzed the data: C. E. M., O. L., L. S. Contributed reagents/materials/analysis tools: S. J. R., P. O., R. H. G., G. D. K., D. L. T., T. C. Q. Wrote the paper: C. E. M., O. L., S. J. R., P. O., J. Q., I. B., R. H. G., G. D. K., D. L. T., T. C. Q., L. S.

Disclaimer. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the NIH, the US State Department, or the Johns Hopkins University.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Madhava V, Burgess C, Drucker E. Epidemiology of chronic hepatitis C virus infection in sub-Saharan Africa. Lancet Infect Dis **2002**; 2:293–302.
2. Lavanchy D. Evolving epidemiology of hepatitis C virus. Clin Microbiol Infect **2011**; 17:107–15.
3. Walusansa V, Kagimu M. Screening for hepatitis C among HIV positive patients at Mulago Hospital in Uganda. Afr Health Sci **2009**; 9:143–6.
4. Modi A, Feld J. Viral hepatitis and HIV in Africa. AIDS Rev **2007**; 9:25–39.
5. Ocama P, Seremba E. Management of HIV and hepatitis C virus infections in resource-limited settings. Curr Opin HIV AIDS **2011**; 6:539–45.
6. Scheiblauer H, El-Nageh M, Nick S, Fields H, Prince A, Diaz S. Evaluation of the performance of 44 assays used in countries with limited resources for the detection of antibodies to hepatitis C virus. Transfusion **2006**; 46:708–18.
7. Seremba E, Ocama P, Opio CK, et al. Poor performance of hepatitis C antibody tests in hospital patients in Uganda. J Med Virol **2010**; 82:1371–8.

8. 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**; 54:318–20.
9. Stabinski L, Reynolds SJ, Ocama P, et al. High prevalence of liver fibrosis associated with HIV infection: a cross-sectional study in rural Rakai, Uganda. Antivir Ther **2011**; 16:405–11.
10. Ray S, Arthur R, Carella A, Bukh J, Thomas D. Genetic epidemiology of hepatitis C virus throughout Egypt. J Infect Dis **2000**; 182:698–707.
11. 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: 447–52.