JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

The best anti-HCV S/Co values for reflecting HCV infection in a university hospital in the eastern part of Turkey

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Abstract

Introduction: The measurement of hepatitis C virus (HCV) RNA is a test that requires high cost, advanced technique, and qualified personnel. Diagnosis and treatment of patients may be delayed due to the high rate of false-positive results. This study aims to predict true antibody positivity and viremia by determining the most appropriate anti-HCV signal-to-cutoff (S/Co) value reflecting HCV infection.

Methodology: The presence of anti-HCV antibodies and HCV RNA levels were examined in 72341 people who applied to the Mengücek Gazi Training and Research Hospital between January 2018 and December 2020. The anti-HCV levels were determined by using the Abbot Architect i2000 SR device (Abbot Diagnostics, Chicago, IL, USA). The levels of HCV RNA were determined in the COBAS AmpliPrep/COBAS, TaqMan 48 (Roche, Diagnostics, Pleasanton, USA) devices using serum samples from patients. Our study is a retrospective and methodological study.

Results: Of the 150 patients with anti-HCV antibodies, 50 (33.3%) were HCV RNA positive, and 100 (66.7%) were HCV RNA negative. Anti-HCV levels of HCV RNA-positive patients were statistically higher than HCV RNA-negative patients. The most appropriate anti-HCV S/Co value for diagnosing hepatitis C patients was 15.4. The sensitivity of this value was 72%, specificity 88%, positive predictive value (PPV) 73.5%, and negative predictive value (NPV) 86.1%. Receiver operating characteristic (ROC) curve was significantly higher than 0.5 (95% confidence interval 0.938-0.827).

Conclusions: Correct approaches can be applied in the diagnosis of HCV infection using the anti-HCV S/Co value found in our study.

Key words: anti-HCV; HCV RNA; S/Co.

J Infect Dev Ctries 2024; 18(1):131-135. doi:10.3855/jidc.18105

(Received 19 February 2023 – Accepted 28 June 2023)

Introduction

Hepatitis C virus (HCV) belongs to the *Hepacivirus* genus of the Flaviviridae family and is the only member of this genus. HCV is an enveloped RNA virus with an average diameter of 50 nm, icosahedral symmetry, spherical structure, single-stranded, positive polarity, and is 9.6 kilobytes in length [1–3].

HCV is transmitted by blood. This transmission occurs while sharing the equipment used to administer intravenous drugs, transfer of infected blood and blood products between people, use of medical equipment with insufficient sterilization and disinfection, unsafe therapeutic injections, risky contact, and sexual practices in contact with infected blood [4].

HCV infection can be of two types: acute hepatitis C virus infection (AHC) and chronic hepatitis C virus infection (CHC), and is generally asymptomatic. The incubation period of AHC infection is between two and six months [4]. If viremia lasts for more than six months, it is an indication that CHC has occurred [5]. Around 20-30% of chronic hepatitis C patients develop cirrhosis and end-stage liver disease within 7-30 years. If the infection is not treated, it causes cirrhosis, liver failure, liver cancer, and death [6].

HCV infection is diagnosed using serological and molecular methods. Serological methods (enzyme immunoassay, EIA) are based on detecting HCV antigen or anti-HCV antibodies occurring in the body against the virus. Molecular methods are based on detecting the presence and amount of HCV RNA belonging to the virus in the serum. Currently molecular methods are the gold standard because they are the most sensitive and reliable method used in diagnosis HCV [7].

This study investigated the relationship between anti-HCV signal-to-cutoff (S/Co) values and HCV RNA positivity. It aimed to determine the most appropriate anti-HCV S/Co value that can guide physicians in predicting viremia in HCV patients to minimize false-positive test results and to reduce unnecessary HCV RNA test requests.

Methodology

Ethics committee

The study titled "Investigation of anti-HCV S/Co value in diagnosing hepatitis C patients who applied to Erzincan Binali Yıldırım University, Mengücek Gazi Training and Research Hospital" was approved by the Binali Yıldırım University Clinical Research Ethics Committee, Erzincan, Turkey (decision date: 4 May 2021; decision number 06/31).

Patient group

Serum samples of 72,341 individuals who applied to Erzincan Binali Yıldırım University (EBYU), Mengücek Gazi Training and Research Hospital (MGEAH) between 1 January 2018, and 31 December 2020, were analyzed. 150 male and female hepatitis C patients over 18 years of age, who were found to have anti-HCV antibodies and had simultaneous HCV RNA requests were included in our study. Our study is a retrospective and methodological study.

Detection of anti-HCV antibodies

The presence of anti-HCV antibodies was detected in the EBYU, MGEAH Microbiology laboratory with the serum samples of the patients and using the Abbott Architect (i2000) SR, (Abbot Diagnostics, Chicago, IL, USA) device. The device works on the principle of chemiluminescent microparticle immunoassay. Architect anti-HCV assay (Abbott, Wiesbaden, Germany) was used. This assay is a two-step immunoassay using chemiluminescent microparticle immunoassay (CMIA) technology to detect anti-HCV antibodies in human serum using the HCV genome's HCr43 and c100-3 proteins.

HCV RNA analysis

The presence and levels of HCV RNA in the serum samples of the patients were determined in the EBYU MGEAH Microbiology laboratory using the COBAS AmpliPrep/COBAS, TaqMan 48, (CAP/CTM), (Roche, Diagnostics, Pleasanton, USA) device. The device works with the real-time (RT) polymerase chain reaction (PCR) method. During RT-PCR, the device performs hybridization in three stages (reverse transcription, cDNA synthesis, and target DNA synthesis from cDNA) and the products are determined quantitatively.

Statistical analysis

While summarizing the descriptive statistics of the data, categorical variables were presented as n (%), and continuous variables were presented as mean ± standard deviation and median (minimum-maximum) values. The assumption of normal distribution in continuous variables was checked with the Kolmogorov-Smirnov test of normality and hypothesis tests were selected according to the distribution type. In the case of normally distributed variables, t-test was used in independent groups and Mann-Whitney U test was used for non-normally distributed variables. Receiver operating characteristic (ROC) analysis was used to determine the numerical value of HCV RNA. Cases with $p < 0.05$ were considered statistically significant. IBM Statistical Package for the Social Sciences (SPSS) v 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) was used for data analysis.

Results

Of the 150 patients included in our study, 67 (44.7%) were female, 83 (55.3%) were male and their mean age was 55.99 (minimum-maximum 22-95) years. Among them, 38 (25.3%) were ≤ 40 years old and 112 (74.7%) were > 40 years old.

Fifty (33.3%) of 150 patients with anti-HCV S/Co level ≥ 1 were found to be HCV RNA positive and 100 (66.7%) HCV RNA negative. The mean HCV RNA level was 978,485 (minimum-maximum 15-9,190,000 IU/mL, with a median of 329,000. In anti-HCV tests, the median was 12.91 (minimum-maximum 1.05-36.83).

Table 1. Hepatitis C virus (HCV) RNA positivity and negativity rates in grouping according to anti-HCV signal-to-cutoff (S/Co) values.

		1.0-5.0	5.1-10.0	10.1-15.0	15.1-20.0	20.1 and above	
HCV	**n**	37	13	35	11	4	100
RNA	**HCV RNA %**	37%	13%	35%	11%	4%	100%
Negative	**anti-HCV %**	100%	92.9%	72.9%	61.1%	12.1%	66.7 %
HCV	**n**	0	1	13	7	29	50
RNA	**HCV RNA %**	0%	2%	26%	14%	58%	100%
Positive	**anti-HCV %**	0%	7.1%	27.1%	38.9%	87.9%	33.3%
	n	37	14	48	18	33	150
Total	**HCV RNA %**	24.7%	9.3%	32%	12%	22%	100%
	anti-HCV %	100%	100%	100%	100%	100%	100%

The mean anti-HCV S/Co levels of patients with positive HCV RNA results were statistically higher than those with negative HCV RNA results ($p < 0.001$). There was no statistically significant correlation between the HCV RNA viral load level and the increase in anti-HCV S/Co values ($p = 0.158$, r = 0.20).

When the patients were grouped according to their anti-HCV S/Co values, and the HCV RNA results were compared, anti-HCV S/Co values between 1.0 and 5.0 were free, between 5.1-10.0 in 1 patient (2%), between 10.1-15.0 in 13 patients (26%), and between 15.1-20.0 in 7 patients (14%). There were 29 patients (58%) in the group with values of ≥ 20.1 (Table 1). It was determined that the anti-HCV S/Co values of the patients grouped according to their anti-HCV S/Co values were also increased (Table 1).

To determine the best cut-off point by ROC analysis, the presence of HCV RNA, which is the gold standard for diagnosing HCV infection, was used. The optimal anti-HCV S/Co value was 15.4 with 72% sensitivity and 88% specificity. Youden Index was used when determining the breakpoint (Table 2). The area under the ROC curve was significantly overestimated at 0.5 (95% confidence interval 0.938-0.827) (Figure 1).

In our study, we determined that in the anti-HCV S/Co 15.1-20 range, which is our most appropriate anti-HCV S/Co value of 15.4, the rate of HCV RNA positive patients was 73.5% and the rate of HCV RNA negative patients was 26.5% (Table 3).

Discussion

HCV infection is currently among the leading causes of mortality and morbidity globally and is an important public health problem [4,8,9]. According to the World Health Organization (WHO) data approximately 290,000 people died due to cirrhosis and

Figure 1. ROC analysis of anti-HCV S/CO value to predict patients' qualitative HCV RNA test results.

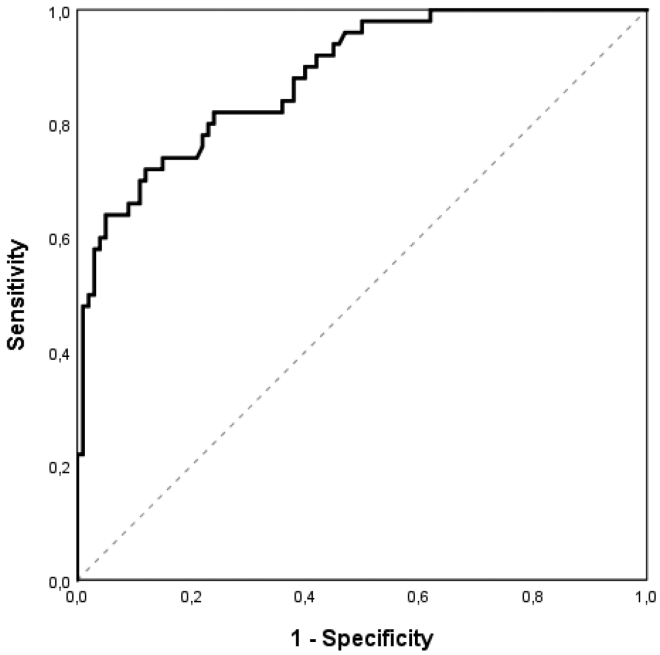

Table 2. Sensitivity and specificity ratios of selected anti-HCV S/Co values according to hepatitis C virus (HCV) RNA according to receiver operating characteristic (ROC) analysis.

S/Co	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Youden Index
14.8	0.74(59.7-85.4)	0.85	71.2	86.7	0.59
15.0	0.72 (57.5-83.8)	0.85	70.6	85.7	0.57
15.2	0.72 (57.5-83.8)	0.86	72	86	0.58
15.3	0.72 (57.5-83.8)	0.87	73.5	86.1	0.59
15.4	**0.72 (57.5-83.8)**	**0.88**	**73.5**	**86.1**	**0.60**
15.5	0.70 (55.4-82.1)	0.89	76.1	85.6	0.58

Bold type indicates the most appropriate anti-HCV S/Co value for diagnosing hepatitis C patients was found to be 15.4.

Table 3. Rates of hepatitis C virus (HCV) RNA positive and negative patients according to the most appropriate anti-HCV signal-to-cutoff (S/Co) value.

Test		HCV RNA		Total
		Negative	Positive	
Negative	n	87	14	101
	Optimal value % (15.4)	86.1%	13.9%	100%
	HCV RNA %	87%	28%	67.3%
Positive	n	13	36	49
	Optimal value % (15.4)	26.5%	73.5%	100%
	HCV RNA %	13%	72%	32.7%
Total	n	100	50	150
	Optimal value % (15.4)	66.7%	33.3%	100%
	HCV RNA %	100%	100%	100%

liver cancer caused by HCV in 2019 [4]. HCV infection is the second most common cause of liver transplantation in our country [10]. In the HCV infection diagnosis algorithm of the Centers for Disease Control and Prevention (CDC), it is recommended to screen for anti-HCV antibodies first and to determine the presence and level of HCV RNA by RT-PCR in cases where reactive antibodies are detected [11]. This algorithm uses HCV RNA testing for each patient which has high cost and the process of diagnosing the real patient and to start treatment is prolonged. These drawbacks can be reduced with the optimal anti-HCV S/Co value determined by examining the relationship between anti-HCV S/Co values and HCV RNA. In addition, the CDC recommends that each laboratory establish its own unique HCV diagnostic algorithm. The CDC reports that the optimal anti-HCV S/Co value can be established for this, and this value can be obtained by analyzing the system data [12]. In our study, the most appropriate anti-HCV S/Co value is 15.4, based on the data of the patients included (Table 2). Optimal values for anti-HCV S/Co varies in different studies. It was determined as 2.7 in the study by Sharifi *et al.* [13], 5 in the study by Şanlıdağ *et al.* [14], 7.13 in the study by Gülden *et al.* [15], 10.9 in the study by Seo *et al.* [16], 12.27 in the study by Kirişçi *et al.* [17], 26 in the study by Sookoyan *et al.* [18] and 25.9 in the study by Balk *et al.* [19]. In terms of epidemiology, if the prevalence of a disease in a population is low, the false positive rate increases and the positive predictive value (PPV), which provides the estimation of the true positivity of the test, decreases. Since anti-HCV positivity is 1% in our country, false positivity is common [20]. The false-positive rate for optimal anti-HCV S/Co 15.4 detected in our study was 13.9%. In the study by Kirişçi *et al*., anti-HCV S/Co was 12.27, while the false-positive rate was 3.4% [17].

Based on the manufacturer's recommendation, no HCV RNA-positive patient with anti-HCV S/Co = 1 was found in our study. HCV RNA-positive patients had an anti-HCV S/Co value of ≥ 10.1, except for only one patient. In our study, sensitivity was 72%, specificity was 88%, PPV was 73.5%, and negative predictive value (NPV) was 86.1% for the most appropriate anti-HCV S/Co value of 15.4. Kirişçi *et al*. determined that for the anti-HCV S/Co value of 12.27, the sensitivity was 94.4%, specificity was 97.4%, PPV was 95.7%, and NPV was 96.6% [17]. Seo *et al.* determined that for the anti-HCV S/Co value of 10.9%, the sensitivity was 94.9%, specificity was 97.3%, PPV was 98.3%, and NPV was 91.4% [16]. The sensitivity and specificity of our study and the sensitivity and specificity of these studies differ. Similar to our study, Gürkan *et al*., did not find any HCV RNA-positive patients with an anti-HCV S/Co value between 1-5, and the number of HCV RNA positive patients with anti-HCV S/Co between 5.1-10 was found to be less than the other groups (16.36%). It was found that there was a significant increase (56.22%) in the number of HCV RNA-positive patients with values of anti-HCV S/Co > 10 [21]. Consistent with our study, the number of HCV RNA-positive patients and the anti-HCV S/Co > 10 value increased significantly in the study by Kirişçi *et al.* [17]. Seo *et al*. reported that all patients with anti-HCV S/Co values > 14.4 were HCV RNA positive [16].

Consistent with our study, the numbers of HCV RNA-positive patients with anti-HCV S/Co level ≥ 1 were lower than those with HCV RNA-negative patients in the studies by Kirişçi *et al.* and Gülden *et al*. [15,17].

In our study, the rate of HCV RNA-positive patients was 73.5% in the anti-HCV S/Co 15.1-20 range with the most appropriate anti-HCV S/Co value of 15.4. Unlike our study, this rate was 3.7% in the study of Kirişçi *et al*. [17].

In our study, there was no significant change in sensitivity and specificity values as the cut-off point changed; however, as the cut-off point increased in the study by Kirişçi *et al.,* sensitivity increased, specificity decreased, PPV decreased and NPV increased [17].

Conclusions

In our study, the most appropriate anti-HCV S/Co value of our laboratory was determined as 15.4. Our anti-HCV S/Co value will be useful in predicting HCV viremia in patients admitted to our hospital. HCV RNA test should be performed in patients with anti-HCV S/Co values above this value. However, since this new anti-HCV S/Co value is high and PPV is not relatively high, it is clear that sick individuals should not be overlooked. Therefore, a second serological test should be performed with a new serum sample two weeks later for those with an anti-HCV S/Co level below this value. The HCV RNA test should be studied considering clinical findings and biochemical parameters. Thus, the false-positive rate can also be reduced. In addition, the most appropriate anti-HCV S/Co value should be determined according to the test kit used by the laboratory. The result report should state this value, and the clinician should be informed about this issue.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Authors' contributions

Conception and design of the study: BG, BD; samples collection: BG, BD, AY, SA; data acquisition: BG, AY; data analysis and interpretation: BG, BD, AY; critical revision of the manuscript for important intellectual content and final approval of the manuscript: BD, BG, AY, SA. All authors read and approved the final manuscript.

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Conflict of interests: No conflict of interests is declared.