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## Prevalence of hepatitis C virus infection among human immunodeficiency virus-1-infected pregnant women in Malawi: The BAN study\*

Charles S. Chasela<sup>a,b,c</sup>, Patrick Wall<sup>b</sup>, Jan Drobeniuc<sup>d</sup>, Caroline C. King<sup>d</sup>, Eyasu Teshale<sup>d</sup>, Mina C. Hosseinipour<sup>a</sup>, Sascha R. Ellington<sup>d</sup>, Mary Codd<sup>b</sup>, Denise J. Jamieson<sup>d</sup>, Rodney J. Knight<sup>e</sup>, Patricia Fitzpatrick<sup>b</sup>, Athena P. Kourtis<sup>d</sup>, Irving F. Hoffman<sup>f</sup>, Dumbani Kayira<sup>a</sup>, Noel Mumba<sup>a</sup>, Deborah D. Kamwendo<sup>a</sup>, Francis Martinson<sup>a</sup>, William Powderly<sup>b</sup>, Charles van der Horst<sup>f</sup>, Saleem Kamili<sup>d,\*</sup>, and the BAN team

<sup>a</sup>UNC Project, Lilongwe, Malawi <sup>b</sup>University College Dublin, Belfield, Dublin, Ireland <sup>c</sup>University of Witwatersrand, School of Public Health, Johannesburg, South Africa <sup>d</sup>Centers for Disease Control and Prevention, Atlanta, GA, USA <sup>e</sup>Principia International, Chapel Hill, NC, USA <sup>f</sup>University of North Carolina, Chapel Hill, NC, USA

### Abstract

**Background**—In Sub-Saharan Africa, prevalence estimates of hepatitis C virus (HCV) vary widely.

**Objectives**—To assess the prevalence of HCV infection among HIV-infected, pregnant women screened for a large clinical trial in Lilongwe, Malawi.

**Study design**—Plasma from 2041 HIV-infected, pregnant women was screened for anti-HCV IgG using a chemiluminiscent immunometric assay (CIA). Specimens with a signal-cut-off ratio 1.00 were considered reactive and those with S/Co ratio < 1.00 non-reactive. All CIA-reactive specimens were tested by a recombinant immunoblot assay (RIBA) for anti-HCV and by PCR for HCV RNA.

**Results**—Of 2041 specimens, 110 (5.3%, 95% CI: 4.5–6.5%) were CIA reactive. Of the 109 CIA reactive specimens available for RIBA testing, 2 (1.8%) were positive, 28 (25.7%) were indeterminate, and 79 (72.5%) were negative. All CIA-reactive specimens were HCV RNA negative ( $n = 110$ ). The estimated HCV prevalence based on the screening assay alone was 5.3%; based on supplemental RIBA testing, the status of HCV infection remained indeterminate in 1.4% (28/2040, 95% CI: 0.1–2.0) and the prevalence of confirmed HCV infections was 0.1% (2/2040, 95% CI: 0–0.4%).

\*The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

\*Corresponding author at: Division of Viral Hepatitis, Centers for Disease Control & Prevention, Atlanta, GA, USA. Tel.: +1 4046394431; fax: +1 4046391563. [skamili@cdc.gov](mailto:skamili@cdc.gov) (S. Kamili).

#### Competing interest

None to declare.

#### Ethical approval

The study was a sub-study of the Breastfeeding, Antiretrovirals, and Nutrition (BAN) study and was approved by the National Health Sciences Research Committee (NHSRC), the University of North Carolina Chapel Hill, and Centers for Disease Control and Prevention.

**Conclusions**—HCV seroprevalence among HIV-infected, pregnant women in Malawi confirmed by supplemental RIBA HCV 3.0 is low (0.1%); CIA showed a high false-reactivity rate in this population.

### Keywords

HIV; HCV; Pregnant women; Malawi

## 1. Background

Worldwide, approximately 170 million are infected with hepatitis C virus (HCV); 40 million are infected with human immunodeficiency virus type-1 (HIV); and, 4.5 million are infected with both viruses.<sup>1,2</sup> Co-infection with HCV and HIV is of particular concern in Sub-Saharan Africa.<sup>3</sup> In Malawi, the estimated prevalence of HCV infection varies widely (0.7–16.5%) by subpopulation and whether supplemental testing confirmed screening results.<sup>5–10</sup> The only study to date among HIV-infected, pregnant women in Malawi estimated the prevalence of non-confirmed, anti-HCV positivity at 16.5%.<sup>4</sup> Only one study in Malawi employed both serologic and supplemental nucleic acid testing for HCV infection, and it reported that only one in 10 HCV screening positive samples was positive for HCV ribonucleic acid (RNA).<sup>7</sup> With the high prevalence of HIV-infection and increasing access to life-prolonging, antiretroviral therapy in Sub-Saharan Africa, liver disease is emerging as a major cause of morbidity and mortality among HIV-infected persons, particularly those co-infected with hepatitis.<sup>10,11</sup> It is imperative that we understand the limitations of the current assays for detection of HCV infection and more accurately estimate the prevalence of HCV and HIV co-infection.

## 2. Objectives

The objective was to assess the prevalence of HCV infection using screening and supplemental confirmatory testing among HIV-infected, pregnant women being screened for a large clinical trial in Lilongwe, Malawi.

## 3. Study design

We analysed plasma specimens from 2041 antiretroviral-naive, HIV-infected, pregnant (<30 weeks gestation) women collected at pre-inclusion screening for the Breastfeeding, Antiretrovirals, and Nutrition (BAN) Study ([www.TheBANStudy.org](http://www.TheBANStudy.org)).<sup>12,13</sup> The study was approved by the Malawi National Health Sciences Research Committee and institutional review boards at the University of North Carolina at Chapel Hill and the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta, GA. All women provided written, informed consent for specimen storage and future laboratory studies. Specimens were shipped from –80 °C storage in Lilongwe (Malawi) to CDC for testing. All specimens were tested for anti-HCV IgG using a chemiluminescent immunoassay (CIA) (VITROS aHCV; Ortho-Clinical Diagnostics, Inc., Rochester, NY) according to manufacturer's instructions. Specimens with a signal-to-cutoff (S/Co) ratio  $\geq 1.00$  were considered reactive, whereas those with an S/Co < 1.00 were considered non-reactive. All CIA-reactive specimens underwent supplemental testing with a recombinant immunoblot assay (RIBA) (RIBA HCV 3.0 SIA; Novartis Inc., New York, NY) and PCR for HCV RNA by a qualitative assay (COBAS AMPLICOR HCV Test v2.0; Roche Molecular Diagnostics, Indianapolis, IN). Using CDC guidelines,<sup>14</sup> a specimen was considered (i) anti-HCV negative if it was CIA non-reactive or RIBA negative; (ii) anti-HCV positive if CIA-reactive and RIBA or HCV RNA positive; (iii) furthermore, since there was no possibility to collect a sample for repeat anti-HCV or HCV RNA testing after >1 month all specimens that were CIA-reactive with an indeterminate RIBA and negative HCV RNA result were considered anti-HCV

indeterminate. Also according to CDC testing guidelines, specimens with a CIA S/Co ratio of >8.0 (established for Ortho HCV CIA) can be considered HCV positive without supplemental testing,<sup>14</sup> and we evaluated the utility of this cut-off for predicting positive RIBA and HCV RNA results in this population. HCV prevalence was estimated using binomial exact methods.

#### 4. Results

The women had a median age of 25 (interquartile range (IQR): 22–29, range: 14–45) years, CD4 count of 425 (IQR: 300–571, range: 100–1218) cells/mm<sup>3</sup>, and alanine aminotransferase (ALT) level of 13 (IQR: 10–15, range: 2–74) IU/L. Overall, the population was of low socioeconomic status, as indicated by a low percentage (18%) with electricity in the home.

Of 2041 specimens, 110 (5.4%, 95% CI: 4.5–6.5%) were CIA reactive (Fig. 1); 98 had S/Co ≤ 8.0, and 12 had S/Co > 8.0. Of the 98 with a S/Co ≤ 8.0, 97 were tested by RIBA; 1 had insufficient volume and was excluded from further analysis. Of the 97, 1 (1.0%) was positive, 27 (27.8%) were indeterminate, and 69 (71.1%) were negative by RIBA; all were HCV RNA negative. Of the 12 samples with S/Co > 8.0, 1 (8.3%) was positive, 1 (8.3%) was indeterminate, and 10 (83.3%) were negative by RIBA; all were HCV RNA negative. The two specimens that tested RIBA positive had CIA S/Co ratios of 15.5 and 4.6. Therefore, a CIA S/Co > 8.0 did not correlate with RIBA and HCV RNA PCR positivity in this population.

Overall, of the 109 CIA reactive specimens tested by RIBA, 2 (1.8%) were positive, 28 (25.7%) were indeterminate, and 79 (72.5%) were negative. None of the CIA-reactive specimens tested positive for HCV RNA. The two women who were RIBA positive had a significantly higher median ALT level (29 IU/L) compared to women with indeterminate (12 IU/L) or negative (13 IU/L) RIBA results ( $p = 0.02$ ).

The RIBA confirmed anti-HCV prevalence among these HIV-infected pregnant women in Malawi was 0.1% (2/2040, 95% CI: 0–0.4%), while 1.4% of participants had anti-HCV indeterminate results. Among the 81 women with reactive CIA and determinate RIBA results, the positive predictive value (PPV) of the CIA for RIBA reactivity was 2.5% (2 of 81, 95% CI: 0.8–8.5%) and the false-positive rate was 97.5% (79 of 81, 95% CI: 91.5–99.2%). The specificity of the CIA assay for evidence of HCV infection among women in this study is uncertain since CIA-non-reactive specimens were not tested by RIBA or HCV RNA. However, following CDC guidelines, we considered CIA-non-reactive specimens ( $n = 1931$ ) anti-HCV negative and excluded the 28 indeterminate results from the denominator (2040) to estimate the CIA specificity at 96.0% (1931/2012, 95% CI: 95.0–96.8%).

#### 5. Discussion

The prevalence of HCV was 5.3% by CIA and 0.1% by supplemental RIBA among this population of HIV-infected, pregnant women in Malawi. The only other study in Malawi that used supplementary testing with RIBA and HCV RNA reported a HCV prevalence of 6.8% by CIA and 0.7% by confirmed HCV RNA among blood donors.<sup>7</sup> These estimates of HCV prevalence in Malawi are consistent with other studies in Sub-Saharan Africa that used supplemental testing.<sup>4,15–17</sup> Together these studies indicate that most HCV prevalence estimates from Sub-Saharan Africa based on screening assays are overestimated. Continued HCV screening without supplemental confirmatory testing of blood donations would result in discarding uninfected blood, and the disclosure of test results may cause unnecessary despair to those misdiagnosed and the permanent withdrawal of uninfected donors from the

small donor pool. Future efforts should focus on improving the HCV testing algorithm in Sub-Saharan Africa.

Our data does not support the use of a CIA S/Co ratio  $\geq 8.0$  for Vitros CIA to screen for HCV infection among HIV-infected, pregnant women in Malawi. Current CDC HCV testing guidelines suggest that CIA-reactive samples with a S/Co ratio  $\geq 8.0$  for Vitros CIA may be reported as anti-HCV positive without further supplemental testing.<sup>14</sup> Although this criterion would reduce screening costs, our study indicates that in such low prevalence settings, S/Co ratio thresholds established for various assays would result in false-positive results and may not reflect true HCV infections.

Our study had some limitations. The number of CIA reactive specimens that gave indeterminate RIBA results was high, but this observation has been noted elsewhere in settings of low HCV seroprevalence<sup>16</sup>; to determine HCV status in persons with such results another sample should be collected for repeat anti-HCV or for HCV RNA testing  $> 1$  month later. Intermittent viremia is known to occur in HCV infection,<sup>14</sup> which could lead to a negative single HCV RNA test. The CDC guidelines are based on studies conducted among different populations in the United States and thus may not be fully applicable to the population of HIV-infected, pregnant women in Malawi. Furthermore, the main BAN trial was not designed to answer questions about HCV and lacks data on history of blood transfusion and injection drug use, which are rare in Malawi.

In conclusion, HCV seroprevalence among HIV-infected, pregnant women in Malawi is low when confirmed by supplemental RIBA HCV 3.0. The CIA showed a high false-reactivity rate. Given the high cost of supplemental testing by RIBA and nucleic acid tests, there is a need to evaluate the performance of available anti-HCV assays (including available rapid tests) in various populations in Malawi and other African settings, including the general population, blood donors, and risk groups.

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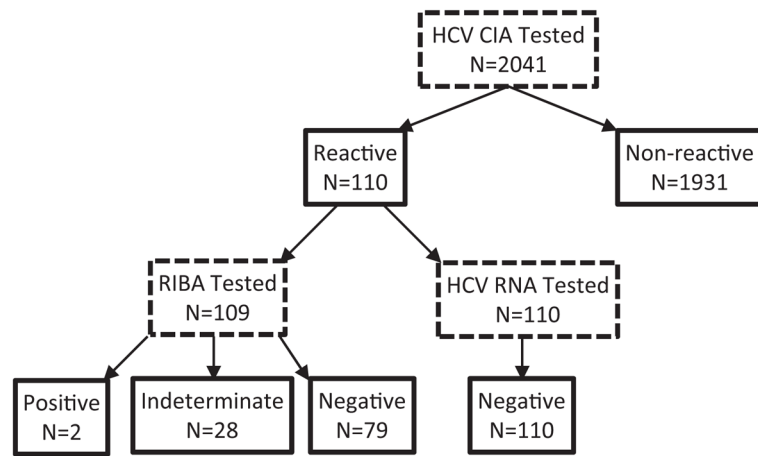
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**Fig. 1.** Hepatitis C CIA with supplemental RIBA and HCV RNA testing of 2041 HIV-infected, pregnant women in Malawi.