Folia Microbiol (2013) 58:343–347
DOI 10.1007/s12223-012-0215-5

Determining immunoassay cutoff value using Western blot results to predict hepatitis C infection in blood donors with low-titer anti-HCV reactivity

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Received: 15 August 2012 / Accepted: 20 November 2012 / Published online: 4 December 2012

Abstract Since the 1990s, blood donors have been scanned for anti-hepatitis C virus (anti-HCV) antibodies, which can be defined by enzyme immunoassay as a screening test. In this population, false-reactive ratios have been high. Recently, some authors have aimed to find a cutoff value for anti-HCV different from those established by test manufacturers to predict HCV infection. In this study, 321 patients, after two repeating tests, had reactive results in s/co <10 titers on anti-HCV test. The patients were 29.6 % (n=95) in women and 70.4 % (n=226) in men. The patients were classified into three groups by Western blot (WB) results (PS, positive; NG, negative; and ID, indeterminate). The average anti-HCV titer of the whole group was 2.61±1.96. Anti-HCV titers of subgroups were 2.43±1.95 in NG, 4.93±2.53 in PS, and 2.50±1.65 in ID (p<0.001). There was a significant difference between NG and PS and between PS and ID subgroups (p<0.001). There was a positive correlation between WB and anti-HCV titers in all patients (r=0.298, p< 0.001), in women (r=0.282, p<0.001), and in men (r= 0.337, p=0.002). According to receiver operator characteristic curve analysis, the cutoff value of anti-HCV titer to predict hepatitis C infection was >2.61 s/co, with 74.1 % sensitivity and 71.6 % specificity (area under the curve, 0.820; 95 % confidence interval, 0.753 to 0.887). We suggest that an effective cutoff value for anti-HCV other than that established by the manufacturer cannot be assigned to predict hepatitis C infection for blood donors in low-prevalence areas.

Abbreviations

Anti-HCV	Anti-hepatitis C virus
EIA	Enzyme immunoassay
HCV	Hepatitis C virus
ID	Indeterminate
NG	Negative
PS	Positive
s/co	Signal to cutoff
WB	Western blot

Introduction

Blood donors have been screened routinely for anti-hepatitis C virus (anti-HCV) antibodies since the 1990s. These antibodies can be detected easily and rapidly by enzyme immunoassay (EIA), and this method is widely used as a screening test (Bar-Shany et al. 1996; Alter et al. 2003). The fact that false-reactive results are common in groups with a low prevalence of infection, e.g., blood donors, suggests that further testing is often required despite the high sensitivity and specificity of EIA tests (Alter et al. 2003; Kiely and Wilson 2000). For this reason, all EIA-reactive samples must be shown to be reactive on two repeat analyses in a second run before being reported as reactive (Dufour et al. 2003). If the duplicate test is reactive, supplemental testing is required (Nelson and Shan 2008). The most commonly used supplemental test is Western blot (WB)

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(Erensoy 2001; Seed et al. 2003; Centers for Disease Control and Prevention 1998). Since these supplemental tests are costly and require qualified equipment and labor, the determination of a positive predictive value for EIA tests is of great importance (Nelson and Shan 2008).

Published studies of anti-HCV-reactive individuals have sought to determine the cutoff value for anti-HCV in addition to those established by test manufacturers to predict HCV infection; however, the characteristics of the patient populations in those studies have varied. This may be the main reason for a lack of consensus regarding appropriate cutoff value to predict HCV infection. The goal of our study was to appraise the cutoff value at our institution using confirmation testing by WB to predict HCV infection among blood donors with low-titer anti-HCV reactivity signal to cut-off (s/co) <10. With this type of study, the validation of cutoff value established by manufacturers to predict HCV infection may be examined in a population with a low prevalence of HCV infection, as is found in Turkey.

Materials and methods

This study was performed retrospectively among 15,540 healthy volunteers in the age range 18–50 years who applied to the blood bank for being blood donors at Ataturk Chest Diseases and Thoracic Surgery Research and Training Hospital in the years from 2004 to 2010. Of 15,540 patients, 327 patients had reactive results on anti-HCV test after at least two repeating tests with the same assay (Abbott Axsym MEIA HCV version 3.0, USA).

All individuals who applied for blood donation were first subjected to anti-HCV test. The patients having a s/co result <1.0 with the AxSYM kit were accepted to be nonreactive; the s/co results ≥1.0 were accepted to be reactive. All reactive anti-HCV results were confirmed with WB (INNO-LIA™ HCV Score, Innogenetics, Belgium) assay. Six patients had s/co >10 titer for anti-HCV. Our study included 321 patients who had reactive anti-HCV titers s/co <10

On WB assay, a total of six regions including three structural (C1, C2, and E2 regions) and three nonstructural (NS3, NS4, and NS5 regions) viral proteins were investigated. If there is no reactive protein band on WB test, the result was accepted to be negative (NG, n=231 patients); a WB pattern with at least two strongly reactive protein bands was accepted to be positive (PS, n=34 patients). The strongly reactive of only one protein band or weakly reactive of two and/or more protein bands were interpreted as indeterminate (ID, n=56 patients).

Table 1 The associated between gender and anti-HCV titers in subgroups

	Female	Male	*p* value
All patients	2.87±2.41	2.61±1.96	0.456
NG	2.57±2.28	2.37±1.80	0.544
ID	2.38±1.59	2.54±1.69	0.776
PS	5.86±2.55	4.54±2.48	0.237

Significant value, $p<0.005$ (Kolmogorov–Smirnov test)
ID indeterminate, *NG* negative, *PS* positive

Among the individuals applying at our institute's blood bank for blood donation, those who underwent surgery, persons who have been tattooed within the last 6 months, individuals having multiple partners, those who had known jaundice, IV drug abusers, persons with chronic diseases, those who had been given any blood products, persons who have been bitten by a suspicious animal, those who had a travel history to a region in which the prevalence of AIDS is high, and individuals with known hepatitis B virus, HCV, and/or human immune deficiency virus infection were not accepted as blood donors.

Statistical analysis

All statistical analyses were performed using the SPSS software package 15.0 (SPSS Inc, Chicago, IL, USA). Data are presented as frequencies and percentages for categorical variables and mean±SD for continuous variables. Differences between two groups were assessed using the chi-square and unpaired t tests. Because parameters were not normally distributed, between-group differences were assessed by the Mann–Whitney U test. Spearman's correlation coefficients were used to test the relationship between the anti-HCV titers and WB results. An anti-HCV titer of additional cut points was performed using receiver-

Table 2 Anti-HCV titer for all individuals who were reactive for s/co <10 in anti-HCV

Anti-HCV (s/co)	Individuals (n)
1.00–1.50	126
1.51–2.00	46
2.01–2.50	41
2.51–3.00	20
3.01–3.50	22
3.51–4.00	12
4.01–5.00	12
5.01–6.00	11
6.01–7.00	9
7.01–8.00	10
8.01–9.92	12

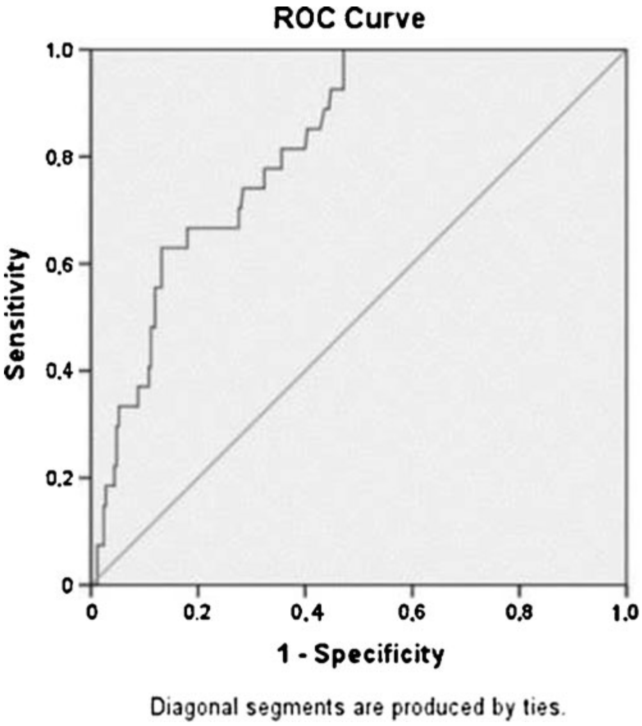

Fig. 1 ROC curve analysis to predict hepatitis C virus infection in 2.61 s/co as cutoff value

operating characteristics (ROC). A value of $p<0.05$ was considered statistically significant.

Results

A total of 327 patients had reactive results on anti-HCV test after at least two repeating tests with the same assay. Our study included 321 patients who had reactive anti-HCV titers s/co <10.

Of the patients, 29.6 % ($n=95$) were women and 70.4 % ($n=226$) were men. The average age of all patients was 32.8± 6.1; the average age was 25.2±5.0 for women and 36.03± 3.03 for men ($p>0.05$).

WB results were NG in 231 patients, PS in 34 patients, and ID in 56 patients. When the patients were classified into three groups by WB results (PS, NG, and ID), there were no differences between the groups for the average age and gender of the patients ($p=0.669$, $p=0.716$, respectively).

The average anti-HCV titer of the whole group was 2.61± 1.96. It was found to be 2.87±2.41 for women and 2.31±1.83 for men ($p=0.456$). Anti-HCV titers were 2.43±1.95 for the NG subgroup, 4.93±2.53 for the PS subgroup, and 2.50±1.65 for the ID subgroup ($p<0.001$). There was a significant difference between NG subgroup and PS subgroup and between PS subgroup and ID subgroup ($p<0.001$), whilst there was no significant difference between NG subgroup and ID subgroup ($p=0.807$). Table 1 shows in-group association between anti-HCV titers by gender. Table 2 presents the number of patients for each anti-HCV titer.

On Spearman's correlation analysis, there was a positive correlation between WB and anti-HCV titers (r, 0.298, $p<0.001$). There was a positive correlation between WB and anti-HCV in women ($r=0.282$, $p<0.001$) and in men ($r=0.337$, $p=0.002$).

According to ROC analysis, the cutoff value of the anti-HCV titer to predict HCV infection was >2.61 s/co, with 74.1 % sensitivity and 71.6 % specificity (area under the curve, 0.820; 95 % confidence interval, 0.753 to 0.887) (Fig. 1). The cutoff value of the anti-HCV titer to predict HCV infection was >3.8 s/co and >6.38 s/co, with 63–66.67 % sensitivity and 86.6–99.4 % specificity, respectively.

On the comparison of positive bands of WB tests between three groups, there was no difference for only the NS5 band ($p=0.093$). There were significant differences for other bands ($p<0.001$) (Table 3).

Discussion

The primary mission of blood banks is to protect the recipients, as much as possible, from transfusion-transmitted infectious diseases. Additionally, their tasks include providing accurate information to blood donors regarding their reactive serologic test results and referring them for further evaluation. Unfortunately, even confirmation tests are not able to provide consistent and decisive results.

In a study with the largest patient series performed in Turkey between 1996 and 2004, the HCV seroprevalence was found to be 1–2.4 % in the general population and

Table 3 *p* values for differences of the reactive band numbers using Western blot in the groups

	C1	C2	E2	NS3	NS4	NS5
Group NG and ID	<0.001*	0.113	0.679	<0.001*	0.316	0.422
Group NG and PS	<0.001*	<0.001*	0.131	<0.001*	<0.001*	0.432
Group PS and ID	0.001*	<0.001*	0.237	0.992	0.562	0.436

ID indeterminate, *NG* negative, *PS* positive

* Significant value, $p<0.005$ (Kolmogorov–Smirnov test)

0.38 % in blood donors. It was reported that parenteral drug abuse was the most significant route of transmission (Gurol et al. 2006). In a study performed by Acar, anti-HCV seroprevalence was found to be 0.50 % among blood donors. This rate declined to 0.07 % after confirmation test results (Acar 2008, unpublished data). Thus, Turkey has a low seroprevalence as compared with other countries; therefore, the proportion of false-reactive results with EIA is expected to be high. Our study has noted similar results.

The sensitivities and specificities of third- and fourth-generation EIA tests that are widely used in blood banks are ≥99 % (Colin et al. 2001). However, the positive predictive values of these tests are low in populations with low prevalence of infection. It was reported that 15–60 % of EIA results were false reactive among low-risk groups, such as blood donors, with a HCV infection prevalence rate <10 % (Alter et al. 2003). If anti-HCV testing is found to be reactive serologically, the Centers for Disease Control recommend verification of the results by WB or nucleic acid testing (NAT) (Centers for Disease Control and Prevention 1998). In a study performed by the American Red Cross in 2005, out of 6,024 blood specimens that were repeatedly reactive on an anti-HCV test, only 37 % were confirmed in WB or NAT tests. In another study conducted in China, 38.9 % of anti-HCV-reactive samples were also positive with WB and NAT (Nelson and Shan 2008). In a multicenter study from Turkey performed by Emektas et al., 67.2 % of 10,050 repeatedly anti-HCV-reactive samples were confirmed by WB (Gurol et al. 2006). In a study conducted by Acar, WB confirmation tests were positive in 0.07 % of anti-HCV-reactive blood donors (Acar 2008, unpublished data). In our study, we used a single WB as a confirmation test for our patients. We referred persons with positive or indeterminate WB results to a tertiary care center for further evaluation of chronic HCV infection. Thirty four (10.59 %) of the samples with anti-HCV s/co <10 were WB positive, and 56 (17.44 %) samples were interpreted as indeterminate. Even if we were to assume all indeterminate results are positive (which it is not acceptable), the proportion of positive results increases to only 28.03 %. As to why the proportion of positive confirmation test results is lower in our study, it may be because the study population consisted only of blood donors and individuals with anti-HCV s/co <10. All patients with anti-HCV titer s/co >10 had positive WB results.

The tests including WB and/or real-time PCR detect 95 % of individuals positively who have anti-HCV titers equal or bigger than 3.8 s/co which was determined by repeated measurements using second-generation Abbot and third-generation Ortho EIA methods. The same study showed a correlation between anti-HCV titers below this value and false-positive results (Contreras et al. 2007). In another study, the rate of positive WB and NAT results was 96.1 % at anti-HCV ≥3.8 s/co with an Ortho third-generation EIA (Nelson and Shan 2008). In a further study from Turkey, confirmation rates by line immunoassay for anti-HCV ≥3.8 s/co and ≥6.38 s/co to predict HCV infection were found to be 98.3 and 100 %, respectively (Acar 2008, unpublished data). In a study with 649 anti-HCV-reactive blood donors, the titer of anti-HCV <4.5 s/co was specific enough to exclude HCV in at least half of the patients. The authors stated that further diagnostic testing was not necessary for individuals with anti-HCV <4.5 s/co (Contreras et al. 2007). In contrast, Alter et al. (2003) recommended confirmation testing with third-generation WB for persons with anti-HCV titers <8 s/co. In another study, it was determined that there was no need for confirmation testing in persons with titers <5 s/co (Oethinger et al. 2005). We found that the most accurate cutoff value to predict HCV infection in our study population was 2.61 s/co. Above this value, test sensitivity and specificity were 74.1 and 71.6 %, respectively. When we accepted a cutoff value of ≥3.8 s/co to predict HCV infection, sensitivity and specificity were found to be 63 and 86.6 %, respectively. A cutoff value of ≥6.8 s/co generated a sensitivity and specificity of 66.67 and 99.4 %, respectively.

The cutoff value for anti-HCV immunoassay reactivity to predict HCV infection of a test with high sensitivity becomes lower in areas with a low prevalence of disease. Turkey has a low anti-HCV seroprevalence, and this cutoff value declines further among blood donors. We believe that an effective cutoff value cannot be assigned to predict HCV infection for blood donors in low-prevalence areas.

References

Alter MJ, Kuhnert WL, Finelli L (2003) Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. MMWR Recomm Rep 52(RR-3):1–13, 15; quiz CE1–4

Bar-Shany S, Green MS, Shinar E (1996) False positive tests for anti-hepatitis C antibodies and the problem of notifying blood donors. Int J Epidemiol 25:674–678

Centers for Disease Control and Prevention (1998) Recommendation for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease. MMWR Recomm Rep 47(RR-19):1–39

Colin C, Lanoir D, Touzet S, Meyaud-Kraemer L, Bailly F, Trepo C (2001) Sensitivity and specificity of third-generation hepatitis C virus antibody detection assays: an analysis of the literature. J Viral Hepat 8:87–95

Contreras AM, Tinoco E, Celis A, Novelo B, Romero MV, Carrada E, Jimenez-Mendez R (2007) Hepatitis C antibody intraassay correlation: is retest in duplicate necessary? Transfusion 47:1686–1690

Dufour DR, Talastas M, Fernandez MD, Harris B (2003) Chemiluminescence assay improves specificity of hepatitis C antibody detection. Clin Chem 49:940–944

Erensoy S (2001) Diagnosis of hepatitis C virus (HCV) infection and laboratory monitoring of its therapy. J Clin Virol 21:271–281

Gurol E, Saban C, Oral O, Cigdem A, Armagan A (2006) Trends in hepatitis B and hepatitis C virus among blood donors over 16 years in Turkey. Eur J Epidemiol 21:299–305

Kiely P, Wilson D (2000) Results of HCV screening of volunteer blood donors with a chemiluminescent immunoassay and a second- or third-generation EIA: overlap of false-positive reactivity and its impact on donor management. Transfusion 40:580–584

Nelson KE, Shan H (2008) Confirmatory testing of hepatitis C virus-positive enzyme immunoassay results in limited-resource countries: should it be attempted? Transfusion 46:1239–1244

Oethinger M, Mayo DR, Falcone J, Barua PK, Griffith BP (2005) Efficiency of the ortho VITROS assay for detection of hepatitis C virus-specific antibodies increased by elimination of supplemental testing of samples with very low sample-to-cutoff ratios. J Clin Microbiol 43:2477–2480

Seed CR, Margaritis AR, Bolton WV, Kiely P, Parker S, Piscitelli L (2003) Improved efficiency of national HIV, HCV, and HTLV antibody testing algorithms based on sequential screening immunoassays. Transfusion 43:226–234