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Poor Performance of Hepatitis C Antibody Tests in Hospital Patients in Uganda

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Most hepatitis C testing in Uganda is performed using commercial rapid strip assays (RSA) to detect antibodies to hepatitis C virus (anti-HCV), rather than enzyme immunoassays (EIA). The prevalence of hepatitis C antibodies in a Ugandan hospital population was determined using both methods to test their accuracy using nucleic acid testing (NAT) as a reference. Sera from 380 consecutive hospitalized Ugandan patients were tested for anti-HCV using an RSA in Uganda, with subsequent automated third-generation EIA testing in the United States, followed by NAT. Recombinant immunoblot assays (RIBA) were used as a supplementary test to detect anti-HCV epitopes. Overall, anti-HCV was detected in 48/380 (13%) by one or both antibody tests. Anti-HCV was detected in 19 (5.0%) patients by RSA and in 33 (8.7%) patients by EIA; only four patients were anti-HCV positive by both methods. Fourteen of the 48 anti-HCV positive patients had detectable serum HCV RNA, 7 each by bDNA assay or by PCR. RSA detected only 7 of 14 HCV RNA positive sera. Of 29 RNA negative but anti-HCV positive patients tested by RIBA, only two were anti-HCV positive; 27 were anti-HCV negative or indeterminate. Anti-HCV testing by RSA and/or EIA was neither sensitive nor specific for detection of ongoing HCV infection in hospitalized Ugandan patients. Our findings underscore the importance of confirmatory nucleic acid testing, which, despite its increased cost, appears essential to manage African patients with HCV. ***J. Med. Virol. 82:1371–1378, 2010.*** © 2010 Wiley-Liss, Inc.

KEY WORDS: rapid slide test; enzyme immunoassay; nucleic acid testing; anti-HCV

INTRODUCTION

The diagnosis of hepatitis C virus (HCV) infection remains a major clinical and public health problem in sub-Saharan Africa where as many as 3% of the population have been exposed [Madhava et al., 2002; Modi and Feld, 2007]. Reported infection rates vary widely, and the true disease burden is not known. One explanation for widely divergent HCV prevalence is the method(s) used to detect HCV infection. Most data are derived from anti-HCV antibody testing. In US populations, the sensitivity and specificity of anti-HCV detection is considered to be very high using third generation assays that require multiple antigens including HCV NS5 protein and these assays have been standard of care for some time [Allain, 1998; Ghany et al., 2009]. The performance of EIAs in Africa has not been well defined [Rouet et al., 2004; Hladik et al., 2006; Scheiblauer et al., 2006; Desbois et al., 2008]. The price and requirement for complex equipment significantly limit wider use of EIA in the developing world. Instead, rapid strip assays (RSA) are utilized throughout Africa because they are inexpensive and give results within 10–20 min. The accuracy of HCV antibody testing in sub-Saharan Africa by either method has not been fully established but has major implications in regard to estimating disease burden and the prevention of transmission of HCV by transfusion [Birungi, 2005]. The purpose of this study was to assess the accuracy of HCV testing in a large panel of sera from an in hospital population in Uganda.

ES, PO, CKO, MMK, HJY, NA and DT all have no conflicts of interest.

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PATIENTS AND METHODS

Patients

This was a cross sectional study in which patients admitted to the Emergency Medical Ward of Mulago Hospital, Kampala, Uganda were recruited in January 2006. The study was approved by the Department of Internal Medicine, Makerere University Medical School, Faculty of Medicine Research and Ethics Committee, Uganda National Council of Science and Technology, and the Institutional Review Board of the University of Texas Southwestern Medical Center at Dallas. Consecutive patients admitted on the ward were approached and briefed on the study. Informed consent was obtained from those who expressed interest in participating. Minor children (n = 12, ages 13–17) were recruited after their assent and consent by their legal guardians had been obtained. Unconscious patients were excluded. Blood samples were collected in serum tubes. Sera were stored in cryo-vials at 4°C for 30–60 min, prior to transfer to a −80°C freezer. In addition to serum specimens, socio-demographic data and information on infection risk factors (including transfusions, surgery, therapeutic cuts, and cosmetic cuts) was collected by administered questionnaire. Questionnaire data were linked to serum samples, and de-identified prior to additional laboratory testing which was performed in blinded fashion.

The HIV status of the study group was defined initially as follows: testing for anti-HIV was performed with both RSA and EIA: 171 were positive by both assays, 23 positive by EIA only, 9 by RSA only and 177 were negative by both assays. Thus, 194 (51.1%) patients were found to be HIV positive (by EIA) on admission and these results were used in the remainder of the analysis (Fig. 1).

On-Site Anti-HCV Antibody Testing by Rapid Strip Assay

In Kampala, anti-HCV antibody testing was performed on all specimens within one to 2 hr of collection according to instructions, using the RSA, Rapidtest® (Cortez Diagnostics, Calabasas, CA) purchased locally [Yuen et al., 2001]. After dipping the test strip in a cryovial containing serum for about 10 sec, the strip was laid flat on a clean, dry and non-absorbent surface for 10–20 min and the result read. The presence of anti-HCV triggers the chemical reaction leading to a pink color observed in the test region, and a similar pink colored band in the control region. In the absence of anti-HCV, only one colored band would appear in the control region and none in the test region. The total absence of color in both regions would constitute an invalid test result. Additional aliquots of each sample were then stored at −80°C and shipped frozen to the University of Texas Southwestern Medical Center at Dallas for further testing.

Detection of Serum Anti-HCV Antibody by EIA

The presence of anti-HCV antibodies was determined using the third-generation ADVIA Centaur HCV assay (EIA; Siemens Diagnostics, Tarrytown, NY), an indirect sandwich immunoassay. The sample is incubated with Solid Phase containing HCV antigen (recombinant C200 and NS5A and synthetic core antigens). Antigen-antibody complexes will form if anti-HCV antibody is present in the sample. Then monoclonal anti-human IgG labeled with acridinium ester is used to detect anti-HCV IgG in the sample. The procedure is performed on the Siemens Centaur® automated analyzer. The relative light units (RLUs) detected are used to calculate the index value from the Master Curve. Samples with an

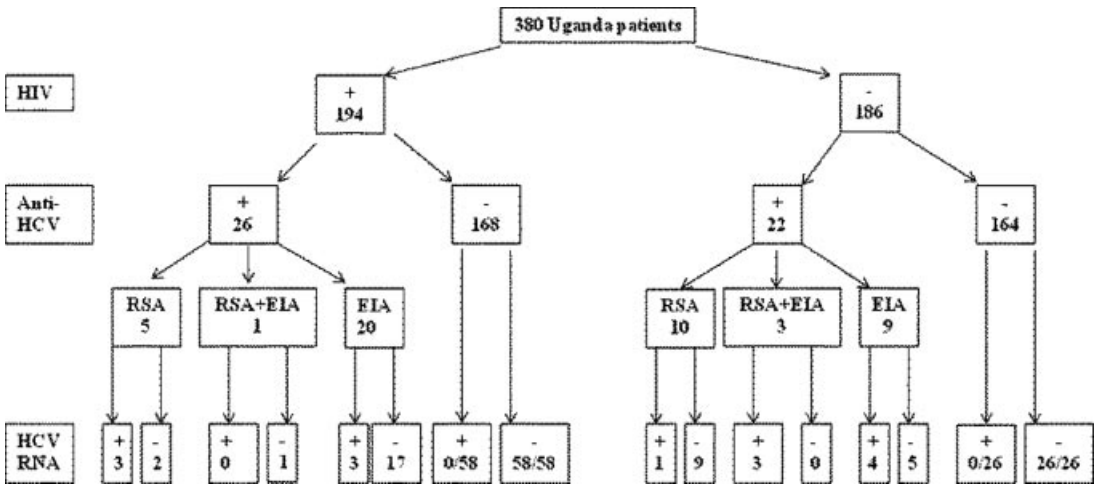

Fig. 1. Flow chart of hepatitis C testing in Ugandan patients: 380 patients were tested, of whom 194 were anti-HIV positive by EIA alone. Next, anti-HCV antibody testing was performed by RSA and then EIA. Patients who were anti-HCV positive were tested for serum HCV RNA by both bDNA and PCR assays. Eighty-four additional patients who were anti-HCV negative by both techniques and HIV positive were randomly selected and tested for serum HCV RNA by bDNA assay only. (+) Positive, (−) negative. A total of 19 patients were positive by RSA and 33 by EIA. A total of 14 patients were positive for HCV RNA, 7 by bDNA and 7 by PCR only. Of the 84 patients screened for HCV RNA by bDNA assay among the anti-HCV negative group, none were found to be HCV RNA positive. Results of RIBA testing are not shown.

index value of less than 0.80 were considered nonreactive for IgG antibodies to HCV. Samples with index values greater than or equal to 1.00 are considered reactive for IgG antibodies to HCV. Samples with index values greater than 0.80 and less than 1.00 were considered equivocal. The sensitivity and specificity for ADVIA Centaur HCV assay on Europe samples have been reported as 100% (449/449), and 99.9% (5,217/5,222) respectively [Dwyer, 2004].

Detection of Serum HCV-RNA by Nucleic Acid Testing (NAT)

Two types of NAT were used for the detection of serum HCV RNA: branched DNA (bDNA) assay (lower detection limit of 615 IU/ml, Versant 3.0: Siemens Diagnostics) and a sensitive polymerase chain reaction (PCR) assay (lower detection limit of ~10 IU/ml, Amplicor®, Roche Diagnostics, Nutley, NJ). Serum HCV RNA levels were measured using bDNA assay on samples that tested positive for anti-HCV antibodies (by either Rapidtest®, EIA as described with at least a 0.8 index value, or both) and on an additional 84 samples that tested HCV antibody negative by both assays. PCR was used for all samples positive by either antibody test: to confirm those samples that were HCV RNA by bDNA as well as all samples HCV RNA negative by bDNA.

Detection of Anti-HCV Antibody by Recombinant Immunoblot Assays (RIBA)

Additional supplementary testing for the presence of serum anti-HCV antibody was performed on 29 with samples available out of 34 who were anti-HCV antibody positive but HCV RNA negative by both NAT methods; recombinant immunoblot (RIBA; Chiron RIBA HCV 3.0 Strip Immunoblot Assay, Mayo Medical Labs, Rochester, MN) was used with subsequent repeat confirmation by Siemens Research Laboratories (Tarrytown, NY).

HCV Genotyping

HCV RNA positive samples were genotyped in the core laboratory according to methods described previously with some modification, based on the sequence of HCV NS5B region [Morice et al., 2001; Sandres-Saune et al., 2003; Laperche et al., 2005].

West Nile Virus Antibodies

To evaluate whether any of the Ugandan test sera had antibodies to West Nile virus that might cross-react in hepatitis C antibody tests, an immunofluorescent assay [Malan et al., 2003] was used to examine 30 sera including 15 patients who were anti-HCV positive by RSA alone, 10 patients who were anti-HCV positive by EIA alone and five who were anti-HCV negative in both assays.

Blocking Antibodies

The presence of blocking antibodies directed at mouse or rabbit components of the slide or EIA tests that might yield false positive tests, were evaluated by subjecting six anti-HCV antibody positive (RSA or EIA) samples to binding using coated test tubes containing appropriate mouse and rabbit antigens (Scantibodies, Santee, CA), followed by repeat testing for the presence of anti-HCV antibodies.

RESULTS

Overall Study Cohort: Patient Characteristics

Of the 380 patients studied, 211 were female. The median age was 38 (13–87) years. Ten patients were from neighboring countries while the majority (232) came from the Central Ugandan Ganda tribe; however, there were 19 other tribes represented. The provisional diagnoses (number) on admission included malaria (59), pulmonary tuberculosis (47), disseminated tuberculosis (19), accelerated hypertension (19), lobar or bronchopneumonia (16), gastroenteritis (15), diabetes mellitus (12), liver failure/cirrhosis (8), hepatitis (6), and hepatocellular carcinoma (2). Forty-seven patients were jaundiced on admission, 32 patients had demonstrable ascites.

Comparison of Antibody Test Results: RSA Versus EIA

Anti-HCV antibodies were found in 19 (5%) patients using the RSA purchased locally (Fig. 1 and Table I). Anti-HCV antibodies tested positive by EIA in 26 (6.8%)

TABLE I. A Comparison of Hepatitis C Antibody Tests Among 380 Patients With Nucleic Acid Tests (bDNA Supplemented by PCR)

Cortez Rapidtest® (Kampala)	Siemens Centaur® EIA (Dallas)	No. of patients in each category	Nucleic acid test positive [bDNA/PCR]	No. RIBA positive/no. tested	No. of false positive samples (anti-HCV pos, NAT neg, RIBA neg)	No. with HIV among HCV false positive (%)
Pos	Pos	4	3	0/1	1	1/1 (100)
Pos	Neg	15	4	0/8	8	1/8 (13)
Neg	Pos	29	7	2/20	18	15/18 (83)
Neg	Neg	332	0/84[a] (0)	N/A	N/A	NA
Total		380	14	2/29[b]	At least 27/48	17/27 (63)

[a]Eighty-four of 332 remaining samples were tested by bDNA and all were negative.
[b]The 2 of 29 found to be HCV antibody positive by RSA or EIA or both were NAT negative but positive by RIBA, indicating resolved hepatitis C infection at low prevalence in the study population.

patients and equivocal in 7 (1.8%) patients. To facilitate the presentation of the data, the seven patients with equivocal anti-HCV were considered as positive. Four patients were anti-HCV positive by both assays. A total of 48 unique patients (13%) were anti-HCV antibody positive by one or both assays. The prevalence of anti-HCV positivity was 13.4% (26/194) in patients with HIV infection and 11.8% (22/186) in patients without HIV infection.

As shown in Figure 2, patients who were anti-HCV positive by both RSA and EIA tended to have higher median index values by EIA compared to the values observed in patients who were anti-HCV positive by EIA alone ($P = 0.064$). Patients who tested positive for anti-HCV by the RSA alone (and were anti-HCV negative by EIA) had comparable index values by EIA assay to the EIA negative/RSA negative group ($P = 0.88$). Retesting with RSA on frozen samples in this study was not feasible.

Determination of Serum HCV RNA

Of 48 patients that tested positive for anti-HCV, seven patients had detectable serum HCV RNA level by bDNA assay (Fig. 4). The median viral load was 2.60×10^5 IU/L with the range of 1.10×10^3 to 8.00×10^5 IU/L. All seven patients were also serum HCV RNA positive by PCR. Seven additional patients were below the threshold level by bDNA but tested HCV RNA positive by PCR. Thus, 14 patients had active HCV infection, giving a prevalence of active HCV infection of 29% among the anti-HCV positive patients. Note that only 3 of 14 were positive by both antibody assays with 7/14 (50%) positive by EIA but negative by RSA (Fig. 3). It is also noteworthy that four patients were positive by RSA and by RNA testing but were negative by EIA. Since the entire 380 patients were not tested, a prevalence figure for the entire study group could not be calculated. HCV genotype 1a was identified in all 11 samples that were subjected to genotyping. Of 33 patients who were anti-HCV positive by EIA, 10 (30.3%) patients were serum HCV RNA positive (Fig. 4). Of 19 patients who were anti-HCV positive by RSA assay, 7 (36.8%) patients were serum HCV RNA positive (Fig. 5).

As shown in Figure 4, six of seven patients who had detectable serum HCV RNA by bDNA assay had an anti-HCV index value by EIA higher than 5.0. The remaining patient with serum HCV RNA of 1.10×10^3 IU/ml had an EIA anti-HCV index value of 0.02. The seven patients who were HCV RNA positive by PCR only had low anti-HCV index values: four were considered borderline or low positive (between 0.80 and 1.54: 0.86, 0.88, 1.19, and 1.54 respectively). The remaining three patients had values below 0.80 and would be considered definitely negative (0.02, 0.02, and 0.12 respectively).

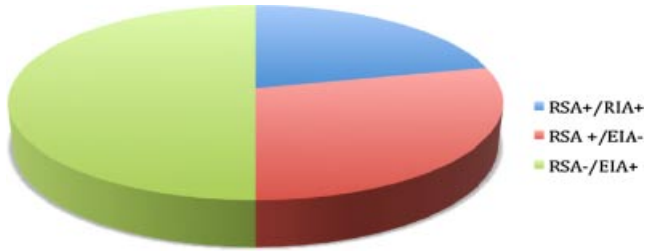

Fig. 3. Antibody results for 14 NAT positive patients. Only 3/14 were positive by both assays; 7/14 were positive by EIA but negative by RSA.

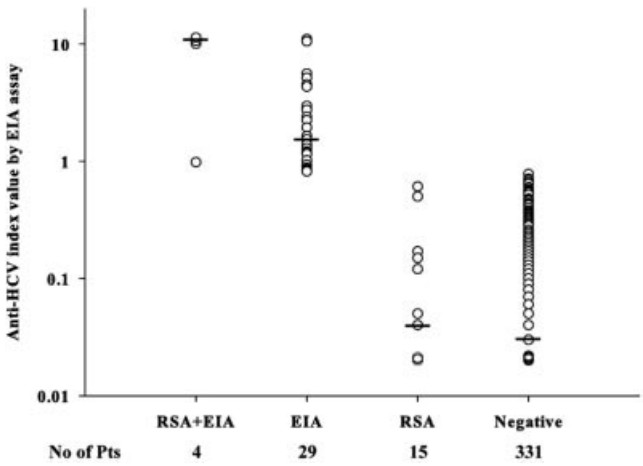

Fig. 2. Comparison of results by EIA index value. Only four patients were positive by both antibody tests. Three with high titer EIA were RNA positive and one with an equivocal index was in fact RNA negative. Of the 29 who were EIA only positive, 7 were HCV RNA positive and 2 of this group were also RIBA positive, yielding 20 remaining patients whose index value was positive but who did not appear to have HCV infection. Those who were RSA positive resembled RSA negative patients in their EIA titers (all were equivocal or negative).

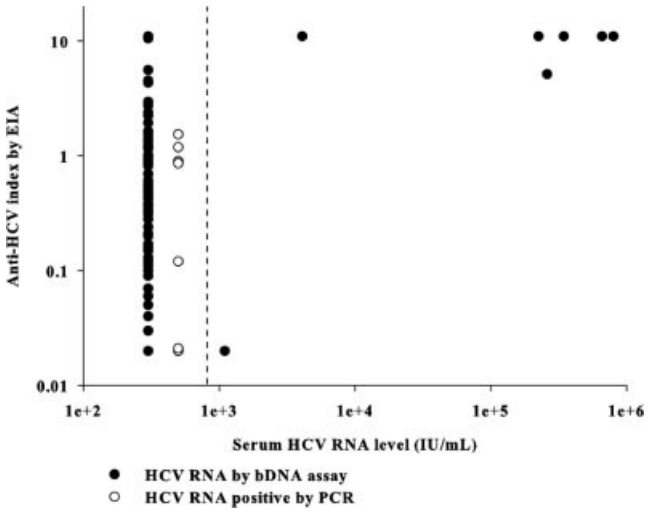

Fig. 4. Level of serum HCV RNA in 132 patients correlated with EIA index value. Patients who were anti-HCV positive (n = 48) were tested by bDNA and by PCR; 84 additional patients who were anti-HCV negative were tested by bDNA only. (●) Serum HCV RNA level by bDNA assay; (○) serum HCV RNA positive by PCR assay only in patients who were anti-HCV positive but had serum undetectable HCV RNA by bDNA assay. Lower limit of detection of bDNA assay is 615 IU/ml. Lower limit of detection for bDNA assay is shown by vertical line. Only antibody positive patients were tested by PCR.

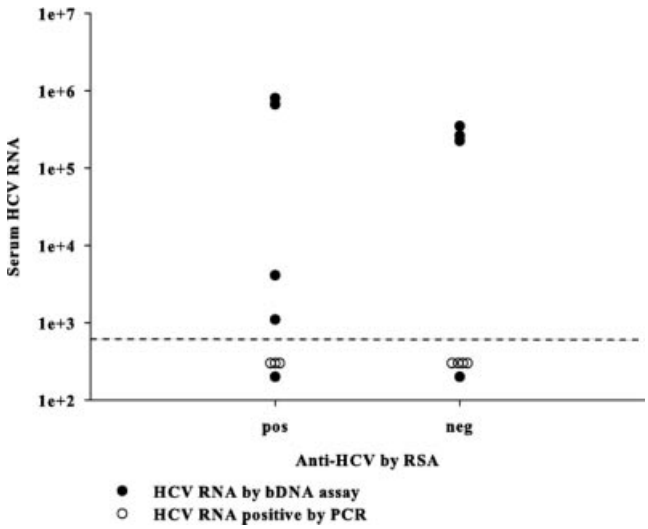

Fig. 5. Serum HCV RNA according to anti-HCV RSA results. A total of 14 positive HCV RNA patients are displayed. The horizontal line shows the lower limit of detection of the bDNA assay. Seven of 19 patients RSA positive patients were RNA positive; however, 7 of the remaining 113 patients who were RSA negative were also HCV RNA positive. RSA negative patients did not have RNA titers differing from those who were EIA positive.

Testing utilizing bDNA was performed on an additional 84 patients who were anti-HCV antibody negative and none were found to have detectable serum HCV RNA. Additional PCR testing could not be performed.

Testing by Recombinant Immunoblot Assay (RIBA)

Recombinant immunoblot assay was performed on 29 of 34 samples that had tested anti-HCV positive by either RSA or EIA assay but had shown no detectable HCV RNA with both nucleic acid tests. Only two samples that were reactive with two of four hepatitis C antigens indicated the presence of hepatitis C antibody (Table I), an additional 12 that were reactive with one of the four test antigens were considered negative for anti-HCV: nine for C33c (NS3) only, one for C22c (core) only, and two for superoxide dismutase. These RIBA results obtained at Mayo Labs were confirmed at Siemens Labs and were essentially identical (data not shown). Sera positive by NAT were not tested by RIBA. Thus, 27 of the 48 patients appeared to be false positive in that they were RNA negative and RIBA negative (5 could not be tested by RIBA so their status is unknown). Among these, only one of eight RSA only false positives were HIV positive (Table I) while 15/18 (83%) of the EIA only false positive patients were HIV positive.

The 14 patients that were serum HCV RNA positive were comparable to patients who were antibody positive but HCV RNA negative in terms of age and risk factors for acquiring infection (Table II). Of note, five of seven patients who were serum HCV RNA positive by PCR only were HIV positive, while only one of seven patients who were serum HCV RNA positive by bDNA assay was HIV positive.

Testing for West Nile Virus Antibodies and Blocking Antibodies

Using an immunofluorescent assay for West Nile virus, none of 30 antibody-positive sera tested positive for antibody against West Nile virus. Similarly, none of six anti-HCV antibody positive sera that were tested demonstrated the presence of any anti-mouse or anti-rabbit interfering or blocking antibodies.

Retesting of Samples

Because of concerns regarding the large number of false positive results, most of the samples were retested on a second automated EIA analyzer (Centaur®; Siemens Laboratories): 16/17 samples that were EIA positive in Dallas were confirmed positive in Tarrytown, with one equivocal (0.91 signal to cutoff ratio). Of the 11 RSA positive but EIA negative sera, 10 were reconfirmed as EIA negative. Among seven EIA equivocal samples retested, 5 were considered positive and 2 equivocal (index values 0.99 and 0.84 respectively).

TABLE II. Comparison of Baseline Demographic Characteristics and Risk Factors for HCV Among the 48 Patients Who Had Anti-HCV Positive Tests

Study variable	RSA pos only, N = 15 (4%)	EIA pos only, N = 29 (8%)	False pos HCV, N = 27 (7%)	RNA pos, N = 14 (4%)
Mean age (years)	44	52	42	51
Gender, n (%)				
Males	3 (20)	14 (48)	8 (30)	4 (29)
Females	12 (80)	15 (52)	19 (70)	10 (71)
Risk factors, n (%)				
Blood transfusion	4 (27)	3 (10)	6 (22)	3 (21)
Surgery	4 (27)	4 (14)	3 (11)	7 (50)
IV drug use/needle sharing	0 (0)	0 (0)	0 (0)	0 (0)
Clinical profile, n (%)				
Jaundice	8 (53)	3 (10)	6 (22)	4 (29)
Splenomegaly	9 (60)	8 (28)	12 (44)	3 (21)
Presence of HIV	7 (47)	16 (66)	20 (74)	5 (36)

EIA and RSA groups overlap with the false positive group.

DISCUSSION

In this cross-sectional study of patients admitted to an urban hospital in Kampala, 14 patients were found to have detectable HCV RNA, giving evidence of only a 29% active HCV infection rate among anti-HCV positive Ugandan patients. Of the remaining 34 patients who were anti-HCV antibody positive but negative for HCV RNA, resolved infection was confirmed by RIBA in only 2, although 10 additional patients displayed a single HCV epitope positive as possible evidence of prior infection. Thus, more than half of the positive HCV antibody tests appeared to be false positives. This study differed from several others [Jeremiah et al., 2008] in screening all patients with both assays, rather than using RSA as the initial screen followed by EIA testing of those found to be RSA positive. It was not possible to test all 380 sera for HCV RNA but did tests were performed on all those found positive by either assay and an additional 84 sera that were negative in both assays.

RSA tests, although used frequently in Uganda for detection of anti-HCV antibodies were found to be unreliable, identifying only 7 of 14 HCV RNA positive sera (50% false negative). In addition, 12 of 19 patients who tested positive by RSA were negative by both NAT and by RIBA (false positives). Thus, the RSA assay by itself appeared to have poor sensitivity and specificity for the detection of serum anti-HCV in this study.

The third-generation Centaur EIA HCV assay has demonstrated remarkable sensitivity and specificity on European samples [Denoyel et al., 2004]. By contrast, like the RSA, it also showed somewhat lower sensitivity among Ugandan patients in this study than expected; among eight patients with low serum HCV RNA levels all eight demonstrated either very low positive or frankly negative EIA testing (Fig. 4).

The performance of third-generation EIA HCV assays has not been fully validated in African patients. No specific diseases or clinical features were identified to be associated with any of the serological subgroups (Table II). Of note, most RSA false positives were HIV negative (87%), while most EIA false positives were HIV positive (83%, Table I). It is possible that low titer antibody levels accompany low viral loads [Maylin et al., 2009]. Failure to mount an appropriate hepatitis C antibody response has been noted during HIV infection [Cribier et al., 1995; George et al., 2002]. Indeed, four of the HCV RNA positive patients had negative tests for HCV antibody by EIA; all of these had been RSA positive; thus, in certain instances this study observed that those negative by EIA were positive by RSA and vice versa. When a further 84 samples negative by both antibody assays were tested for the presence of HCV RNA, no additional active infections were detected. The overall role that HIV infection, malnutrition and chronic disease play in these results remains speculative. An alternate hypothesis to explain the apparent low viral loads and infrequent positive tests would be that genomic differences in African strains result in poor sensitivity and therefore unreliable detection by assays developed in the US or Europe. HCV genotype 1a was determined in this group of Ugandan patients, the predominant genotype in US and Europe; HCV genotype 1 and well as genotype 4 predominate in much of central Africa [Rustgi, 2007; Kamal and Nasser, 2008]. Phylogenetic analysis shows that HCV genotypes 1 and 4 share a common ancestry [Kamal and Nasser, 2008]. A larger community-based study will be needed to clarify both the prevalence and distribution of HCV genotypes in Uganda. It seems likely that there are significant genomic differences between continents within the same genotype and these differences might explain the observation that most of our HCV RNA positive patients demonstrated only low-level viremia. Low viral loads may represent a situation unique to this African patient population, or might reflect poor primer homology and therefore low level of detectability in our nucleic acid tests.

The third generation Centaur EIA HCV assay also showed a high false positivity rate in Ugandan patients. Twenty-one of 33 patients who were anti-HCV positive by EIA were considered to be false positives based on a negative serum HCV RNA and negative RIBA (Table I). To confirm the main findings, positive sera were re-tested on a separate EIA analyzer at a different site and the results confirmed those obtained at the initial test site. Among the false positive group, a higher proportion (15/18, 83%) had HIV co-infection, as compared to 51% in the overall cohort. EIA testing is generally considered to be very reliable; so much so that confirmatory testing by RIBA is rarely needed in the United States. The high false positive test rates have been observed previously in African patients and appear to be unique to this population [Callahan et al., 1993; Hladik et al., 2006]. False positive tests observed in earlier studies have been attributed to nonspecific antibody binding or possibly to cross-reactivity with other tropical pathogens, such as other flaviviruses [McFarlane et al., 1990; Sonmez et al., 1997]. When RIBA testing was unrevealing, a subset of samples were tested for evidence for West Nile virus, one of the most common flaviviruses in tropical Africa, using an established immunofluorescent assay with appropriate controls. None of the samples tested positive for West Nile infection, suggesting that cross-reacting antibodies at least to this flavivirus were unlikely. Recent vaccination for influenza as well as autoimmune conditions may also impact HCV-EIA results [Imarengiaye et al., 2006]. No further evidence of autoimmune disease or recent vaccination could be found in the test positive subjects; however, a sizable proportion of patients, particularly those with false positive EIAs (but not RSAs), were infected with HIV. Further, the presence of blocking antibodies could not be detected in the small subset of the antibody positive, RIBA and RNA negative patients that were tested. It seems likely that antibodies resulting from antigens unique to Uganda that cross-react with HCV in some fashion might be at play here; their identification will be needed to improve the performance of these assays.

Another explanation for variable results might be shipping and storage of serum samples [Rowan et al., 1994]. As shown in Figure 4, utilizing a higher cut-off value than is standard in the US might significantly improve the specificity of the EIA assay used in this study, but would also lower the number of true positive infections detected, given that four of our patients had equivocal or weakly positive antibody tests [Hladik et al., 2006; Ghany et al., 2009].

The 3.6% in-hospital prevalence of hepatitis C infection may not be a reliable measure of community prevalence. It is within the estimated ranges for Sub Saharan Africa [Madhava et al., 2002; Erhabor et al., 2006; Imarengiaye et al., 2006], but is somewhat higher than that observed in previous Uganda-based outpatient studies [George et al., 2002]. Nevertheless, the prevalence of hepatitis C in the overall Ugandan population would appear to be much lower than that seen in the Egyptian Republic where hepatitis C is highly endemic [Frank et al., 2000].

This study has several limitations. Given the high HIV prevalence (>50%), in this study population, false negative anti-HCV antibody tests could have occurred, however this seems unlikely since none of the 84 randomly selected HCV antibody negative but HIV infected patients demonstrated the presence of HCV RNA. The presence of HIV may promote non-specific immunoglobulin production which might be responsible for the increased number of false positive antibody tests seen [Cadogan and Dalgleish, 2008]. Shipping samples long distance could possibly compromise sample quality but the samples arrived at their destination well frozen and there was no indication that they had thawed *en route*.

In summary, these data indicate that hepatitis C antibody tests, whether RSA or EIA, do not reliably diagnose chronic hepatitis C in this Ugandan hospital population. Lower HCV RNA viral loads were observed in this patient group for unclear reasons. Although RSA may well continue to be used due to cost considerations, these findings underscore the importance of considering EIA in place of, or in addition to, RSA, followed by confirmatory nucleic acid tests. Given the low viral loads detected in many samples, NAT methods will need to be as sensitive as possible to identify all those with hepatitis C viremia. A test algorithm that uses a PCR technique for any RSA/EIA positive samples would be preferred since false positive antibody tests could cause needless alarm and anxiety among patients, and result in mislabeling of certain patients or blood donors as HCV positive [Musana and Yale, 2005]. If this study is confirmed, more expensive NAT will be required to accurately identify hepatitis C patients in sub-Saharan Africa, with implications for blood banking as well as patient care. The clinical significance of low titer HCV infection and of the large number of apparent false positive HCV antibodies detected remains unclear.

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