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Importance of EIA Signal-To-Cut-Off (s/co) Ratio for HCV Donor Notification in Resource Poor Settings

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Abstract

Introduction: Screening of blood donors for HCV is vital for ensuring the safety of blood. A number of highly sensitive & specific assays based on detection of antibodies against recombinant HCV polypeptides are available. These screening assays exhibit high false positivity which creates issues for donor notification & unnecessary discarding of blood components.

Objective & Aims: This study was conducted with an aim to identify whether s/co ratio of EIA assay could help in distinguishing the HCV viremic donors from the false positive donors.

Methods: Whole blood (WB) donors were screened by 3rd generation EIA for a period of 9 months & simultaneously tested by RT-PCR, followed by viral load in PCR reactive donors. Sensitivity, specificity, PPV and NPV for EIA was calculated & s/co ratio of EIA reactive samples was analyzed for HCV viremia.

Results: HCV prevalence was 0.61% among 17017 WB donors with 42 donors having detectable viral load. Lower s/co ratio (<5) was associated with high false positivity (viremia detectable in 11.76% EIA reactive donors). EIA was found to be highly sensitive &specific for donor screening with low PPV.

Conclusion: EIA s/co ratio could be used to notify donors of their HCV status & to determine their further referral and reentry into blood donor pool. Discard rate due to high false positivity should be acceptable keeping in mind the high degree of safety of blood products offered.

Keywords: Blood donors; HCV; S/co ratio; EIA; Donor notification

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Introduction

Hepatitis-C virus (HCV) has emerged as a major public health problem across the world with approximately 160 million individuals, i.e. 2.35% of the world population estimated to be are chronically infected with HCV worldwide [1]. The modes of transmission of the virus include injection drug use, unsafe injection practices, blood transfusion, healthcare related procedures (occupational exposures like needle stick injuries), tattooing, perinatal transmission and sexual transmission.HCV is one of the few viruses for which transfusion services screen the blood donors so as to prevent its transmission.

Screening of blood donors across most of the centers is based on the detection of antibodies (IgG) against recombinant HCV polypeptides by two main methods: Enzyme immunoassay (EIA) and Chemiluminescence immunoassay (CIA). Anti-HCV EIA results are interpreted by comparison of absorbance readings with a defined cutoff value. Any value above the cut off is reported as reactive and the blood unit collected is discarded. The EIA resultshave also been interpreted in the form of signal (or sample) to cut off ratio (s/co ratio) to express the results quantitatively. Though the assays currently used for screening of blood donors are highly sensitive and specific yet there still exists a high prevalence of false-positive results to the range of 15% to 60%, especially among populations with low prevalence of HCV such as blood donors [2].

The high false positivity of current HCV screening assays creates dilemma for the transfusion services as to how to notify the donors of their HCV status. As per CDC, HCV antibody reactive sample may be due to current HCV infection, or past HCV infection that has resolved, or biologic false positivity for HCV antibody and test for HCV RNA should be done to identify current infections [3]. Supplemental testing for HCV RNA in case of donors may not be possible in resource poor

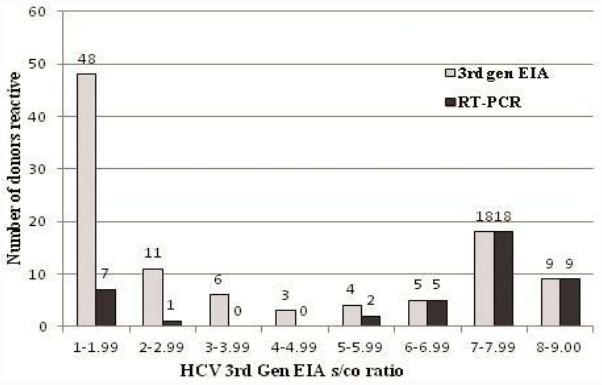

Figure 1: Distribution of RT-PCR positive donors among different EIA s/co groups.

setting. In this study we thus aimed to identify whether s/co ratio of EIA assay could help in distinguishing the HCV viremic donors from the false positive donors.

Material and Methods

The study was conducted in the Department of Transfusion Medicine of a tertiary care hospital of north India from Dec 2013 to August 2014.

Study population

Whole blood donors including both voluntary and replacement donors presenting during the study period were included in the study. All the donors were selected as per the guidelines for blood banks laid out by the Drugs and Cosmetics Act of India, 1940 [4].

Method

All the blood donors were screened for markers of HBV, HCV, HIV and syphilis as per departmental SOPs. HCV screening was done in fully automated DAVINCI Quattro system by third generation EIA using Biorad Monolisa Anti-HCV plus assay version 2 kits. All the samples with s/co ratio >1 were tested in duplicate in a separate batch and marked as reactive when s/co ratio is >1 in at least one well. Plasma from the respective blood donors was stored at <-40˚C till further testing. All the donors including EIA positive donors were further tested individually by RT-PCR using Roche MPX assay. The donors found to be reactive by RT-PCR were further tested by Cobas Taqman HCV test v2.0 to find out the HCV viral load. Donors found to be reactive for other Transfusion Transmitted Infections (TTI) were excluded from the study.

Statistical analysis

The data obtained were coded and analyzed using SPSS v.20 (IBM Corporation). Descriptive statistics and chi square test were done where necessary. A p value >0.05 was considered to be significant. Sensitivity, specificity, PPV and NPV was calculated using a 2x2 table.

Results

A total of 17017 donors donated whole blood during the study period of which 16530 (97.1%) donors were male and 487 (2.9%) donors were female. Of the 17017 donors who were screened104 (0.61%) donors were reactive for anti-HCV by EIA (s/co ratio>1). All these donors were further screened by RT-PCRof which 42 donors (0.25%) were reactive for HCV RNA. All the 42 donors were also reactive by EIA. The remaining 16975 donors were non-reactive for HCV RNA. Among these, 62 donors though reactive by EIA were negative for HCV RNA by RT-PCR testing.

Table 1: 2x2 contingency table to calculate sensitivity, specificity, PPV and NPV.

EIA	PCR reactive	PCR non-reactive
reactive	42	62
non-reactive	0	16975

S/co ratio of EIA reactive samples ranged from 1.002 to 8.466. We observed that of the 104 reactive samples 68 samples had an s/co ratio of <5 and only 8samples were reactive by RT-PCR for HCV in this group. The viral load of the samples with s/co ratio <5 range from 25 –34 IU/ml. As the s/co ratio increased the number of samples reactive by RT-PCR testing increased in each s/co ratio group (Figure 1). We found that at s/co ratio of <5, viral load was detectable only in 11.76% samples whereas at s/co ratio >5, viral load was detectable in 94.5% of the samples. The viral load of the samples with s/co ratio >5 range from 1400 IU/ml to 8319756 IU/ml.

The sensitivity and specificity of 3rd generation EIA was found to be 100% and 99.75% respectively. Similarly the positive predictive value and negative predictive value was 40.38% and 100% respectively (Table 1).

Discussion

Screening the blood donors for transfusion-transmissible infections (TTIs) is a critical step in ensuring the safety of blood and its products intended for transfusion to patients. It is recommended that for the screening of blood donors, both sensitivity and specificity of the selected assay should be the highest possible or available (WHO 2010) [5]. Sensitivity and specificity determines the reliability of a test yet they do not give the predictive values. In our study we found that third generation EIA for HCV was sufficiently reliable as a screening test. It had high sensitivity and specificity as well as a high NPV of 100%. The discard rate due to false positive EIA was 1 unit every 275 units collected.

Though, screening test with high sensitivity may ensure a safer blood supply yet it presents dilemma to the transfusion services while notifying the blood donors of their test results due to the high false positivity. In our study we found that ~40% of donors with a reactive anti-HCV assay had viremia suggestiveof active hepatitis whereas the remaining 60% of the donors represented false positive anti-HCV assay.Thefalse positivity was more pronounced at lower s/co ratios with only 11.76% of reactive donors having viremia below s/co ratio of 5. It has been suggested that this false positivity at lower s/co ratios may be non-specific and can be attributed to cross reactivity with other antigens possibly from respiratory viruses [6]. Moreover a higher false positivity is also associated with lower prevalence of HCVas seen in our study.

Although diagnostic algorithms have been devised based on s/co ratio to guide supplemental testing in patient population [3] but for notifying blood donors no algorithmbased on the results of screening assayshave been proposed. Notification of a false positive result creates unnecessary mental stress to the donor and leads to permanent exclusion fromthe donor pool. We thus believe that s/co ratios may be used to notify donors of their HCV status as it is been used as a guide to supplemental testing in patients. Donors with s/co ratio of <5 should be notified specifically mentioning the s/co ratio and they should be again tested by more specific supplemental or a qualitative assay. However donors with a high s/co must be notified to visit their physician and toabstain from donating blood in the

future. The chances of having viremia at s/co ratio >5 was ~95% and thus the donors with s/co >5 may be advised to be tested directly by a quantitative assays to minimize the cost as well as the time taken in starting the management. This approach would be helpful to the centers, specifically in resource poor settings where supplemental tests are not easily available. Based on the findings it is suggested that s/co ratio might be used to notify the donors more reassuringly. However centers using different screening assays need to evaluate their own cut offs for s/co ratio before adopting this approach for notification.

Conclusion

S/co ratio of EIA for HCV could serve as an important tool in notifying the blood donors of their HCV status in resource poor setting where there is absence of supplemental testingAlgorithmsderived on the basis of s/co ratio could also be used for guiding the blood donors for further referral and for reentry purposes.

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