ORIGINAL ARTICLE

Comparison of Anti HCV Signal-to-Cutoff Ratio with HCV RNA Results

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SUMMARY

Background: **Anti-HCV antibody level screening is used in the diagnosis of HCV. However, a positive (S/Co > 1) result in the anti-HCV test does not always reflect true positivity. Antibody level results of S/Co ratio > 1 have to be validated through HCV RNA. In this study, we aimed to compare the signal-to-cutoff ratios of patients with positive (> 1) HCV antibody levels with the results of HCV RNA by PCR.**
Methods: **In total, 17,021 samples were tested for anti HCV between January 2017 and December 2019. HCV antibody (anti HCV) was performed with a fully automated chemiluminescent microparticle immunoassay (CMIA, Abbot®, Architect System). Real Time PCR test (Anatolia Geneworks HCV, Turkey) was used as nucleic acid amplification method.**
Results: **Of the 17,021 patients, 16,706 (98.15%) tested negative and 315 (1.85%) tested positive in the anti-HCV assay. An additional HCV RNA test was requested for these 315 patients with positive anti-HCV assay results (S/Co ≥ 1) of which 23.81% (75/315) were positive for HCV RNA in serum, with a median (IQR): 5.43 $\log_{10}$ IU/mL (4.75 - 6.01 $\log_{10}$ IU/mL). Patients who tested positive for HCV RNA had significantly higher S/Co values compared to patients who tested negative (median (IQR): 13.38 (12.30 - 14.57) vs. 1.79 (1.34 - 1.79), p < 0.001).**
Conclusions: **When S/Co ratios of patients who tested anti-HCV positive and HCV RNA positive were evaluated, it was assumed that high S/Co values were more relevant to true positivity. It was also concluded that low S/Co ratios needed to be verified through PCR.**
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KEY WORDS

INTRODUCTION

Hepatitis C virus (HCV) is a flavivirus with a single-stranded RNA genome that infects about 1% of the world's population [1]. HCV infection is still a concern for mortality and morbidity. In 2016, the World Health Organization set the course for the global health sector to eliminate hepatitis infection by 2030 and to reduce chronic hepatitis C cases by 90% and hepatitis C deaths by 65% [2]. Accurate and early diagnosis of HCV infection is crucial in achieving these goals. HCV can cause acute infections in 15% and chronic infections in 50 - 80% of the patients with the infection. Early diagnosis

in HCV infections also reduces the risk of fibrosis, cirrhosis or hepatocellular carcinoma due to chronic HCV infection [3]. In the diagnosis of HCV infection, first the level of HCV antibody in the serum is analyzed. Serological tests with positive results must be validated by further tests. Approximately 15 - 30% of individuals infected with HCV heal spontaneously without viremia in the patient. In patients who are suspected to have viremia, HCV RNA viral load is analyzed through molecular methods [4].

HCV antibody in serum can be positive in cases of acute infection, chronic infection or past infection. The distinction between these conditions is possible by the quantitative detection of the HCV RNA genome through polymerase chain reaction (PCR), which is a nucleic acid amplification method [5,6]. Positivity with low levels of HCV antibody are not always tested along with HCV RNA. Even after a certain period of time, the HCV antibody may become negative. False positive screening test results are a concern for the patient. False positive screening test results are alarming for the patient.

In this study, we aimed to compile the PCR-based HCV RNA results of patients with positive HCV antibody levels.

MATERIALS AND METHODS

Patients

A total of 17,021 samples brought for anti-HCV test were included in the study at the microbiology laboratory of a tertiary hospital between January 2017 and December 2019. The study consisted of two phases. In the first phase, anti-HCV antibody levels of the patients were analyzed. In the second phase, HCV RNA PCR test was performed on patients who tested positive in the first phase. Repeated blood samples, follow-up of patients who had previously received antiviral treatment, and cases which could not be verified through HCV RNA (PCR) were not included in the study.

Anti-HCV tests

HCV antibody (anti HCV) was performed with a fully automated chemiluminescent microparticle immunoassay (CMIA, Abbot®, Architect System). While ≥ 1.00 signal-to-cutoff (S/Co) anti-HCV antibody test results were considered as reactive, < 0.90 S/Co results were considered as non-reactive and ≥ 0.90 S/Co and < 1.00 S/Co results were considered as limit values. The S/Co ratio of the device was ≥ 1. The capture antigen in the ARCHITECT test is a recombinant antigen containing the HCr43 fusion protein (core, NS3) and the c100-3 protein (NS4). Chemiluminescent microparticle immunological test determines if there is antibody in the serum. It interacts with the recombinant antigen in the kit to form an antigen antibody complex. This complex generates light through chemical reactions, and the measurement is based on the light that it generates.

HCV RNA PCR

RNA isolation from the samples was performed using the automatic isolation system (Anatolia Geneworks HCV, Turkey) according to the recommendation of the manufacturer. Verification was performed through the HCV RNA polymerase chain reaction test (Anatolia Geneworks HCV, Turkey) with the lowest detection limit of 15 IU/mL and quantitative limit between 70 and 1×10^6 IU/mL.

Statistical analysis

Continuous variables were summarized by median and inter-quartile range (IQR). HCV RNA concentration values in serum were converted to $\log_{10}$ values. Relationships between continuous variables were assessed by Pearson's correlation. The statistical analyses were performed by using R version 3.6.1 (R Development Core Team, 2010; www.R-project.org) and run in RStudio (www.rstudio.com). Figures were generated by using the ggplot2 package in R [7].

Data

The data was obtained from a retrospective cohort of 17,201 patients who had been requested to take anti-HCV tests from January 2017 to December 2019 at the Bolu Abant Izzet Baysal Training and Research Hospital.

RESULTS

The anti-HCV S/Co values obtained from 17,021 patients were compared with HCV RNA results. Of the 17,021 patients, 16,706 (98.15%) tested negative and 315 (1.85%) tested positive using the ARCHITECT anti-HCV assay. An additional HCV RNA test was requested for the 315 patients who had positive results in anti-HCV assay (S/Co ≥ 1) and 23.81% (75/315) of these 315 patients were tested positive for HCV RNA in serum, with a median (IQR): 5.43 $\log_{10}$ IU/mL (4.75 - 6.01 $\log_{10}$ IU/mL). Of the patients who previously tested positive in an anti-HCV assay, 1.41% (240/17,021) tested negative based on the HCV RNA test.

The patients who tested positive for HCV RNA, had significantly higher S/Co values compared to the patients who tested negative (median (IQR): 13.38 (12.30 - 14.57) vs. 1.79 (1.34 - 1.79), p < 0.001) (Figure 1). Additionally, a positive correlation was observed between anti-HCV (S/Co) results and the levels of HCV RNA in serum ($\log_{10}$ IU/mL) (r = 0.244, p = 0.035) (Figure 2).

DISCUSSION

There are still more than 71 million patients with HCV infection in the world and 399,000 patients die each year due to HCV-related cirrhosis or liver cancer [8]. As there is no protective vaccine against HCV, the number
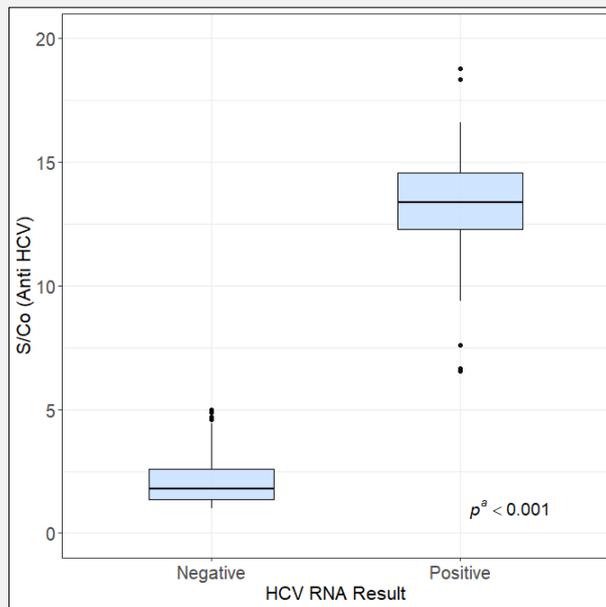
Figure 1. Box plot of S/Co by HCV RNA results.
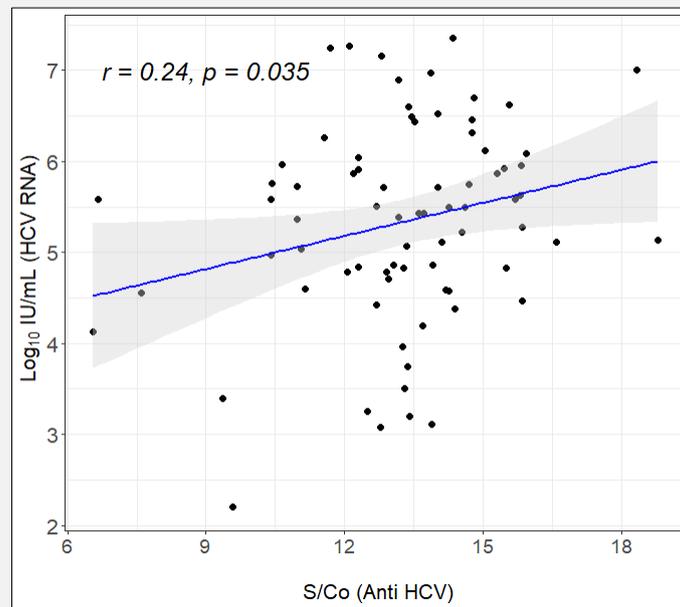
[a] **Mann Whitney U test.**

Figure 2. Correlation of the anti-HCV (S/Co) results and the levels of HCV RNA (log_{10} IU/mL) (r = 0.244, p = 0.035).

of patients infected with HCV increases every day [9]. Anti-HCV antibody level screening test is used in the diagnosis of HCV. However, a positive (S/Co > 1) result in the anti-HCV test does not always reflect true positivity [10]. In this study, anti-HCV testing was performed on a total of 17,201 patients of which 1.85% (315/17.021) tested positive. However, 240 of the patients with positive antibody test results, tested negative in HCV RNA PCR test. In other words, false positivity was found to be 1.41% (240/17.021) in this study. The overall true positivity was found to be 0.43% (75/17.021). Kileng et al. [11] initially validated 1.0% (217/20.946) anti-HCV positivity; however, by using a different method later, they concluded negative results in 83 of 217 patients. The seroprevalence of HCV in Turkey ranges from 0.3 to 1.8% [12]. False positivity ratio is high in our country [13]. In their study, Yenilmez et al. [14] showed that at least two of the three cases were false anti-HCV positive. CDC recommends low S/Co antibody level results to be validated through a more specific complementary test such as HCV RNA [15,16]. The manufacturer (Abbot®, Architect System; Germany) states that the test can report ≥ 95% true positive results when the S/Co ratio is taken as ≥ 5 [17,18]. The mean value of anti-HCV tests of the patients with negative HCV RNA results was found to be 1.79. Şafak et al. [13] found HCV RNA positivity in 4 of 69 samples (5.8%) with low S/Co ratio. Ecemiş et al. [19] observed HCV RNA positivity in 2 of 52 patients (3.8%) with an S/Co ratio ≤ 5 for HCV antibodies. Similarly, in another study, HCV RNA positivity was reported in 3 of 215 (0.9%) samples (1.4%) [19]. In this study, HCV RNA tests of patients with S/Co ratio ≤ 5 were found negative (Figure 1). The mean of anti-HCV value in HCV RNA negative cases was found to be 1.79 (S/Co 1 - 5). In many studies, the S/Co ratio in anti HCV positive cases varies according to the method used [20,21]. As a result, also by considering the studies conducted, it was concluded that the possibility of false positivity was high in patients with HCV antibody S/Co ratio of < 5, regardless of the method used. Therefore, it was deliberated that all positive results must be validated through the HCV RNA test. When the S/Co ratios of anti-HCV positive and HCV RNA positive patients were analyzed, the S/Co ratio of all the results was > 8. The average value was 13.38. These results show us that high S/Co values are more relevant with true positivity. Evidence for this can be shown as a statistically positive correlation between anti-HCV S/Co values and HCV RNA values.

A limitation of this study was that anti-HCV positive patients were only confirmed with HCV RNA. In other words, the accuracy of the results could be supported through similar methods such as RIBA. In addition, detailed clinical history of HCV positive patients could be examined.

In conclusion, this study demonstrated that not all anti HCV positive results were true positivity. Based on a certain S/Co ratio, it can be stated that a positive or negative value varies according to the method and the manufacturer. It was also demonstrated that there was a positive correlation between S/Co ratio and HCV RNA.

Declaration of Interest:
None.

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