



# The Efficiency of Hepatitis C Virus Core Antigen Test in the Diagnosis of Hepatitis C Infection

Hepatit C Enfeksiyonunun Tanısında Hepatit C Virüsü Kor Antijen Testinin Etkinliği

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## ABSTRACT

**Objective:** It was aimed to investigate diagnostic value of hepatitis C virus (HCV) core antigen test in patients with positive or negative anti-HCV assay by comparing with HCV ribonucleic acid (RNA) assay.

**Materials and Methods:** Serum samples obtained from 189 patients who were admitted to Necmettin Erbakan University Meram Faculty of Medicine between December 2010 and February 2012, and HCV RNA assay were carried out for various reasons. Two mL of samples were stored under suitable conditions and anti-HCV, HCV core antigen and strip immunoblot assay [Commercial INNO LIA™ HCV Score (Innogenetics NV in Ghent, Belgium)] were performed. Genotyping was performed in the amplicons of the samples with positive HCV RNA test.

**Results:** The diagnostic sensitivity specificity, negative predictive value and positive predictive value of HCV core antigen test were 96.2%, 100%, 97.3%, and 100%, respectively. Sixty-five serum samples were genotyped and their distribution were detected: Fifty-nine samples were genotype 1b, 2-genotype 1a/1b, 1-genotype 3a, 1-genotype 4, 1-genotype 2a/2c, and 1 was genotype 1a.

**Conclusion:** It was concluded that HCV core antigen assay is highly specific, sensitive, reliable, reproducible, and easy to perform. It may be applied as a supplemental and confirmatory test in anti-HCV assays in the diagnosis of HCV.

**Keywords:** Hepatitis C virus, core antigen, hepatitis C virus ribonucleic acid, strip immunoblot assay

## ÖZ

**Amaç:** Hepatit C virüsü (HCV) kor antijen testinin tanı değerinin anti-HCV testi pozitif veya negatif olan hastalarda HCV ribonükleik asit (RNA) ile kıyaslanarak araştırılmasıdır.

**Gereç ve Yöntemler:** Necmettin Erbakan Üniversitesi Üniversitesi Meram Tıp Fakültesi'ne Aralık 2010- Şubat 2012 tarihleri arasında başvuran ve çeşitli nedenlerle HCV RNA testi çalışılan 189 hastadan elde edilen serum örnekleri bu çalışmaya dahil edildi. İki mL serum örneği uygun koşullar altında saklandı ve anti HCV, HCV kor antijen ve strip immünblot testi [Ticari INNO LIA™ HCV Score testi (Innogenetics NV in Ghent, Belçika)] testleri çalışıldı. HCV RNA pozitif olan örnekler genotipleme yapıldı.

**Bulgular:** Çalışmamızda HCV kor antijen testinin sensitivite, spesifite; negatif prediktif değer ve pozitif prediktif değerleri sırayla %96,2, %100, %97,3 ve %100 olarak tespit edildi. Genotipleme yapılan 65 örneğin 59'u genotip 1b, 2'si genotip 1a/1b, 1'i genotip 3a, 1'i genotip 4, 1'i genotip 2a/2c ve 1'i genotip 1a olarak tespit edildi.

**Sonuç:** HCV kor antijen testi sensitivitesi ve spesifitesi yüksek, kolay uygulanabilir, güvenilir bir testtir. Bu test HCV enfeksiyonunun tanısında anti-HCV test sonuçlarının confirmasyon ve tamamlayıcı testi olarak kullanılabilir.

**Anahtar Kelimeler:** Hepatit C Virüsü, kor antijen, hepatit C virüsü ribonükleik asit, strip immünblot testi

## Introduction

Hepatitis C virus (HCV) is classified within the genus Hepacivirus in the Flaviviridae family. It is a single-stranded RNA virus with positive polarity (1,2). There are approximately 200 million individuals infected with HCV throughout the world. Moreover, HCV is considered as the most important reason for liver diseases in both developed and developing countries (3). HCV prevalence varies greatly in geographic distribution depending on the level of development of the country. High prevalence is found in Africa and Asia, whereas the ratio is lower in industrialized countries, such as Australia, North America, and Northern and Western Europe (4). It has been divided into six main genotypes and more than 80 different subtypes according to the nucleotide sequences of HCV (2). There is a close relationship between the genotypes and subtypes of HCV and pathogenesis and epidemiology of the disease (5,6).

Nowadays, anti-HCV assays, which detect antibodies against HCV and used as a screening test to detect HCV in blood and blood products, are performed in order to prevent transmission of HCV. Although screening tests are highly effective in reducing the risk of hepatitis C, they can give false-positive test results in some individuals without any clinical or laboratory findings related to HCV infection (7,8). However, strip immunoblot assay (SIA) is used as a supplementary test for positive anti-HCV assay results. This test has some disadvantages, such as difficulty of performing, high cost and a high percentage of indeterminate results (9). Although molecular methods are currently the most reliable method for determining HCV infection, they are time consuming and expensive and require high-technical equipment (10).

HCV core antigen is a protein which has 191 amino acids and its molecular weight is 21 kDa (11). HCV core antigen can be detected approximately 1-2 days after the emergence of HCV RNA and before the formation of anti-HCV antibodies in serum (12).

The aim of the study was to evaluate the diagnostic efficiency of HCV core antigen assay among anti-HCV-seropositive and seronegative individuals by comparing with HCV RNA assay.

## Materials and Methods

This investigation was designed as a cross-sectional study. The study sample was collected from sera in which HCV RNA testing was performed for various reasons in our laboratory. One hundred eighty nine sera were obtained between December 2010 and February 2012. One hundred nine samples were taken from the HCV RNA-positive sera. As a control group, 80 samples were collected from HCV RNA-negative sera. The control group patients were admitted to the hospital for reasons not related to hepatitis infection.

The clinical research Ethics Committee of Meram Faculty of Medicine approved the study.

Anti-HCV assay was studied using commercial Architect® i2000SR chemiluminescence immunoassay (CIA) system (Abbott laboratories, diagnostics division, abbott Park, IL, USA) chemiluminescent microparticle immunoassay technology.

SIA was performed using Commercial INNO LIA™ HCV Score (Innogenetics NV in Ghent, Belgium).

HCV core antigen assay was carried out using Architect® i2000SR CIA system (Abbott laboratories, diagnostics division,

and Abbott Park, IL, USA). This test is a new generation HCV core antigen assay. The test was performed following the manufacturer's instructions. 110 µl of each sample was used in the study and test period was 36-40 min. for each sample. The cut off value was 3.00 fmol/liter (0.06 pg/mL) according to manufacturer's instructions. Thus, <3.00 fmol/liter was considered as non-reactive and ≥3.00 fmol/liter was considered as reactive. The test results that were between ≥3.00 fmol/liter and <10.00 fmol/liter were retested. If both assays were nonreactive, test result was considered as nonreactive in terms of HCV core antigen. If one or both of the repeated tests were ≥3.00 fmol/liter, the test was considered as reactive (13).

Commercial COBAS® AmpliPrep/COBASv TaqMan® HCV Test (Roche Molecular Systems, USA), a nucleic acid amplification test, was applied in HCV RNA quantification. The assay features are low limit of detections (15 IU/mL) and quantification of HCV RNA, all genotypes, with a linear range of 43 to 69.000.000 IU/mL.

The samples were prepared using COBAS® AmpliPrep device, and amplification and detection was performed automatically by using COBAS® TaqMan® 48 analyzer device (Roche Molecular Systems, USA).

HCV genotyping was performed by a commercial Ampliquity HCV-TS (AB ANALITICA, Padova, Italy).

### Statistical Analysis

Statistical analysis was carried out with SPSS version 16.0 (SPSS Inc, USA, IL). p value of less than 0.05 was considered statistically significant. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for statistical analysis of the data. Spearman's correlation coefficient was used to assess the linear relationship between HCV core antigen and HCV RNA concentrations. Pearson's correlation coefficient was used to assess the linear relationship between HCV core antigen concentrations and viral load values after log transformation.

## Results

The study included 189 patients (80 male and 109 female). The mean age of the patients was 51.37±18.4 years.

Anti-HCV assay revealed that 148 serum samples were reactive and 41 were non-reactive.

Two samples were detected to be anti-HCV positive, HCV core antigen positive, SIA positive and, HCV RNA negative. When the HCV RNA assay was repeated with new serum samples 3 weeks later, positive HCV RNA results were obtained.

When the HCV core antigen test results of 8 samples with the values of ≥3.00 fmol/liter and <10.00 fmol/liter were retested, 6 were evaluated as non-reactive and 2 were evaluated as reactive. According to the results of SIA (Commercial INNO LIA™ HCV Score), 78 samples were positive, 103 were negative, and 8 samples were found to be indeterminate.

Study results are given in Table 1.

The HCV core Ag results are given according to HCV viral load in Table 2.

HCV RNA levels of three samples detected to be HCV RNA-positive and HCV core antigen-negative were 1.5 x10<sup>1</sup> IU/mL, 2.7 x10<sup>1</sup> IU/mL and 2.57 x10<sup>3</sup> IU/mL.

Spearman's correlation coefficient was calculated as 0.874, and a linear association was found between HCV RNA and HCV core antigen (p<0.01).

The correlation between the levels of HCV RNA and core antigen was significant ( $r=0.840$ ,  $p<0.01$ ).

The relationship between concentrations of HCV RNA and HCV core antigen is shown in Figure 1.

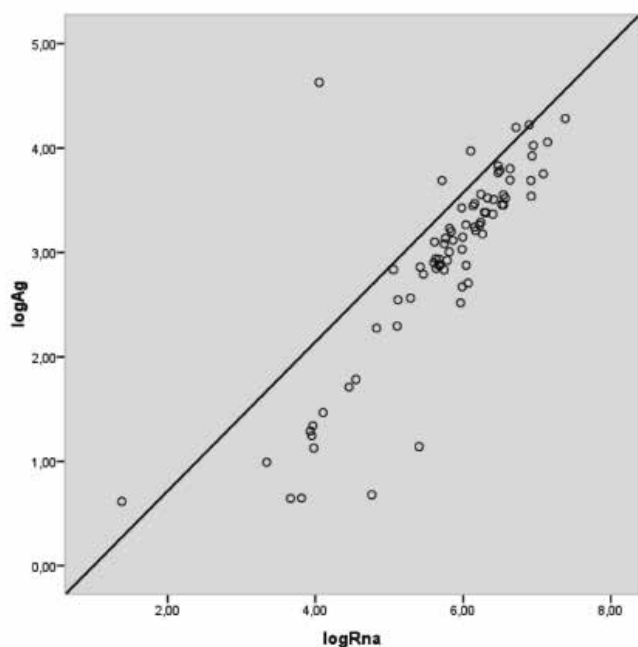
The results of the HCV core antigen test were compared to HCV RNA. Sensitivity, specificity, NPV, PPV, and accuracy of HCV core antigen test were 96.2%, 100%, 97.3%, 100%, and 98.4%, respectively.

Only 65 serum samples were genotyped. Their distribution were defined as follows: 59 were genotype 1b, 2 - genotype 1a / 1b, 1 - genotype 3a, 1 - genotype 4, 1 - genotype 2a/2c and 1 was genotype 1a.

## Discussion

CIA and enzyme immunoassay (EIA), the most widely used methods in the diagnosis of HCV infection, have been used as a screening test (2). An important disadvantage of anti-HCV assay is that the rate of false-positive results is high especially at low anti-HCV value (8,14,15). According to the CDC guidelines, if anti-HCV results are low S/Co, a supplemental test is required (16).

Another disadvantage of anti-HCV assay is false-negative results. The reason for these cases are severe immunosuppression, hemodialysis, AIDS and agammaglobulinemia (17,18,19). In this study, HCV RNA ( $5.2 \times 10^6$  IU/mL), and HCV core antigen ( $1.58 \times 10^4$  fmol/L) were positive in serum of a chronic liver disease patient with a negative anti-HCV assay (0.12). Moreover, SIA (Commercial INNO LIA™ HCV Score) result of this sample was evaluated as negative. It was accepted as immunosuppression.



**Figure 1.** Correlation between hepatitis C virus ribonucleic acid (HCV RNA) and core Ag levels. Pearson's correlation coefficient showed a high correlation between the levels of HCV RNA and core antigen ( $r=0.840$ ,  $p<0.01$ )

In recent years, confirmation of HCV replication has been shown to be possible by detecting and measuring HCV core antigen. While detection limit of the first generation HCV core antigen tests has been 1.5 pg/mL, that of the second generation test has been 0.06 pg/mL (3 fmol/L) (12,18,20).

HCV RNA levels have correlated with serum HCV core antigen. However, when the level of serum HCV RNA has decreased, number of false-negative results of HCV core antigen assay have increased (21,22,23,24). In this study, three serum samples with a low level of HCV RNA ( $1.5 \times 10^1$  IU/mL,  $2.7 \times 10^1$  IU/mL and  $2.57 \times 10^3$  IU/mL) were detected with positive HCV RNA and negative HCV core antigen. The correlation between HCV RNA and HCV core antigen was significant ( $r=0.874$ ,  $p<0.01$ ).

In this study, two samples were detected to be anti-HCV-positive, HCV core antigen-positive, SIA-positive and, HCV RNA-negative. When the HCV RNA assay was repeated with new serum samples 3 weeks later, we obtained positive HCV RNA results. These results may appear in non-viremic HCV RNA period. In the presence of such a situation, HCV RNA should be repeated with new samples after several weeks (25,26)

In our study, SIA (Commercial INNO LIA™ HCV Score) was detected indeterminately and HCV RNA was detected negative in eight serum samples with positive anti-HCV assay. Anti-HCV S/Co results were  $\leq 4.39$  in the sera with indeterminate SIA results (Commercial INNO LIA™ HCV Score). These results suggest that results of positive anti-HCV and indeterminate SIA should be confirmed with HCV RNA. Nevertheless, the results of these sera samples with HCV core antigen assay were also non-reactive.

In this study, HCV core antigen sensitivity, specificity, PPV and NPV were found to be 96.2%, 100%, 100%, and 97.3%, respectively. These results were consistent with that of similar previous studies. Daniel et al. (27) found 85.3% sensitivity and 95.8% specificity, Miedouge et al. (23) found 100% sensitivity and 99.2% specificity,

| Table 1. The results of SIA, HCV core antigen and anti-HCV assay |         |          |             |     |
|--|---------|----------|-------------|-----|
| Test result  | HCV RNA | Anti-HCV | HCV core Ag | SIA |
| Positive   | 80      | 148      | 77          | 78  |
| Negative   | 109     | 41       | 112         | 103 |
| Indeterminate  | -       | -        | -           | 8   |

HCV: Hepatitis C virus, SIA: Strip immunoblot assay

| Table 2. Hepatitis C virus ribonucleic acid core ag results according to hepatitis C virus ribonucleic viral load |                 |             |          |
|---|-----------------|-------------|----------|
|   |                 | HCV core Ag |          |
|   |                 | Negative    | Positive |
| HCV RNA   | Negative        | 109         | 0        |
|   | Positive        | 3           | 77       |
| Viral load (IU/mL)  | 15-20.000       | 3           | 10       |
|   | 20.000-100.000  | 0           | 4        |
|   | 100.000-500.000 | 0           | 14       |
|   | 500.000-800.000 | 0           | 9        |
|   | >800.000        | 0           | 40       |

HCV RNA: Hepatitis C virus ribonucleic acid, HCV: Hepatitis C virus ribonucleic

Kesli et al. (25) found 96.3% sensitivity and 100% specificity, Yuksel et al. (28) found 94.3% sensitivity and 97.9% specificity, Ergünay et al. (29) found 75.8% sensitivity and 95.1% specificity, and Ardoğan et al. (30) found 86.5% sensitivity and 100% specificity.

PPV and NPV values in other studies were as follows: Daniel et al. (27) found 96.4% PPV and 83.1% NPV, Kesli et al. (25) found 100% PPV and 89.7% NPV, Yuksel et al. (28) found 99.1% PPV and 87% NPV, and Ardoğan et al. (30) found 100% PPV and 59.4% NPV.

In this study, HCV core antigen sensitivity rate (96.2%) was higher than the results of Daniel et al. (27) (85.3%), Ergünay et al. (28) (75.8%), and Ardoğan et al. (30) (86.5%), and was very close to the results of Kesli et al. (25) (96.3%), Yuksel et al. (28) (94.3%), and was lower than that of Miedouge et al. (23) (100%). In our study and other studies, specificity and PPV of HCV core antigen test were found to be quite high. This result suggests that positive core antigen test results are an important parameter in predicting the presence of the disease.

In conclusion, HCV antigen assay is highly specific, sensitive, reliable, reproducible, and easy to perform. It may be useful as a complementary and confirmatory test in anti-HCV assays.

#### Ethics

Ethics Committee Approval: The study were approved by Necmettin Erbakan University Meram Faculty of Medicine Ethics Committee, Informed Consent: Verbal consent was obtained.

Peer-review: External and Internal peer-reviewed.

#### Authorship Contributions

Surgical and Medical Practices: Mehmet Emin Demircili, Mehmet Özdemir, Bahadır Feyzioğlu, Bülent Baysal, Concept: Mehmet Emin Demircili, Mehmet Özdemir, Bahadır Feyzioğlu, Bülent Baysal, Design: Mehmet Emin Demircili, Mehmet Özdemir, Bahadır Feyzioğlu, Bülent Baysal, Data Collection or Processing: Mehmet Emin Demircili, Mehmet Özdemir, Analysis or Interpretation: Mehmet Emin Demircili, Mehmet Özdemir, Bahadır Feyzioğlu, Bülent Baysal, Literature Search: Mehmet Emin Demircili, Writing: Mehmet Emin Demircili

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