Original Article

Establishing a sample-to cut-off ratio for lab-diagnosis of hepatitis C virus in Indian context

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Abstract:

Introduction: Lab-diagnosis of hepatitis C virus (HCV) is based on detecting specific antibodies by enzyme immuno-assay (EIA) or chemiluminescence immuno-assay (CIA). Center for Disease Control reported that signal-to-cut-off (s/co) ratios in anti-HCV antibody tests like EIA/CIA can be used to predict the probable result of supplemental test; above a certain s/co value it is most likely to be true-HCV positive result and below that certain s/co it is most likely to be false-positive result. A prospective study was undertaken in patients in tertiary care setting for establishing this "certain" s/co value. **Materials and Methods:** The study was carried out in consecutive patients requiring HCV testing for screening/diagnosis and medical management. These samples were tested for anti-HCV on CIA (VITROS® Anti-HCV assay, Ortho-Clinical Diagnostics, New Jersey) for calculating s/co value. The supplemental nucleic acid test used was polymerase chain reaction (PCR) (Abbott). PCR test results were used to define true negatives, false negatives, true positives, and false positives. Performance of different putative s/co ratios versus PCR was measured using sensitivity, specificity, positive predictive value and negative predictive value and most appropriate s/co was considered on basis of highest specificity at sensitivity of at least 95%. **Results:** An s/co ratio of ≥6 worked out to be over 95% sensitive and almost 92% specific in 438 consecutive patient samples tested. **Conclusion:** The s/co ratio of six can be used for lab-diagnosis of HCV infection; those with s/co higher than six can be diagnosed to have HCV infection without any need for supplemental assays.

Key words:

Anti-hepatitis C virus tests, Center for Disease Control, chemiluminescence immuno-assay, polymerase chain reaction, testing algorithm

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Introduction

Hepatitis C virus (HCV) is a major public health problem worldwide with around 3% of the world's population infected with HCV.[1] It is responsible for chronic liver disease,[2] may lead to cirrhosis and/or hepatocellular carcinoma and is a leading cause of liver transplantation.[3] Asymptomatic infection or nonspecific symptoms and varied clinical presentations make it difficult to diagnose this infection. Routine diagnosis of HCV infection is based on detecting specific antibodies by enzyme immuno-assay (EIA) or chemiluminescence immuno-assay (CIA). These anti-HCV tests are used widely for clinical diagnosis and screening of asymptomatic persons. Among populations with a low (<10%) prevalence of HCV infection, false-positive results do occur as these tests are very sensitive.[4-11] These false positive results are there despite published reports exhibiting high specificity of commonly used test-kits.[12] Center for Disease Control (CDC), United States, has recommended that a person should be considered to have serologic evidence of HCV infection only after an anti-HCV screening-test-positive result has been confirmed by a reflex serologic test which is more specific like recombinant immunoblot assay (RIBA) or nucleic acid test (NAT).[13]

Center for Disease Control reported that the value of signal-to-cut-off (s/co) ratios in the anti-HCV antibody tests like EIA and CIA can be used to predict the probable result of the supplemental test-which means that above a certain s/co value it is most likely to be true-HCV positive result and below that certain s/co it is most likely to be false-positive result. CDC guidelines of 2003[14] indicate that for CIA (Ortho Clinical Diagnostics) this "certain s/co" value is eight (8). Test value above this specific s/co ratio of eight would predict a true antibody-positive result >95% of the time, regardless of the HCV prevalence or characteristics of the population being tested. Supplemental testing of screening-test-positive samples could then be limited to those with a low (<8) s/co ratio, while screening test-positive samples with high s/co ratio can be reported as anti-HCV antibody positive without reflex supplemental testing. Since there is no such study on an appropriate s/co ratio for anti-HCV antibody testing in India, a prospective study was undertaken in hospital

patients in a tertiary care setting to ascertain similar s/co value and whether such value will work in our context.

The aim of this study was to determine s/co ratio for screening CIA testing kit routinely used in our lab that would predict a true antibody positive result 95% of the times in the patient population.

Materials and Methods

Study settings and duration

The study was carried out in a multi-disciplinary tertiary care hospital from November 2012 to December 2012.

Study population

Patients of all ages requiring HCV testing for screening/diagnosis and medical management of their clinical condition were included in the study. Most of these were admitted patients who were evaluated according to the standard presurgical work-up, which includes tests for human immunodeficiency virus (HIV) 1 and 2, hepatitis B and hepatitis C.

Samples

Consecutive serum (clotted whole blood) samples from admitted presurgical patients tested for anti-HCV on CIA during the period of study were included for calculating and establishing a significant s/co value, which can be used as a predictor of positive HCV antibody status.

Screening test

The anti-HCV screening test used was enhanced CIA (VITROS® Anti-HCV assay, Ortho-Clinical Diagnostics, Raritan, New Jersey). Specimens with a single reactive result are considered screening test-positive and do not require retesting (unlike EIA). Vitros EciQ Immunodiagnostic system is a fully automated, random and stat access immunodiagnostic analyzer, which works on the principle of enhanced chemiluminescence. The sensitivity and specificity of Vitros anti-HCV reported in the kit-insert is 100% and 99.75%, respectively.

Supplemental tests (nucleic acid test)

The supplemental NAT used was an Abbott real-time polymerase chain reaction (RT-PCR). The Abbott RT HCV assay uses RT-PCR 17 to generate amplified product from the RNA genome of HCV in clinical specimens. In addition, an RNA sequence that is unrelated to the HCV target sequence is introduced into each specimen at the beginning of sample preparation. This unrelated RNA sequence is simultaneously amplified by RT-PCR and serves as an internal control to demonstrate that the process has proceeded correctly for each sample. The amount of target sequence that is present at each amplification cycle is measured through the use of fluorescent labeled oligonucleotide probes on the Abbott *m*2000*rt* instrument. The probes do not generate a signal unless they are specifically bound to the amplified product. The amplification cycle at which the HCV-specific fluorescent signal is detected by the Abbott *m*2000*rt* is proportional to the log of the HCV RNA concentration present in the original sample.

Classification of test results

The gold standard for this study was this supplemental PCR test-result. Results of supplemental tests were considered final and true negatives, false negatives, true positives, and false positives were calculated accordingly. Samples negative with CIA and PCR were considered true negatives while samples with negative CIA and positive PCR were considered as false negatives. Samples that were positive by CIA and PCR were considered true positives while sample that was reactive by CIA but negative on PCR was considered false positive.

Data collection and analysis

All the data were stored in Microsoft excel sheets (Microsoft corporation, USA) and finally was analyzed using the the Statistical Package for the Social Sciences (SPSS), version 20.0 (SPSS Inc., Chicago, IL, USA . The performance of CIA versus the supplemental tests was measured using following statistical parameters:[15]

- Sensitivity (Sn): True positive/true positive + false negative × 100
- Specificity (Sp): True negative/true negative + false positive × 100
- Positive predictive value (PPV): True positive/true positive + false positive × 100
- Negative predictive value (NPV): True negative/true negative + false negative × 100.

To assess the efficacy of s/co threshold versus PCR, Sn and PPV of different putative s/co ratios were listed and calculated. The most appropriate s/co was considered on the basis of highest specificity at the sensitivity of at least 95%. Further, statistical analysis was performed to find receiver operator characteristics (ROC) of the s/co ratio and calculating the area-under-curve (AUC).

Ethical clearance

The study was approved by the Institutional Review Board and an independent Ethics Committee.

Results

A total of 438 consecutive patient samples were tested with both CIA and PCR. As shown in Table 1, 116 (true positives) out of 142 CIA positive samples were confirmed positives, while 26 CIA positive samples were not confirmed (false positives) with a supplemental test. 290 (true negatives) out of 296 samples were negative with both CIA and PCR, while 6 (false negatives) out of these 296 were missed by CIA. This gives the calculation of sensitivity as 95.1% and specificity of 91.8%. The predictive value of the positive test (PPV) was 81.7%, and the predictive value of negative test (NPV) was as high as 98%.

When the sensitivity and specificity values of various putative s/co values were calculated, the s/co value of both five (5) and six (6) had a sensitivity of over 95% and specificity of 91.7% as shown in Table 2. Over this cut-off value of six, the sensitivity

Table 1: Testing results of 438 consecutive patients tested with CIA and PCR

CIA results	Detected by PCR (%)	Not detected by PCR (%)	Total
CIA positives	116 (95.1)	26 (8.2)	142
CIA negatives	6 (4.9)	290 (91.8)	296
Total	122	316	438

CIA: Chemiluminescence immuno-assay, PCR: Polymerase chain reaction

started declining with specificity gaining ground at higher s/co ratios. Erring on the side of caution, six (6) seems to be the most appropriate s/co ratio to predict a true antibody-positive result >95% of the time, in the patient population. It also means that at a cut-off of higher than six the results can be released as positive without the need for doing supplementary test. This change in algorithm reduces the number of unnecessary supplemental tests.

The diagnostic accuracy of this s/co ratio of six was also confirmed by plotting a ROC. The AUC of the ROC curve was 0.985 (95% confidence interval: 0.971-1.000) (Figure 1).

Discussion

The factors unique to HCV testing for detection of HCV infection are comparatively long window period of around 60 days, before antibodies are detectable and rapid multiplication of the virus with a doubling time of 0.45 days causing high viremia within a short period of few days. The combination of slow development of antibody marker and rapid viral replication results in high viral load and higher probability of HCV transmission as compared to other transfusion transmissible infections like HIV and hepatitis B virus, through blood component transfusion, sharing needles amongst intra-venous drug users and unsafe sexual practices. Moreover, the probability of false-positive result with the screening tests is higher with anti-HCV antibody detection.[4-11] In Indian context also, it has been reported that the specificity of HCV antibody detection in serology tests is low. In this report nearly 45% of the screening assay reactive samples, tested negative by confirmatory tests (Immunoblot and Line Immuno-assay).[16] It is, therefore, not correct to label a patient as HCV positive on the basis of a single anti-HCV antibody detection test, irrespective of the kit/technique used. It can possibly be used in patients with known viral hepatitis for monitoring the response to treatment along with viral-load assay and estimation of liver enzymes. However, the screening test reactive result in asymptomatic cases like patients going for various surgeries, will have limited accuracy in predicting the true HCV infection status. It is, therefore, prudent to follow-up the initial anti-HCV screening tests with more specific supplemental assay like RIBA/NAT. Such confirmation of the presence of anti-HCV antibody by supplemental tests minimizes unnecessary medical visits and psychological harm for persons who test false positive by screening assays and ensures that counseling, medical referral, and evaluation are targeted for patients serologically confirmed as infected with HCV.[13]

Table 2: Sensitivity and specificity of various putative s/co ratios for deciding the appropriate cut-off

CIA values	Sensitivity	Specificity
5.0	0.951	0.917
6.0	0.951	0.917
7.0	0.943	0.917
8.0	0.943	0.917
9.0	0.943	0.920
11.0	0.934	0.920
12.0	0.926	0.920

CIA: Chemiluminescence immuno-assay, s/co: Signal-to-cut-off

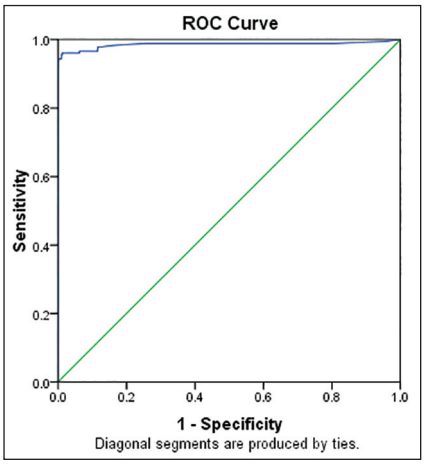

Figure 1: The receiver operator characteristics curve for signal-to-cut-off ratio of six (6)

In the armamentarium of supplemental assays — RIBA and NATs, RIBA is no longer available with world-wide shortages. Food and Drugs Administration (FDA), US has issued a guidance saying that "when current donor test-results are repeatedly reactive on an anti-HCV screening test and reactive on HCV NAT, the reactive NAT acts as a positive supplemental test and it is not necessary to perform a licensed multi-antigen supplemental test for anti-HCV."[17] This explains the use of PCR as the confirmatory supplemental tests in the present study. The latest Morbidity and Mortality report update on testing for HCV infection states that "Chiron RIBA HCV 3.0 Strip Immunoblot Assay (Novartis Vaccines and Diagnostics) that was recommended for supplemental testing of blood samples after initial HCV antibody testing is no longer available." It adds that "As a result, the only other FDA-approved supplemental tests for HCV infection are those that detect HCV viremia."[18]

The suggested use of elevated s/co ratio of eight (8) in the "Guidelines for Laboratory Testing and Result Reporting of Antibody to HCV"[14] is based on American Red-cross data where 64% of 24,700 Ortho anti-HCV EIA repeatedly reactive donations were RIBA positive, and of those, 94% of samples having an EIA s/co value of at least 4.0 tested RIBA positive. Similarly, the s/co ratio for CIA was computed at eight (8). The present study on CIA found the s/co ratio of six (6) to be the most appropriate. This s/co ratio of six is very similar to eight. These results are consistent with those of several previous studies.[19-25] According to Seo *et al*., the anti-HCV s/co ratio accurately predicts HCV viremia in patients positive for anti-HCV. Using anti-HCV EIA (Architect i2000; Abbott Laboratories, Abbott Park, IL, USA) in 487 patients anti-HCV s/co ratio cut-off value of 10.9 had high sensitivity and specificity of 94.4% and 97.3%, respectively.[26]

Moreover, the ROC-curve further substantiated six (6) as the appropriate s/co, since AUC of 0.95 was quite close to the ideal AUC of one (1) as illustrated in Figure 1.

This allows for a newer algorithm of lab-diagnosis of HCV for patients. Those with an s/co higher than six can be said to have HCV infection. It goes without saying that a physician would see this in light of the clinical picture comprising of medical history, physical examination and other investigations such as liver enzymes, viral-loads, etc.

Conclusion

The s/co ratio of six (6) can be used for diagnosing HCV infection; those with s/co higher than six can be diagnosed to have HCV infection without any need for supplemental assays. This changed algorithm would benefit the hospital and health-care settings to reduce the turn-around-time without compromising on the accuracy of lab-diagnosis of HCV in patients.

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