Original Article

Role of signal-to-cut-off ratios of anti-hepatitis C virus antibody by enzyme immunoassays along with ID-NAT for screening of whole blood donors in India

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Abstract:

Background: The use of elevated signal-to-cut off ratios (S/CO) as an alternate to further supplemental testing (i.e., RIBA) has been included in the guidelines provided by the Centres for Disease Control and Prevention for HCV diagnostic purposes since 2003. With availability of screening by NAT and non availability of RIBA, further confirmation of HCV infection has been possible at the molecular level (RNA). **Aims:** To study the role of S/CO ratios of anti hepatitis C virus antibody detection by enzyme immunoassays (EIA) along with ID-NAT for screening of whole blood donors. **Methods:** In this study we reviewed the donor screening status for anti HCV from January 2013 to May 2014. All the donations were screened for anti HCV with fourth generation ELISA (BioRad Monolisa Ag-Ab Ultra) as well as with ID NAT (Procleix Ultrio). The S/CO ratio of all the anti-HCV reactive samples were analysed for their presence of HCV RNA. **Results:** On screening 21,115 donors for HCV, 83 donors (0.39%) were found reactive on pilot tube and repeat plasma bag testing (S/Co ratio ≥1) by ELISA. 41 donors were HCV RNA reactive with ID-NAT. 4 samples out of 41 were NAT yields and 37 were concordant reactive with ELISA. The S/Co ratio of anti-HCV reactive samples ranged from 0.9-11.1 [mean = 5.1; SD ± 2.9] whereas S/Co ratio of anti HCV and NAT reactive samples (concordant positives) ranged from 4.1-11.1 [mean 7.3]. In our analysis we found that S/CO ratio of 4 showed positive predictive value (PPV) and sensitivity of 100%. **Summary/ Conclusions:** Our study showed that S/CO of 4 for anti HCV on ELISA would have maximum positive predictive value of having donor with HCV RNA. S/CO ratio of 4 is very close to 3.8 which was the CDC guideline. The presence of anti-HCV does not distinguish between current or past infections but a confirmed anti-HCV–positive result indicates the need for counseling and medical evaluation for HCV infection.

Key words:

ELISA, hepatitis C virus, nucleic acid testing, signal-to-cut-off ratio

Introduction

Screening for hepatitis C virus antibody (anti-HCV) on donated blood is a recommended practice across the blood banks around the world. Screening for HCV on the donated blood was made mandatory in the year 2002 in our country. This test is also used for initial testing in people with clinical manifestations of HCV infection and chronic liver disease. False positivity in anti-HCV antibody screening is reported to be around 15-62%[1,2] with third generation immunoassays, hence role of supplemental testing by recombinant immunoblot assay (RIBA) has been approved for confirmation of anti-HCV for reactive donations in the USA.

Elevated signal-to-cut-off-ratio (S/CO ratio) as an alternate to further supplemental testing (for confirmation by RIBA) has been included in the guidelines provided by the Centers for Disease Control and Prevention (CDC) for HCV diagnostic purposes since 2003.[3] With availability of screening of HCV RNA by nucleic acid testing (NAT) further confirmation of HCV infections in donors have been possible. As per Food and Drug Administration guidance, certain licensed HCV NAT assays have been labeled with a "limited supplemental claim"; that is, "when current donor test results are repeatedly reactive on an anti-HCV screening test

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and reactive on HCV NAT, the reactive NAT acts as a positive supplemental test and it is not necessary to perform a licensed multiantigen supplemental test for anti-HCV."[4]

In this study, we reviewed the S/CO ratios for anti-HCV repeat reactive donations for anti-HCV screening test by Bio-Rad (Monolisa) Ag-Ab Ultra, qualitative enzyme immunoassay (EIA), to assess their value in the context of their ID-NAT reactive status for HCV RNA.

Materials and Methods

In this study, we reviewed the donor screening data for anti-HCV from January 2013 to May 2014. All samples from the donation were screened for anti-HCV with fourth generation ELISA and with ID-NAT as per the algorithm 1 [Figure 1].

Sample collection

As per the routine practice at our blood bank three pilot tube samples were collected with each whole blood donation. Two samples out of three were collected in EDTA vacutainer and one in plan vacutainers (two plasma sample and one serum). The serum sample was used for serology testing (ELISA) for anti-HCV. EDTA samples (plasma) were used for blood group confirmation and for ID-NAT screening. For all repeat testing, for initial reactive (IR) sample, samples were considered from the donated bag (plasma bag).

Screening for hepatitis C virus

All donations were tested for anti-HCV and HCV RNA (by ID-NAT) as per algorithm 1. All donations were tested in parallel and if results of NAT and ELISA do not match the samples for further evaluation were stored from the plasma bag. Any IR result was repeated again on the sample from the bag and pilot tube before labeling it as repeat reactive (RR). Any sample which was not RR on ELISA by pilot tube and bag, was considered as contamination.

All ELISA and NAT nonreactive samples were considered as concordant nonreactive for HCV whereas ELISA and NAT reactive donor sample was considered as concordant positives. Bag and samples were quarantine and discarded.

Any sample which was HCV NAT reactive (discriminatory HCV RNA reactive) but ELISA nonreactive was considered as NAT yield for HCV where as a ELISA reactive and NAT nonreactive sample was referred as sero-yield. All sero-yield samples were further tested with a rapid assay, fourth generation, quantitative immunoassay (Flaviscreen), for anti-HCV detection.

Serology testing (anti-hepatitis C virus screening)

Anti-HCV screening was done by ELISA, Monolisa Ag-Ab Ultra (Bio-Rad), and a qualitative EIA. This assay includes microplate solid phase coated with monoclonal antibody against capsid protein of HCV, 2 recombinant protein produced by *Escherichia coli* from NS3 region (genotype 1 and 3a), one recombinant antigen from nonstructural region NS4 and a mutated peptide from the capsid of structural area of HCV genome.

The S/CO ratio was obtained by measuring the signal strength of sample and the signal strength of an internal cut-off. Samples with an S/CO ratio of 1.0 are defined by the manufacturer as positive.

In order to confirm the reactivity of anti-HCV with ELISA all reactive were tested with a fourth generation assay based on the principle of immunochromatography on nitrocellular membrane. This membrane consisted of recombinant antigens derived from core, NS3, NS4, and NS5 regions of multiple HCV genotypes (apart from genotype 1).

Individual donar nucleic acid testing (ID-NAT)

For ID-NAT, Procleix Ultrio kit was used based on TMA. The assay contains reagents which are used for simultaneous detection of all three viruses initially. Initial NAT assay was done on the pilot tube sample and if found reactive then the sample from the bag was repeated twice. The repeat sample testing if found reactive, was further tested by discriminatory testing for HBV, HCV, and HIV, respectively. A positive discriminatory test confirmed the presence of the respective virus. The clinical sensitivity for the Procleix Ultrio Assay has been demonstrated for specimens with HIV-1 or HCV viral RNA concentrations equal to or >100 copies/ml or HBV viral DNA concentrations equal to or >15 IU/ml.

Results

Anti-hepatitis C virus screening

On screening 21,115 donors, 83 samples (0.39%) were found RR (S/CO ratio ≥1) by ELISA for anti-HCV [Table 1]. The S/CO ratio of RR samples ranged from 1.0 to 11.1 with mean value S/CO ratio of 5.1 (SD: ±2.9). The S/CO ratio of anti-HCV and NAT reactive samples (concordant positives) ranged from 4.1 to 11.1, with mean value of 7.3. As per the algorithm, 55.4% of total ELISA reactive samples were also found reactive by rapid testing [Table 1].

Hepatitis C virus screening by ID-NAT

On screening 21,115 donor samples by NAT, 41 samples (0.19%) were found to be reactive for HCV RNA. Out of 41 HCV NAT

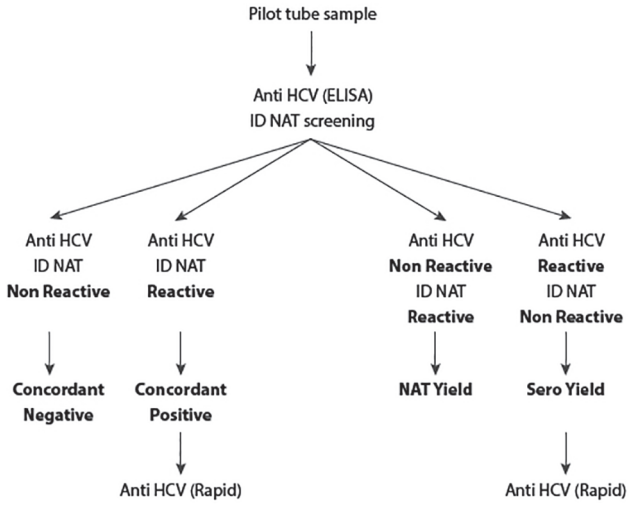

Figure 1: Algorithm for hepatitis C virus antibody screening for blood donors

Table 1: Anti-HCV screening by ELISA of 21,115 donors

Parameter	*n*
Total anti-HCV reactive by ELISA	83 (0.3% over all)
Anti-HCV reactivity by ELISA and rapid testing (confirmed anti-HCV reactive)	46 (out of 83)
NAT reactivity among confirmed anti-HCV reactive	37 (out of 46)
NAT reactivity among ELISA (R) rapid (NR)	Nil

HCV: Hepatitis C virus, NAT: Nucleic acid testing

reactive, 4 samples were NAT yields and 37 were concordant reactive with ELISA [Table 1]. All the concordant reactive samples were ELISA as well as rapid reactive.

Correlation between anti-hepatitis C virus S/CO Ratio and hepatitis C virus RNA

Out of 83 anti-HCV reactive samples, HCV RNA was identified in 37 samples (44.5% RNA reactivity among anti-HCV reactive donors). RNA identification was considered as confirmatory test for HCV infection in our donor population. S/CO ratio for anti-HCV by ELISA of confirmed positive and negative donors were assessed. The S/CO ratio of HCV RNA reactive and nonreactive showed a clear demarcation (box and whisker plot; Figure 2). S/CO ratio values clearly clustered toward a higher ratio with ID-NAT reactive status.

The diagnostic sensitivity and specificity, the positive predictive value (PPV) and negative predictive value (NPV), were analyzed at different S/CO ratios [Table 2]. Sensitivity and PPV was 100% at S/CO ratio 3, 3.8, and 4, whereas specificity and NPV was >75% above S/CO ratio of 4.5.

Discussion

The unavailability of the RIBA in the market and implementation of NAT technology for donor screening prompted this investigation to assess application of raised S/CO ratio of anti-HCV by ELISA and its correlation with ID-NAT. RIBA is gold standard for confirmation of anti-HCV ELISA reactivity but with its absence the initial anti-HCV reactivity can also be confirmed by ELISA from a different manufacturer or on a different platform.[5] Confirmation of anti-HCV status is of great importance, for appropriate donor management as well as for diagnostic perspective hence; in the absence of RIBA the S/CO ratio of reactive HCV antibody by EIA has gained importance.

As per CDC guideline in 2003 the screening tests with high S/CO ratios have demonstrated to predict a supplemental test positive in >95% of the time. As well as screening test positive samples with high S/CO ratios can be reported as anti-HCV positive without supplemental testing. In one of the study[6] on role of S/CO ratio for anti-HCV screening relative to a RIBA positive result showed, an S/CO of 3.80 or higher using the Ortho ELISA had a PPV of 88% and sensitivity of 96.3%. Similarly our results also indicate that an elevated S/CO ratio of above 3.8 shows yield of nearly 100% sensitivity [Table 3]. Similarly, another study[7] showed that the PPV for use of an elevated anti-HCV S/CO value relative to a RIBA positive result ranged from 89.1% to 95% (S/CO of 5.00 for Ortho and 3.20 for Abbott, respectively) with sensitivities of 88.7% to 93.1% (Abbott and Ortho, respectively).

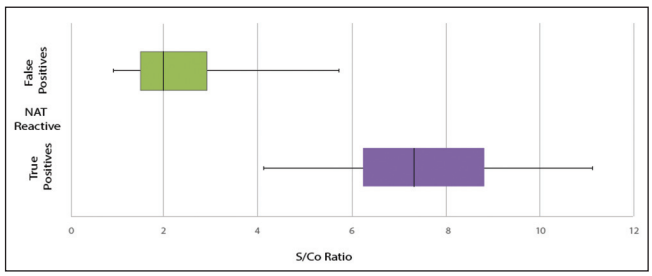

Figure 2: Distribution of hepatitis C virus antibody signal-to-cut-off-ratio and RNA (nucleic acid testing) results

Although the presence of anti-HCV does not distinguish between current or past infection, a confirmed anti-HCV-positive result indicates the need for counseling and medical evaluation for HCV infection, including additional testing for the presence of virus, and liver disease (e.g., alanine aminotransferase). Most of the studies have shown that almost all (99.82%) EIA-repeat reactive, RIBA-negative donations were NAT-nonreactive similarly in our study all ELISA reactive rapid nonreactive were also NAT nonreactive.

With availability of NAT for screening of donated blood and unavailability of RIBA in the market, NAT is considered[4] as a supplementary test to confirm HCV infection. In one of the study[6] proposing addition of NAT for diagnostic algorithm for HCV showed that 38.5% of RR EIA showed presence of HCV RNA whereas it was 44.5% in our study. In that study RIBA confirmed RR EIA samples showed presence of RNA in 98.7% whereas it was 80.4% (37 out of 46) in our study by using rapid testing to confirm RR EIA.

Several studies have been published about the ability of S/CO ratio of screening test (CIA) to predict the supplemental test results.[8-10] Lai *et al.*[9] concluded that for Ortho CIA, it is not necessary to confirm negative or positive values if the S/CO ratio is ≤ 3.0 or ≥20.0 because of the high rate of true-negative and true-positive. Even with ELISA, one of the study[11] demonstrated good correlation between S/CO ratios and supplemental positive results. It showed that a uniform S/CO ratio cannot be used to predict a 95% confirmatory positive rate for these different manufactured kits.

High sero-yield of anti-HCV (presence of anti-HCV and absences of HCV RNA) as in our study can be due to various reasons. First, serum HCV RNA levels fluctuate during chronic infection with intermittent viremia yielding false-negative NAT results.[12,13] In such cases, the viral load may be insufficient to elicit the full host response, resulting in a low level of anti-HCV antibodies. Second, antibody-positive and RNA nonreactive donors may be viremic below the NAT detection level, or may represent cases where HCV RNA exists intrahepatically, and cannot be detected in circulation.[13,14] Finally, the virus can also spontaneously clears out of the body after infection in 15-20% individuals, who then remain negative for HCV RNA for a long time and show positive antibody test results in the absence of circulating virus. Although antibody reactivity declines over time after spontaneous resolution of infection, T-cell responses might be maintained.[15]

Limitations of our study

The kits used for ELISA were only from a single manufacturer hence results of predictive value may change when other kits

Table 2: Anti-HCV screening and ID-NAT results

Total donations	Serology and NAT concordant reactive	Serology reactive	ID-NAT reactive	Sero-yield	NAT yield
21,115	37	83	41	46	4

HCV: Hepatitis C virus

Table 3: Diagnostic performance of the anti-HCV ELISA screening test in the prediction of HCV RNA ID-NAT result status

Parameter	Percentage of correct diagnosis at S/Co ratio of				
	3	3.8	4	4.5	5
Sensitivity	100	100	100	94.5	89.1
Specificity	61.7	70.2	70.2	76	78.2
PPV	100	100	100	94.5	90
NPV	66.6	72	72	76	76.7

PPV: Positive predictive value, NPV: Negative predictive value, HCV: Hepatitis C virus, ID-NAT: Individual nucleic acid testing, S/Co ratio: Signal-to-cut-off-ratio

are used. Even confirmation of ELISA anti-HCV status with other ELISA platforms should have been performed instead we confirmed the infection status with NAT. An ideal study design would have been to use an unscreened population for all available kits and then perform supplemental tests and S/CO analysis on those repeat reactive.

Conclusion

We tried to formulate the predictive value to confirm HCV infection. As per our study, anti-HCV S/CO ratio above 4 shows 100% sensitivity and PPV of HCV infection and presence of HCV RNA, which also coincides with CDC guidelines. Studies like ours will help to formulate better algorithm for donor management in a place like India where NAT is still not mandatory. These supplemental tests are of more importance for framing guidelines and policies for confirmatory testing on initial reactive screening assays, donor management, donor notification, recipient tracing, and donor re-entry as well as for HCV look back studies. Further an elaborative multi-institution study, screening large number of samples with all manufacturers in the market is necessary before formulating a guideline for India.

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Conflicts of interest

There are no conflicts of interest.

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