

Comparison of samples found positive by anti-HCV screening test with line immunoassay and determination of threshold value

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SUMMARY

OBJECTIVE: This study aimed to compare the serum samples found reactive (≥ 1 – ≤ 20 signal-to-cutoff ratio) with Elecsys antibodies to hepatitis C virus screening test with innogenetics-line immunassay hepatitis C Virus Score test and to determine the most appropriate threshold value for our country, since positive results close to the cutoff value cause serious problems in routine diagnostic laboratories.

METHODS: Antibodies to hepatitis C virus-positive samples from 687 different patients were included in the study. Antibodies to hepatitis C virus antibody detection was performed using Elecsys antibodies to hepatitis C virus II kits (Roche Diagnostics, Germany), an electrochemiluminescence method based on the double-antigen sandwich principle, on the Cobas e601 analyzer (Roche Diagnostics) in accordance with the recommendations of the manufacturer. Samples that were initially identified as reactive were studied again. Samples with ≥ 1 – ≤ 20 signal-to-cutoff ratio reagents as a result of retest were included in the study to be validated with the third-Generation Line immunassay kit (innogenetics-line immunassay hepatitis C Virus, Belgium).

RESULTS: A total of 687 samples with antibodies to hepatitis C virus positive and levels between 1–20 S/Co were found to be 56.1% negative, 14.8% indeterminate, and 29.1% positive by innogenetics-line immunassay hepatitis C Virus confirmation test. When the cases with indeterminate innogenetics-line immunassay hepatitis C Virus test results were accepted as positive, the signal-to-cutoff ratio value for antibodies to hepatitis C virus was determined as 5.8 (95% confidence interval) in distinguishing the innogenetics-line immunassay hepatitis C Virus negative and positive groups.

CONCLUSION: It was concluded that with further studies on this subject, each country should determine the most appropriate S/Co value for its population, and thus it would be beneficial to reduce the problems such as test repetition and cost increase.

KEYWORDS: Hepatitis C virus antibodies. Enzyme immunoassay. Hepatitis C virus. Immunoassay.

INTRODUCTION

Early detection of hepatitis C virus (HCV) antibodies is the first step in the management of chronic hepatitis and identification of patients who are in need of treatment^{1,2}. First-generation anti-HCV tests were developed in 1990 using the recombinant c100-3 epitope of the NS4 protein and have limited sensitivity and specificity^{1,3}. A second-generation assay was soon developed using a multi-antigen format,

including epitopes from the core, NS3 and NS4 proteins^{1,4}. In the early 2000s, third-generation tests were introduced to detect the presence of antibodies against recombinant core, NS3, NS4, and NS5 antigens of the virus. The test format had also changed from an enzyme immunoassay (EIA) method to a chemiluminescent immunoassay (CLIA) method, with a marked improvement in performance^{1,5}. The Elecsys anti-HCV II test (Roche Diagnostics, Germany) works with the

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electrochemiluminescence (ECLIA) method and is based on the double-antigen sandwich principle⁵. The INNO-LIA HCV score test (Innogenetics, Belgium) is a line immunoassay (LIA) method recommended for use as an additional test for samples found reactive in the anti-HCV screening procedure. The INNO-LIA HCV Score assay uses well-defined antigens derived from HCV immunodominant proteins from the core, the E2 hypervariable region (HVR), the NS3 helix region, and the NS4A, NS4B, and NS5A regions⁶.

In societies with low prevalence of HCV, such as our country, and in those with autoimmune disease, it has been reported that tests used to detect anti-HCV antibodies give high false-positive results⁷. Positive results close to the cutoff value cause serious problems in routine diagnostic laboratories (such as reporting problems, increased costs due to repeat testing or HCV-RNA testing). In contrast, the presence of HCV-RNA often does not accompany close to the cutoff value or low anti-HCV positivity⁸. In this study, it was aimed to compare the samples with positive results (≥ 1 – ≤ 20 S/Co) with the Elecsys anti-HCV screening test with the INNO-LIA Score test and to determine the threshold value for the anti-HCV screening test.

METHODS

A total of 687 anti-HCV-positive (≥ 1 – ≤ 20 S/Co) serum samples from different patients sent to Erciyes University Medical Faculty Central Laboratory Serology Unit between January 2018 and April 2021 were included in the study. Then, the same sera were studied with the Third-Generation LIA kit (INNO-LIA, HCV Score, Innogenetics, Belgium). The results of both tests were evaluated retrospectively.

Anti-HCV Antibody Detection

Anti-HCV antibody detection was performed using Elecsys anti-HCV II kits (Roche Diagnostics), an ECLIA method based on the double-antigen sandwich principle, on the Cobas e601 analyzer (Roche Diagnostics) in accordance with the recommendations of the manufacturer. Samples with < 1 S/Co value were considered nonreactive, while samples with ≥ 1 S/Co value were considered reactive. Samples that were initially identified as reactive were studied again. Samples with ≥ 1 and ≤ 20 S/Co reagents as a result of retest were included in the study to be validated with the Third-Generation LIA kit.

Line Immunoassay

Anti-HCV-positive samples were studied with the Third-Generation INNO-LIA HCV score test (Innogenetics, Belgium) kits containing the C1, C2, E2, NS3, NS4, and NS5 regions of the HCV genome in accordance with the recommendations

of the manufacturer and were interpreted as negative, indeterminate, and positive.

Negative: All HCV antigen bands have a negative reactivity degree or one of the HCV antigen bands, except that NS3 has \pm reactivity.

Positive: Reactivity of \pm or higher in at least two HCV antigen bands.

Indeterminate: Any HCV antigen line has a reactivity rating of 1+ or higher, or the NS3 band has reactivity of \pm or more, while all other antigen lines are negative.

Statistical analysis

Data were evaluated using statistical package program IBM SPSS Statistics for Windows, version 26.0 (IBM Corp. Released 2019, Armonk, NY, USA). In comparisons according to the INNO-LIA HCV score verification test result categories, which had more than two subcategories, the Elecsys anti-HCV II screening test continuous measurement value distribution was evaluated by the Kruskal–Wallis test based on the normality test result. The Bonferroni test was used as a multiple comparison test. A $p < 0.05$ value was considered statistically significant. In addition, the analysis of the data was performed with the MedCalc 15.8 program. As a result of the application of the INNO-LIA HCV score confirmation test, the Elecsys anti-HCV II screening test results of patients with anti-HCV positive; receiver operating characteristic (ROC) curve analysis was applied to determine the cutoff point as negative group with (indeterminate+positive) group and positive group with (negative+indeterminate) group.

RESULTS

A total of 687 samples were anti-HCV positive with results ranging from 1–20 S/Co, 385 (56.1%) were negative with the INNO-LIA HCV score confirmatory test, 102 (14.8%) were indeterminate, and 200 (29.1%) was found positive. It was found that the distribution of anti-HCV-positive values by categories as a result of the INNO-LIA HCV score verification test did not provide the normal distribution assumption. The descriptive statistics and median value and the 25th and 75th percentile values of continuous measurement values that are anti-HCV positive and whose value varies between 1–20 S/Co according to each INNO-LIA HCV score test result categories are given in Table 1. The distribution of the measurement values of the anti-HCV-positive group according to the Kruskal–Wallis test result shows statistically significant differences according to the INNO-LIA HCV score result categories ($p < 0.001$).

ROC curve analysis was used to determine the cutoff point of the negative group with (indeterminate+positive) group and the positive group with (indeterminate+negative) group as a result of

application of the INNO-LIA HCV score confirmation test to the results found positive with the Roche Elecsys anti-HCV II test (Figures 1 and 2). As a result of the analysis, when the cases that were indeterminate according to the INNO-LIA HCV score test result were considered positive, the S/Co value for anti-HCV was determined as 5.8 (95% confidence interval) in distinguishing the INNO-LIA HCV score test negative and positive groups. At S/Co values >5.8, sensitivity was 79.1%, specificity 78.2%, positive predictive value 73.8%, and negative predictive value 82.6%. When the cases that were indeterminate according to the INNO-LIA HCV score test result were considered negative, the S/Co value for anti-HCV was determined as 7.3 (95% confidence interval) for the INNO-LIA HCV score test in distinguishing the negative and positive groups. At S/Co values >7.3, the sensitivity was 81%, the specificity was 79.1%, the positive predictive value was 61.1%, and the negative predictive value was 91%.

DISCUSSION

The diagnosis of HCV infection usually begins with the detection of anti-HCV using EIA and a CLIA screening methods⁹. Direct HCV-RNA testing is recommended in anti-HCV-positive patients with clinically acute or chronic liver disease due to the possibility of false-positive results in populations where prevalence is low¹⁰. However, high costs, labor-intensive procedures, and the need for specialized equipment and qualified personnel limit the widespread use of molecular techniques^{9,11}. Furthermore, deciding on a reliable, easy-to-use, and cost-effective test to predict true HCV infection status or HCV viremia in anti-HCV reactive patients remains controversial. Although it is recommended to confirm with tests such as Recombinant Immunoblot Assay (RIBA) when a low S/Co result is obtained in the classical diagnosis algorithm of HCV, these tests are likely to yield “indeterminate” results¹². The Centers for Disease Control and Prevention (CDC) removed the RIBA test from the new algorithm and explained that the cutoff value of ≥ 1 S/Co should be adjusted according to the characteristics of

the population⁸. In addition, the CDC has proposed predictive cutoff values for some commercially available anti-HCV screening tests. For example, Architect (Abbott Laboratories, USA) has set a threshold value of ≥ 5 S/Co for the anti-HCV screening test, but these values have not yet been specified for the Roche Elecsys anti-HCV II tests^{9,13}. Lai et al.¹⁴ reported that when the S/Co ratio is < 3.0 or ≥ 20.0 , there is no need for anti-HCV confirmatory testing with RIBA because of the high true negative and high true positive rate, respectively. They also reported that the RIBA confirmatory test is required for patients with an S/Co ratio of 3.0–19.9, due to possible false-positive results given by the ECLIA. Results between 1.0 and 20 S/Co with the Elecsys anti-HCV II screening test in our laboratory are confirmed by the INNO-LIA HCV score test.

In our study, a total of 687 samples with reactive anti-HCV results (1–20 S/Co) were tested with the INNO-LIA HCV score confirmation test. It was found that 56.1% were negative,

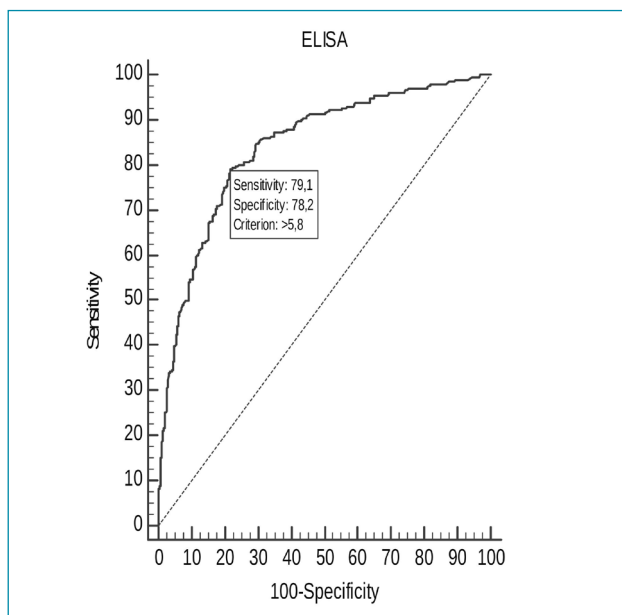


Figure 1. Receiver operating characteristic curve analysis of negative group with (indeterminate+positive) group.

Table 1. Evaluation of the distribution of Elecsys Antibodies to hepatitis C virus II screening test measurement values of patients with Antibodies to hepatitis C virus positive according to the confirmatory test Innogenetics- line immunassay hepatitis C virus score test findings.

INNO-LIA HCV score	n (%)	$\bar{X} \pm SS$ M (Q ₁ -Q ₃)	p-value*	Pairwise comparisons
Negative	385 (56.0)	4.21 ± 3.74 2.66 (1.54–5.40)	$\chi^2=274.140$; $p<0.001$	1–2: $p<0.001$
Positive	200 (29.2)	12.59 ± 5.20 12.86 (8.36–16.83)		1–3: $p<0.001$
Indeterminate	102 (14.8)	7.29 ± 4.56 6.53 (3.63–10.00)		2–3: $p<0.001$

*Kruskal Wallis Test. INNO-LIA HCV score: innogenetics-line immunassay hepatitis C virus

14.8% were indeterminate, and 29.1% were positive. It has been reported that a total of 47041 samples, which were found to be anti-HCV reactive by EIA method, was found to be positive in 49.3%, indeterminate in 17.1%, and negative in 33.5% by RIBA method¹⁵. In another study, two different anti-HCV systems (Cobas e411 Elecsys anti-HCV II and Vidas anti-HCV Biomerieux) were compared with the INNO-LIA HCV score test using 1931 serum samples. It has been reported that the performance agreement of Vidas and INNO-LIA for discrepant samples is 65%, and the percentage agreement is 80% for Vidas-negative samples and 28% for Vidas-positive samples. It was stated that Cobas had a performance agreement of 41% with INNO-LIA in discrepant samples, and the percentage agreement was 28% for Cobas negative samples and 72% for Cobas positive samples¹⁶. In a study where Architect i2000SR (Abbot Laboratories) and Vidas systems were used as anti-HCV screening test, 70 serum samples with low positive ($1 \leq S/Co < 8$) were compared with the INNO-LIA HCV score assay. It has been reported that the agreement between

the Architect i2000SR and the INNO-LIA HCV score assay is 42.6%, and the percentage agreement between Vidas and the INNO-LIA HCV score assay is 79.4%¹⁷. A multicenter study conducted in Turkey reported that 67% of 10050 anti-HCV-positive serum samples were positive with RIBA¹⁸. In another study, this rate was found to be 61.4%⁸. In our study, when the indeterminate results were considered positive, this rate was found to be 49.5%.

Yang et al.¹ reported that in the Elecsys anti-HCV II assay, an S/Co ratio of 20.0 predicted a true positive result $\geq 95\%$ of the time. On the other hand, Wu et al.¹⁹ found that this value was 12.0 for the InTec test (InTec products, China) and 5.0 for the Architect test in their study in which they investigated the appropriate S/Co thresholds. Saribas et al.⁸ determined the S/Co value of 7.2 (95% confidence interval) for Architect anti-HCV in distinguishing LIA positive and negative groups when LIA indeterminate cases were considered negative. In our study, when the results found indeterminate by the INNO-LIA HCV score assay were considered positive and negative, the S/Co ratios for anti-HCV were found to be 5.8 and 7.3, respectively.

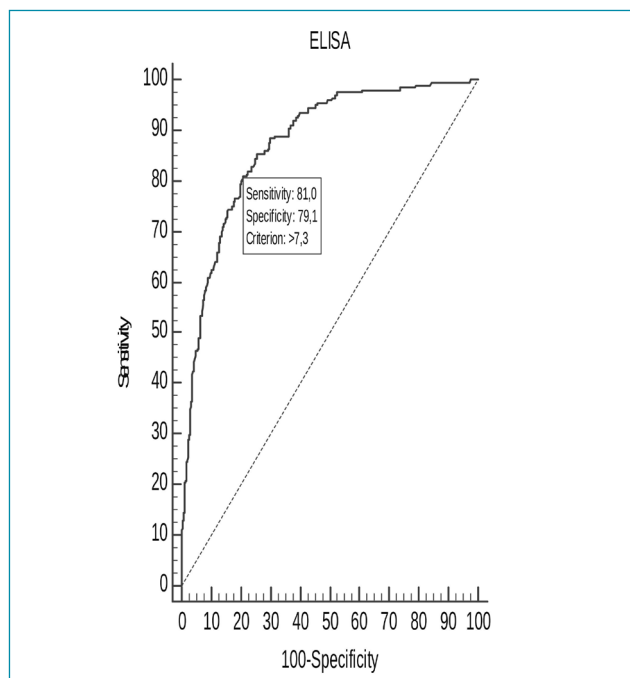


Figure 2. Receiver operating characteristic curve analysis of positive group with (indeterminate+negative) group.

CONCLUSIONS

The lack of HCV-RNA results in each patient limited us to make a comparison in this respect. Although the INNO-LIA HCV score assay is used as a complementary test in the detection of anti-HCV antibodies, it has the disadvantage of visual evaluation and highly uncertain results. As a result, it is necessary for each country to determine the most appropriate S/Co value for its population, with more studies to be done on this subject. This will reduce patient victimization due to reporting problems and problems such as increased cost due to repeated testing or the need for HCV-RNA testing.

AUTHORS' CONTRIBUTIONS

MAA: Conceptualization, Investigation, Writing – review & editing. **PS:** Conceptualization, Data curation, Writing – review & editing. **MO:** Data curation, Writing – original draft, Writing – review & editing. **BE:** Formal analysis.

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