DOI: 10.4274/jarem.galenos.2023.53254
J Acad Res Med 2023;13(2):95-9

Optimizing Signal to Cut-off Values (S/Co) of Anti-HCV in Prediction of Viremia: A Retrospective Analysis with HCV-RNA

Yasemin Derya Gülseren, Ali Korhan Sığ, Muradiye Yarar

University of Health Sciences Turkey, Balıkesir Atatürk City Hospital, Clinic of Medical Microbiology, Balıkesir, Turkey

Cite this article as: Gülseren YD, Sığ AK, Yarar M. Optimizing Signal to Cut-off Values (S/Co) of Anti-HCV in Prediction of Viremia: A Retrospective Analysis with HCV-RNA. J Acad Res Med 2023;13(2):95-9

ABSTRACT

Objective: The prevalence of chronic hepatitis C worldwide is defined in three categories: low (<2.5%), medium (2.5-10%) and high (>10%) while the prevalence of it is seen between 0.5-1.9% in Turkey. This study aims to identify the most appropriate signal to cut-off (S/Co) value to observe viremia and decrease the number of unnecessary hepatitis C virus-ribonucleic acid (HCV-RNA) analyzes due to false positive anti-HCV results.

Methods: A retrospective analysis was performed regarding the relationship of HCV-RNA and anti-HCV results between January 2019-September 2021. Adult patients (18+ age) with reactive anti-HCV and HCV-RNA results were included in the study.

Results: A total of 626 patients were included (median age 49.7; standard deviation ±18.2), of which 352 (56.2%) were female and 274 (43.8%) were male. Despite their seropositivity, 595 (95%) patients were HCV-RNA negative and only 31 (%5) patients were positive. Non-parametric Mann-Whitney U test was used to compare anti-HCV results regarding HCV-RNA positive and negative patients. Anti-HCV levels in the HCV-RNA positive group of patients (median =13.7) were significantly higher compared with the HCV-RNA negative group (median =1.1) (p=0.001). HCV-RNA was accepted as the gold standard in receiver operator characteristic (ROC) analysis to define the most accurate cutoff value which was found to be 8.9 S/Co. Sensitivity and specificity were 93% and 91%, respectively [area under the curve (AUC): 0.94] (95% confidence interval: 0.907-0.974). AUC was significantly over 0.5 (p=0.001).

Conclusion: It is essential for laboratories to modify reference values according to their patient populations and prevalence. 8.9 S/Co was defined as the most appropriate value for anti-HCV in our laboratory and below this, additional interventions and retesting should be performed prior to the report.

Keywords: Hepatitis C, serological diagnosis, PCR, seropositivity

ORCID IDs of the authors: Y.D.G. 0000-0002-7877-5960; A.K.S. 0000-0003-2907-257X; M.Y. 0000-0001-7747-3103.

Corresponding Author: Ali Korhan Sığ,
E-mail: dr_korhan@hotmail.com

Received Date: 22.02.2023 **Accepted Date:** 25.07.2023

J Acad Res Med 2023;13(2):95-9

INTRODUCTION

Hepatitis C virus (HCV), first identified in 1989, remains a public health issue, annually affecting almost 2 million individuals worldwide. It is one of the major causes of chronic liver disease, including hepatocellular carcinoma (1,2). Only in the USA, there are 3.2 million individuals estimated to be living with HCV infection, whereas half a million citizens are infected in Turkey (approximately 1%), suggesting the second leading reason for liver transplantation (2-4). Its prevalence is at 45%, 8-17%, and 6% in intravenous (IV) drug addicts, imprisoned individuals, and hemodialysis patients, respectively (4).

HCV is a member of the Flaviviridae family in the hepacivirus genus with a single-strand ribonucleic acid (RNA) and envelope. The most frequent type worldwide (including Turkey) is genotype 1 subtype 1b (2,4). The whole pathogenesis has not been totally understood; however, it is known that the natural progression of the disease takes a long time. Almost all untreated acute cases turn into chronic viral hepatitis, of which 5-15% convert to eventually cirrhosis and 1-4% cause hepatocellular carcinoma (5). Symptomatic cases rarely become chronic and most patients are diagnosed incidentally because individuals in the subclinic phase (transforming progress from acute to chronic) are usually not aware of their infected status (4-6).

HCV infection is diagnosed by testing for specific antibodies, which actually do not state whether the infection is acute, chronic, or resolved. In addition, an "antibody-undetectable" window period was defined in the first weeks of infection (5). Screening is another terminology, since The Centers for Disease Control and Prevention (CDC) recommends it at least once in a lifetime for all adults during each pregnancy (7). In HIV-infected individuals, persons with persistently abnormal alanine aminotransferase (ALT) levels, hemodialysis patients, children born from HCV-infected mothers, IV drug addicts, and blood donors, routine periodic testing is strongly recommended (7). As stated above, additional testing is required to distinguish between a resolved and current infection to consider antiviral treatment. Hence, nucleic acid testing [HCV-RNA polymerase chain reaction (PCR)] is a good marker to observe current viremia, and the CDC published an algorithm to diagnose the infection (8).

In the stated algorithm, CDC particularly pointed to possible false positivity of antibody tests (8). Enzyme immunoassay (EIA) and chemiluminescent immunoassay (CLIA) are routinely used with highly beneficial potential to predict viremia, since high signal to cut-off (S/Co) ratios were reported to be associated with HCV-RNA positivity, however, it is noted that especially high rates of false positivity can be observed in low prevalence (<2.5%) areas (9). In such cases, the usage of S/Co ratios in reflex supplemental testing algorithms is recommended by CDC (1). This study aims to identify the most appropriate and accurate S/Co value to observe viremia and decrease the number of unnecessary HCV-RNA analysis due to false positive anti-HCV results. With this S/Co value, we plan to provide self-algorithms for our laboratory.

METHODS

Materials: A retrospective-analysis was performed regarding the relationship of HCV-RNA and anti-HCV results between January 2019-September 2021 in Balıkesir Atatürk City Hospital. Adult patients (18+ age) whose anti-HCV was reactive and who were simultaneously investigated for HCV-RNA were included in the study. Patients that did not fit these criteria were excluded.

Methods: Anti-HCV analysis was performed with the CLIA method using an Architect i2000 analyzer (Abbott Diagnostics, Abbott Park, IL, USA). Isolation of HCV-RNA was done with a Magnesia 16 device and kit (Anatolia Geneworks, Istanbul, Turkey), and PCR was applied with Bosphore HCV Quantification Kit v2.0 by a Montania 4896 Real Time PCR device (Anatolia Geneworks, İstanbul, Turkey).

Statistical Analysis

Median S/Co values were calculated for both HCV-RNA positive and negative groups. Due to non-normal distribution of anti-HCV results, a nonparametric Mann-Whitney U test was used to compare anti-HCV results regarding HCV-RNA positive and negative patients. HCV-RNA was accepted as the gold standard in receiver operator characteristic (ROC) curve analysis to define the most accurate cut-off value.

Ethical approval: Approved by the Ethical Board of İstanbul Medipol University Non-Invasive Clinical Research (decision no: 1306, date: 23.12.2021).

RESULTS

A total of 626 patients were included (median age 49.7; standard deviation ±18.2), of which 352 (56.2%) were female and 274 (43.8%) were male. Almost all patients were under a follow-up schedule for infectious disease and gastroenterology services.

Five hundred ninety five (95%) of patients were HCV-RNA negative, while 31 (5%) patients were positive. Anti-HCV levels were significantly higher in the positive HCV-RNA group (median: 13.7) compared with the negative HCV-RNA–group (median: 1.1) (p=0.001) (Figure 1).

In the ROC analysis, the most accurate cut-off value was found to be 8.9 S/Co (Table 1). Sensitivity was 93%, specificity was 91%, and area under curve (AUC) was found to be 0.94 (confidence interval: 95%; 0.907-0.974), which was significantly higher from 0.5 (p=0.001) (Figure 2).

DISCUSSION

At present, isolation and culture of HCV from clinical specimens are extremely difficult, and HCV-RNA is accepted as a gold standard in order to show viremia; especially the quantitative one, which is important for treatment monitoring. However, the test requires experienced staff, is time-consuming, and costs high, and for these reasons, serological analysis has come forward as a simpler and cheaper screening procedure (10,11). On the other hand, anti-HCV IgMs could be detected in patients with acute

J Acad Res Med 2023;13(2):95-9

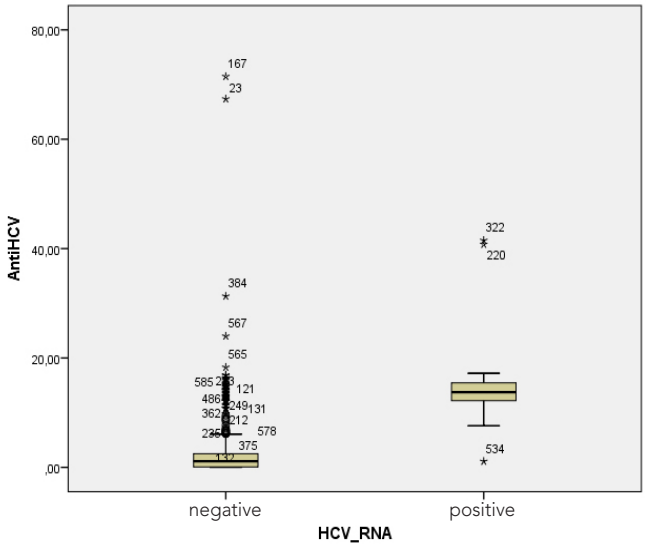

Figure 1. Median analysis of anti-HCV levels
HCV-RNA: hepatitis C virus-ribonucleic acid

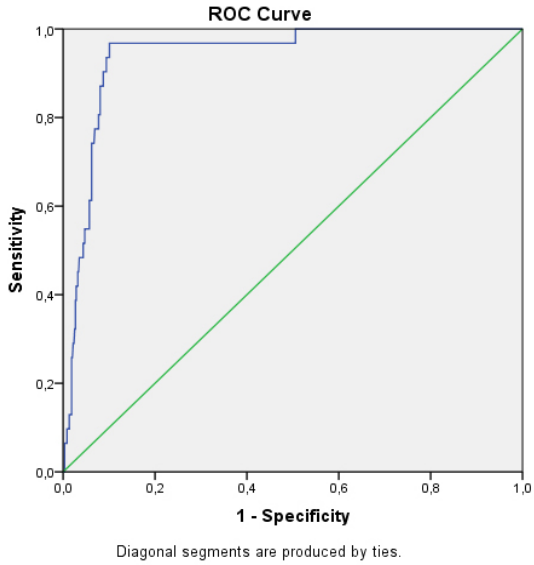

Figure 2. ROC analysis
ROC: receiver operator characteristic

Table 1. ROC analysis results

Anti-HCV S/Co	Sensitivity, %	Specificity, %
1.01	100	46
2.02	96	70
3.05	96	79
4.06	96	83
5.08	96	85
6.02	96	87
7.03	96	89
8.9	**93**	**91**
9.07	90	91
10.1	90	92

ROC: receiver operator characteristic, HCV: hepatitis C virus, S/Co: signal to cut-off

hepatitis C and chronic patients. Thus, anti-HCV IgM cannot be used as a reliable marker for active infection, which prevents its routine usage for screening purposes. Currently, screening and diagnostic assays via EIA or CLIA for anti-HCV total antibody are widely used in clinical practice (11). In general, the CLIA shows better specificity than the EIA, but both methods have high false-positive rates, especially in low-prevalence populations (1). In addition, as previously stated, anti-HCV total antibody seropositivity may indicate previous exposure to the virus, active infection, or false positivity. A window period of false negativity was also reported. These conditions strongly limit "diagnostic" value of the total antibody (5,10). The World Gastroenterology Organization Global Guideline on Diagnosis, Management and Prevention of Hepatitis C states that acute hepatitis C diagnosis depends on "marked elevation of alanine aminotransferase (ALT; more than 10x)", "with or without jaundice", "detectable serum HCV-RNA" and "followed by anti-HCV seroconversion weeks later" (12).

Serology has an important mission as a screening procedure, which the CDC strongly recommends at least once in an adult's lifetime, especially in particular patient groups (7). Screening tests are actually slightly different from diagnostic tests because a high negative predictive value (negative likelihood ratio) is mainly focused on "slightly tolerated" false positives. The main expectation from a screening test is not to miss even a single case, so suspected positive results are exposed to confirmatory tests such as HCV-RNA, which are mainly used as diagnostic tests with high specificity (2,11). The accuracy of a test is not based only on sensitivity and specificity, since overall accuracy generally shows a prevalence-dependent feature, which is a problematic descriptor of test validity. Other terms to describe the validity of tests were defined, such as positive likelihood ratio, negative likelihood ratio, and AUC, which do not vary with disease prevalence (13).

CDC notified the importance of S/Co value, particularly in low prevalence countries (like Turkey), to optimize the interpretation of assay results and recommended laboratories to define their own ratio (2). In such cases, no further testing is required for diagnosis. Studies have reported that higher S/Co value correlates with higher positive predictive value (1). Regarding this, the S/Co values for Abbott Architect (Abbott Diagnostics, Abbott Park, IL, USA), Ortho Vitros (Ortho Clinical Diagnostics, Raritan, NJ, USA), and the Siemens Advia Centaur (Siemens Healthineers AG, Erlangen, Germany) anti-HCV assays were stated by CDC as 5.0, 8.0, and 11.0 respectively (3). Kim et al. (3) suggested 19.0 for Elecsys assay (Roche Diagnostics International AG, Basel, Switzerland) (ELISA vs recombinant immunoblot assay-RIBA and HCV-RNA). Similarly, there are studies from Turkey indicating

J Acad Res Med 2023;13(2):95-9

S/Co values, such as Altuğlu et al. (14) (3.27 with CLIA vs line immunoassay), Şanlıdağ et al. (15) (5.0 with CLIA vs HCV-RNA), Karakoc et al. (16) (8.1 with Ortho EIA and 3.4 with microEIA vs RIBA and PCR), and Aydın et al. (2) (7.13 with CLIA vs HCV-RNA). Confirmatory tests other than PCR may be evaluated as "overdated". On the other hand, results compared with HCV-RNA, including this study (8.9 with CLIA vs HCV-RNA), show variability. Differences between these values would not reflect any differences in terms of analytical performances, but they can be based on assay differences (methods and molecules utilized for signal generation and detection) and/or sample size. In a wide study by Lai et al. (17), below 3.0 S/Co values were indicated as negative with CLIA vs RIBA, along with 3.0-19.9 values as the gray zone, which requires additional confirmatory tests.

Defining valid S/Co value is important to increase accuracy. However, as shown, there is a hidden danger in creating an individual laboratory-based S/Co value, since this might corrupt standard procedures at the national base and might also cause confusion in interpretations. This is because of not only variations between laboratories even when the same devices are used but also because of high-fixed S/Co values that can cause the missing of true positives, which is totally undesirable for a screening test. On the other hand, low S/Co values can make the assay less specific, which makes the test insufficient to establish the clinical diagnosis of the disease (18), since the CDC recommended additional testing for laboratories with low S/Co values (1). A wide study from Turkey directly supports this suggestion that high S/Co values are more appropriate (9). On the other hand, there are also studies indicating that S/Co definition does not sufficiently optimize the tests and thus additional tests are required (1,19). Furthermore, HCV antibody assays vary according to their antigens, test platforms, and performance characteristics, so it is hard to suggest a standard S/Co value (19).

Study Limitations

The major limitation of this study was the lack of all HCV-RNA results of all patients during the study period. Our center became active in 2017 and in our investigation time zone, we could not reach the HCV-RNA data of all seropositive patients. Secondly, our study was based on a single CLIA device, and hence we could not conduct multiple device antibody testing.

CONCLUSION

Even though there are controversial points about assay interpretations via S/Co values, the CDC still recommends laboratories to do so, especially when focusing on a population-based perspective. In Turkey, anti-HCV false positivity is a common condition that creates unnecessary workload, costs, and a requirement for molecular analysis. In this study, a high S/Co value of 8.9 showed a direct correlation with true positivity without any further testing to detect viremia. For values below this value, additional sampling and testing are recommended before HCV-RNA PCR.

Acknowledgements: The authors wish to declare special thanks to İlkay Bozdağ, M.D., Onur Irmak, M.D., and Osman Kılınç, M.D. (University of Health Sciences Turkey, Balıkesir Atatürk City Hospital, Clinic of Medical Microbiology, Balıkesir, Turkey) for their precious support.

Ethics Committee Approval: Approved by the Ethical Board of İstanbul Medipol University Non-Invasive Clinical Research (decision no: 1306, date: 23.12.2021).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Author Contributions: Surgical and Medical Practices - Y.D.G., A.K.S., M.Y.; Concept - Y.D.G., A.K.S., M.Y.; Design - Y.D.G., A.K.S.; Data Collection and/or Processing - Y.D.G., A.K.S., M.Y.; Analysis and/or Interpretation - Y.D.G.; Literature Search - Y.D.G., A.K.S., M.Y.; Writing - Y.D.G., A.K.S., M.Y.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

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J Acad Res Med 2023;13(2):95-9

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