

The Usefulness of Anti-HCV Signal to Cut-off Ratio in Predicting Viremia in Anti-HCV in Patients With Hepatitis C Virus Infection

Fahimeh Ranjbar Kermani¹; Zohreh Sharifi^{1,*}; Fereshteh Ferdowsian¹; Zahrah Paz¹; Farzaneh Tavassoli¹

¹Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, IR Iran

*Corresponding author: Zohreh Sharifi, Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, P.O. BOX: 14665-1157, Tehran, IR Iran. Tel/Fax: +98-218860150130, E-mail: z.sharifi@ibtto.ir

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Background: Hepatitis C Virus (HCV) infection is diagnosed by antibody and RNA based methods. Patients with anti-HCV sample rate/cutoff rate (S/CO) ratios > 1 are reported as anti-HCV positive. RNA based methods are introduced to confirm positivity in seropositive samples.

Objectives: The current study aimed to assess relationship between S/CO rates and HCV-RNA levels in the laboratory to identify HCV viremia in patients with a positive anti-HCV.

Patients and Methods: All serum samples were assayed for anti-HCV by ELISA method. A total of 265 anti-HCV positive patients were tested for HCV-RNA testing by quantitative method using Artus HCV RG Real-time Polymerase Chain Reaction (RT-PCR) kit. Statistical analysis was done by SPSS version 16.

Results: Of the 265 patients with HCV infection, 204 (77%) were male and the mean age was 43.53 ± 13.17 years, ranging 1 - 81 years. No correlation was found between S/CO ratios and HCV-RNA levels. There was significant difference in S/CO ratio between viremic and non-viremic subjects. The sensitivity, specificity, negative predictive value, and positive predictive value were 100%, 81.4%, 100%, and 77.2%, respectively in the S/CO ratio of 2.7.

Conclusions: The present study indicated that anti-HCV S/Co ratio is useful to predict non-viremic patients. A cut-off value of 2.7 can determine the usefulness of HCV-RNA testing. Patients with S/CO < 2.7 are not viremic; therefore, HCV-RNA testing is not recommended. It is suggested that laboratories report S/CO ratio along with anti-HCV results to manage HCV infection better, especially in countries that quantitative HCV testing is expensive or not available.

Keywords: Hepatitis C Antibodies; Hepatitis C Infection

1. Background

Hepatitis C Virus (HCV) is a major cause of post transfusion hepatitis and liver transplantation in many countries. It is estimated that approximately 3% of the world's population is infected with HCV and 60% to 85% of the patients develop chronic infection that leads to cirrhosis, and hepatocellular carcinoma, and need liver transplantation (1, 2). Precise detection of HCV is very important in the infection management. Three groups of HCV testing are available: 1) serological assays to detect anti-HCV. 2) Molecular assays to detect and quantify HCV-RNA. 3) Viral genotyping to determine HCV genotype. HCV tests are used to diagnose HCV and to guide therapy and/or monitor the treatment of HCV infection (1, 3-5).

The most common type of HCV antibody testing is the enzyme immunoassay by methods of enzyme linked immunosorbent assay (ELISA), and chemiluminescence immunoassay (CIA). ELISA testing is a semi-quantitative assay that its results are reported as positive or negative based on comparison of absorbance reading of each sam-

ple with a cut-off value defined for the lot used expressed as a signal to cutoff ratio (S/CO), and shows the quantity of antibody in blood (6). Although HCV antibody assays are highly sensitive and specific in patients with chronic HCV infection, there are frequent false positive in anti-HCV results. In addition, anti-HCV assays detect current active or past HCV infection. They failed to distinguish these two forms of HCV infection. Quantitative viral load tests are used to measure the amount of HCV-RNA in one milliliter of blood. PCR-based HCV RNA methods are used as the gold standard to confirm the HCV positivity in anti-HCV assays and monitor the treatment (1, 4, 7-9).

2. Objectives

The current study aimed to evaluate the relationship between quantitative anti-HCV (S/CO ratio) and quantitative HCV-RNA levels, also to determine a specific S/CO ratio with a routine commercial HCV-Ab kit used in the laboratories to identify viremic from non-viremic anti-HCV positive patients.

Table 1. Subjects Based on PCR Results ^a

Variables	All Subjects	Non-Viremic Group	Viremic Group	P Value
Male	204 (77)	57 (76)	147 (77.4)	> 0.05
Age, y	43.52 ± 13.169	43.05 ± 13.472	43.7 ± 13.08	> 0.05
Anti-HCV S/CO ratio	3.92	2.7	4.10	< 0.05

^a Data are presented as No. (%) or mean ± SD.

3. Patients and Methods

3.1. Patients

In the current cross sectional study, from May 2012 to October 2013, 378 patients suspected with HCV who referred to Iranian Blood Transfusion Organization (IBTO) Research center were included. Patients had no history of Hepatitis B Virus (HBV) and Human Immunodeficiency Virus (HIV) infections. The written consent was signed by the patients to include in the study and the questionnaire was filled out for all subjects. All subjects also were tested for anti-HCV assay; HCV viral loads were performed on all anti-HCV positive patients.

3.2. ELISA Assay

All fresh serum samples were assayed for anti-HCV (Hepanostika HCV Ultra, UK) by ELISA method, according to the manufacturer's protocol. The results were expressed as S/CO ratio calculated by dividing the signal detected on each sample to the cut-off value. The S/CO ratios ≥ 1.00 were reported as positive.

3.3. RNA Extraction

Briefly, viral RNA was extracted from one milliliter plasma using the QIAamp UltraSense virus kit (Qiagen, Germany) according to the manufacturer's protocol. The extracted RNA was eluted in elution buffer and used as the template for the quantitative RNA PCR.

3.4. Quantitative RNA PCR

Quantitative RNA PCR was performed using Artus HCV RG RT-PCR kit (Qiagen GmbH, Qiagen Strasse, Germany) with lower detection limit of 34 IU/mL.

20 μ L of the extracted RNA was added to 30 μ L of Master Mix in each 0.1 mL microtube and test was performed in Rotorgene-Q apparatus. A standard curve was automatically drawn with the Rotorgene-Q software using five quantification standard concentrations of HCV-RNA to analyze the viral RNA load. The results expressed in IU/ μ L were determined in IU/mL based on Artus HCV RG RT-PCR kit handbook. In each run, plasma negative sample and internal control were used to prevent false positive and negative results, respectively.

3.5. Statistical Analysis

Statistical analysis was conducted using SPSS version

16. Quantitative group variables were described as mean, standard deviation, and range. To compare variables, χ^2 and Mann-Whitney tests were used. P value < 0.05 was considered as significant.

4. Results

Of the 265 HCV-Ab positive patients, 204 (77%) were male and the mean age was 43.53 \pm 13.17 years. HCV-RNA was detected in 190 (71.7%) subjects. There were significant differences in S/CO ratio between viremic and non-viremic patients. The S/CO ratio was higher in viremic group than the non-viremic group (P < 0.05) (Table 1).

The sensitivity, specificity, negative predictive and positive predictive values were 100%, 81.4%, 100%, and 77.2%, respectively in the S/CO ratio of 2.7 (Table 2).

Table 2. Profile of HCV Antibody Assay ^a

Variables	Values
Anti-HCV S/CO Ratio	2.7
Sensitivity	100
Specificity	81.4
Negative Predictive Value	100
Positive Predictive Value	77.2

^a All of the data are presented as (%) except Anti-HCV S/CO ratio.

5. Discussion

Hepatitis C infection is a major health problem since it can cause chronic disease. It is estimated that 20% of people with chronic HCV infection develop cirrhosis after 25 years (1, 4, 8-10). Diagnostic testing for HCV has been improved over the past decade. The initial testing for HCV detects anti-HCV in blood samples. A positive result of anti-HCV by ELISA method may represent active viremia, infection in the past or false positive. Although Recombinant Immunoblot assay (RIBA) is used to confirm results in ELISA method, it cannot detect viremia to follow treatment. Qualitative and quantitative assays for HCV-RNA are introduced as gold standards to confirm viremia in patients with positive anti-HCV (1, 4, 8, 9). Quantification of HCV-RNA is important to determine disease status and is used before and during anti-viral therapy (9-14).

Although using quantitative HCV-RNA RT-PCR to detect and monitor the treatment of HCV infection is approved, it consumes time and money especially in patients with no viremia; also, its high cost makes it unavailable in

many laboratories. Recently, the necessity to use confirmatory testing in anti-HCV low S/CO ratio was suggested by the Centers for Disease Control (CDC). They also introduced S/CO ratio ≥ 3.8 as a cut-off value and suggested that S/CO ratio < 3.8 determines low positive. An anti-HCV S/CO ratio ≥ 3.8 determines a true anti-HCV positive result in 95% of cases. There is low possibility of HCV viremia in low positive patients. In contrast, in patients with S/CO ratio ≥ 3.8 the mentioned possibility is high (15). Several studies conducted to detect a cut-off point to distinguish low positive from high positive subjects reported that the majority of subjects with low positive anti-HCV results, by ELISA method, were negative in HCV-RNA testing (6, 12, 13).

Several studies are conducted to introduce S/CO value to distinguish viremic and non-viremic patients. In several published studies, different S/CO values ranging from 3 to 34 were determined in the third generation of anti-HCV assays (6, 10, 13, 16-20). The result of the current study showed that in low positive anti-HCV ELISA results, the frequency of false positivity was high. According to the obtained result, using 2.7 as a cut-off for S/CO ratio, the sensitivity was 100%. The study found that all HCV-Ab positive patients with S/CO cutoff ratio < 2.7 were not detectable HCV-RNA. Positive results in HCV-Ab assay may represent a past infection or false positive result. In the current study the majority of the patients with HCV-Ab positive, S/CO cut-off ratio ≥ 2.7 were viremic.

Due to differences in sample size, the study population, and the kit used to detect HCV-RNA, there are discrepancies in the S/CO ratios introduced as cut-off point in different studies. Further studies with common approaches are necessary to predict using anti-HCV S/CO ratio as a cut-off value.

In conclusion the present study indicated that anti-HCV S/CO ratio can be used as a useful tool to manage HCV infection. A cut-off value of 2.7 can determine the need to HCV-RNA testing. Therefore, for patients with S/CO < 2.7 , HCV-RNA viral load is not recommended. It is suggested that laboratories should report S/CO ratio along with anti-HCV results.

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Authors' Contributions

Zohreh Sharifi: the corresponding author; Fahimeh Ranjbar Kermani: experiments performing, data analysis, and the manuscript preparation. Fereshteh Ferdowsian, Zahrah Paz and Farzaneh Tavassoli performed the experiments. All authors read and approved the final manuscript.

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References

- Majid AM, Gretch DR. Current and future hepatitis C virus diagnostic testing: problems and advancements. *Microbes Infect.* 2002;**4**(12):1227-36.
- Ghany MG, Strader DB, Thomas DL, Seeff LB, American Association for the Study of Liver D. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology.* 2009;**49**(4):1335-74.
- Richter SS. Laboratory assays for diagnosis and management of hepatitis C virus infection. *J Clin Microbiol.* 2002;**40**(12):4407-12.
- Bossi V, Galli C. Quantitative signal of anti-HCV by an automated assay predicts viremia in a population at high prevalence of hepatitis C virus infection. *J Clin Virol.* 2004;**30**(1):45-9.
- Galel SA, Strong DM, Tegtmeier GE, Holland PV, Kuramoto IK, Kemper M, et al. Comparative yield of HCV RNA testing in blood donors screened by 2.0 versus 3.0 antibody assays. *Transfusion.* 2002;**42**(11):1507-13.
- Balk M, Saydam G, Cengiz D, Tuerkmen A, Simsek H, Himmetoğlu T. The utility of ANTI-HCV S/CO ratio, HCV-RNA and ALT test in predicting viremia in ANTI-HCV positive patients. *Turk J Biochem.* 2007;**32**(2):51-4.
- Sugitani M, Inchauspe G, Shindo M, Prince AM. Sensitivity of serological assays to identify blood donors with hepatitis C viraemia. *Lancet.* 1992;**339**(8800):1018-9.
- Strader DB, Seeff LB. Hepatitis C: a brief clinical overview. *ILAR J.* 2001;**42**(2):107-16.
- Schiff ER, de Medina M, Kahn RS. New perspectives in the diagnosis of hepatitis C. *Semin Liver Dis.* 1999;**19** Suppl 1:3-15.
- Sookoian S, Castano G. Evaluation of a third generation anti-HCV assay in predicting viremia in patients with positive HCV antibodies. *Ann Hepatol.* 2002;**1**(4):179-82.
- Yuki N, Hayashi N, Hagiwara H, Naito M, Ohkawa K, Kasahara A, et al. Hepatitis C virus antibodies and virus replication in asymptomatic blood donors. *Vox Sang.* 1994;**67**(3):280-5.
- Dufour DR. Lot-to-lot variation in anti-hepatitis C signal-to-cutoff ratio. *Clin Chem.* 2004;**50**(5):958-60.
- Dufour DR, Talastas M, Fernandez MD, Harris B, Strader DB, Seeff LB. Low-positive anti-hepatitis C virus enzyme immunoassay results: an important predictor of low likelihood of hepatitis C infection. *Clin Chem.* 2003;**49**(3):479-86.
- Lee PL, Wang JH, Tung HD, Lee CM, Lu SN. A higher than expected recovery rate from hepatitis C infection amongst adolescents: a community study in a hepatitis C-endemic township in Taiwan. *Trans R Soc Trop Med Hyg.* 2004;**98**(6):367-72.
- Alter MJ, Kuhnert WL, Finelli L, Centers for Disease C, Prevention. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. Centers for Disease Control and Prevention. *MMWR Recomm Rep.* 2003;**52**(RR-3):1-13.
- Seo YS, Jung ES, Kim JH, Jung YK, Kim JH, An H, et al. Significance of anti-HCV signal-to-cutoff ratio in predicting hepatitis C viremia. *Korean J Intern Med.* 2009;**24**(4):302-8.
- Kim HS. Anti-HCV signal-to-cutoff ratio in predicting hepatitis C viremia. *Korean J Intern Med.* 2009;**24**(4):299-301.
- Abdelaziz H. HCV signal to cutoff ratio in predicting Hepatitis C viremia. *J Blood Disord Transfus.* 2013;**4**(5):23-5.
- Lai KK, Jin M, Yuan S, Larson MF, Dominitz JA, Bankson DD. Improved reflexive testing algorithm for hepatitis C infection using signal-to-cutoff ratios of a hepatitis C virus antibody assay. *Clin Chem.* 2011;**57**(7):1050-6.
- Altuğlu İ, Gürsel D, Aksoy A, Orman M, Erensoy S. The importance and role of anti-HCV signal/cutoff ratio in diagnosis of hepatitis C virus infection. *Ege J Med.* 2011;**50**:223-8.