Original Article

Evaluation of biochemical, hematological, RIBA and PCR assays in predicting viremia in anti-HCV positive patients

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Abstract

Introduction: The detection of HCV-RNA by PCR assays is considered to be the gold standard for confirming the presence of HCV viremia. However, high costs, long and laborious procedures limit their widespread usage. This retrospective study was conducted to assess the predictive performances of biochemical and hematological parameters, anti-HCV signal-to-cutoff (S/CO) ratios and RIBA assay for HCV viremia.

Methodology: Medical records of 210 patients with positive anti-HCV results were analyzed. Samples were tested for anti-HCV by the Roche Elecsys assay. RIBA and PCR assays were performed with Inno-Lia HCV Score test, and Roche Cobas TaqMan HCV test, respectively.

Results: Anti-HCV positive patients were categorized into two groups: positive HCV-RNA(viremic) group (n = 94) and negative HCV-RNA(non-viremic) group (n = 116). All viremic patients had positive RIBA results, while in the non-viremic group, 80 (69%) patients had negative/indeterminate RIBA results and 36 (31%) patients had positive RIBA results. Compared with the non-viremic group, the viremic group had significantly higher alanine aminotransaminase (ALT), aspartate aminotransferase, gamma-glutamyl transferase, mean platelet volume, platelet distribution width and anti-HCV levels, and significantly lower platelet count and plateletcrit levels (p < 0.05). With multivariate logistic regression analysis, serum ALT and anti-HCV levels were found to be strong predictive factors for HCV viremia. A S/CO ratio of ≥ 12.34 was identified as the optimal anti-HCV level to predict viremia.

Conclusions: An anti-HCV S/CO ratio of 12.34 can determine the necessity for PCR assay, when carefully evaluated together with the biochemical and hematological evidence. This approach may reduce the cost of diagnosis particularly in low-resource settings.

Key words: antibody titer; hepatitis C; polymerase chain reaction; viremia.

Introduction

Hepatitis C virus (HCV) infection is a major public health problem affecting over 200 million people globally [1,2]. In Turkey, the prevalence of HCV infection is reported as 0.3-1.8%, and approximately 600.000 individuals are estimated to be infected with HCV [3-5]. The most common HCV genotype in our country is genotype 1b [2,4]. Until the beginning of 2010s, the standard treatment of chronic HCV infection has been based on the combination antiviral therapy with pegylated interferon alfa and ribavirin. In recent years, direct-acting antivirals, including second-generation NS3-4A protease inhibitors, NS5A inhibitors, and NS5B polymerase inhibitors have been launched, and have improved treatment options for HCV infection dramatically [6]. Current standard therapies in our country are applied according to the published guidelines in Europe and Turkey [4,5,7]. There are differences in the recommended regimens depending on the HCV genotype, the presence/absence of resistance-associated substitutions or compensated cirrhosis. However, the most preferred treatment protocol in our hospital is paritaprevir/ritonavir/ombitasvir plus dasabuvir or sofosbuvir and ledipasvir combination for genotype 1b-infected, treatment-naive patients without cirrhosis.

Laboratory methods for the diagnosis of HCV infection are mainly based on serologic assays determining specific antibodies to HCV (anti-HCV) and molecular techniques detecting viral nucleic acid (HCV-RNA). Previous studies have suggested that patients with suspected acute or chronic HCV infection should be first tested for anti-HCV by enzyme immunoassays (EIAs) or automated chemiluminescence immunoassays (CLIAs), which are commonly available, rapid and relatively cost-effective screening tests [1,8,9]. A positive anti-HCV test result may indicate current active infection, past infection, or false positive reaction [10]. Although EIAs and CLIAs are highly sensitive and specific for detecting patients

with HCV infection, false-positive results are not infrequent, and more likely to occur in low-prevalence (< 3%) populations such as Turkey [3,8,11]. Therefore, positive anti-HCV screening test results require confirmation with other more specific supplementary tests such as recombinant immunoblot assay (RIBA) or molecular assays [9,10]. A negative RIBA result generally indicates a false positive anti-HCV screening test with the exceptions of the early phase of acute infection and immunosuppression status, while a positive RIBA result represents current or past infection [8,12]. Determination of HCV-RNA by polymerase chain reaction (PCR) is considered to be the gold standard for confirming the diagnosis of HCV infection and assessing viremia in patients during antiviral therapy and follow-up [11,13-15]. However, due to the higher costs, labor intensive and time-consuming process of supplementary tests, these methods are not suitable for widespread use in many laboratories, especially in developing countries [9,13,16].

The laboratory diagnosis of HCV infection should be as reliable as possible because a positive anti-HCV screening test result has a deep impact on the life of the affected individual. Precise diagnosis is important to inform people about whether they are currently infected and infectious or not [9,10]. Hence, clinicians need to know the factors that may be helpful to predict current HCV infection while waiting for the supplementary test result. The concentration of anti-HCV antibody is expressed as a signal-to-cutoff (S/CO) ratio, and it was suggested that lower levels could be associated with false-positive results and higher levels could reflect true infection status or viremia [13,16,17]. Several studies have confirmed the usefulness of S/CO ratios in predicting HCV viremia in anti-HCV positive patients [11,13,15,18]. However, optimal S/CO values indicating true infection status may differ from one manufacturer to another. Hence, the differences in the significant S/CO values determined by using diverse commercial kits should be taken into account when evaluating HCV viremia.

The objective of this retrospective study was to evaluate the predictive performance of biochemical parameters, hematological parameters, anti-HCV S/CO ratios and RIBA assays for HCV viremia in anti-HCV positive patients and to guide clinicians in the diagnosis of HCV infection.

Methodology

Medical records of subjects who were found to be anti-HCV positive at the Medical Microbiology Laboratory of Suleyman Demirel University Research and Practice Hospital between September 2017 and November 2018 were analyzed retrospectively. Decisions on performing anti-HCV screening, RIBA and PCR assays were largely based on clinical evaluation by clinicians working at different departments in our hospital. The indications for HCV testing (abnormal liver tests, suspected sexual contact, intravenous drug abuse, receiving hemodialysis or donated blood or organs, long-term hospitalization, surgical operation, etc.) and laboratory methods for the diagnosis of HCV infection were generally determined according to the current published guidelines [4,5,7]. All patients with suspected HCV infection were evaluated for HBV and HIV coinfections. Anti-HCV screening by CLIA was the initial step for HCV testing and anti-HCV positivity was confirmed by PCR assay alone or by both PCR and RIBA assays depending on clinician's decision. HCV genotyping assay was usually ordered from clinicians before the management and treatment of HCV infection.

Patients without supplementary testing request (RIBA and PCR assays), patients previously diagnosed with HCV infection, or patients coinfected with hepatitis B virus (HBV)/human immunodeficiency virus (HIV) were excluded from the study. According to HCV-RNA PCR assay results, anti-HCV positive patients were divided into two groups: positive HCV-RNA (viremic) group and negative HCV-RNA (non-viremic) group.

Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey.

After providing informed verbal consent, 5-8 mL of venous blood was taken from each subject, and the blood specimens were centrifuged prior to testing. Fresh serum samples were analyzed for anti-HCV screening by using an automated CLIA method (Roche Cobas e601 analyzer, Roche Diagnostics, Mannheim, Germany). An improved version of the Roche Elecsys Anti-HCV kit (Elecsys Anti-HCV II assay, Roche Diagnostics, Mannheim, Germany) was used to detect the anti-HCV levels. All samples with borderline or positive test results were retested in duplicate. Anti-HCV test results of ≥ 1.00 S/CO were considered positive, while results of < 0.90 S/CO were considered negative and results of ≥ 0.90 S/CO and < 1.00 S/CO were considered borderline, as per the manufacturer's guidelines.

For RIBA and HCV-RNA PCR assays, serum samples were stored at -20°C until testing (within 10-15 days after the screening test). According to the manufacturers' instructions, third-generation RIBA and

HCV-RNA PCR assays were performed with the Inno-Lia HCV Score test (Fujirebio, Gent, Belgium), and Roche Cobas AmpliPrep/Cobas TaqMan HCV test (Roche Molecular Diagnostics, Pleasanton, USA), respectively. For RIBA assay, specimens were considered positive when ≥ 2 bands showed reactivity, indeterminate when only 1 band was reactive, and negative when no reactivity was detected. The lower detection limit of the Cobas AmpliPrep/Cobas TaqMan HCV assay (viral load) was 15 IU/mL. Specimens with reactive anti-HCV screening test results but negative HCV-RNA results and negative or indeterminate RIBA results were evaluated as false positive. Patients with positive HCV-RNA test result were considered viremic.

For HCV genotyping assay, serum samples were shipped on dry ice to Ankara, and genotype determination was performed using real-time PCR assay (Sacace Biotechnologies, Como, Italy) at Synlab Laboratory, Ankara, Turkey.

Biochemical analysis (alanine aminotransaminase; ALT, aspartate aminotransferase; AST, alkaline phosphatase; ALP, gamma-glutamyl transferase; GGT, albumin, total bilirubin), complete blood count (CBC) analysis (leukocyte count; WBC, hemoglobin; Hb, platelet count; PLT, mean platelet volume; MPV, plateletcrit; PCT, platelet distribution width; PDW) and coagulation analysis (prothrombin time; PT, international normalized ratio; INR, activated partial thromboplastin time; aPTT) were performed using

Beckman Coulter AU 5800 chemistry analyzer (Beckman Coulter, Brea, USA), Beckman Coulter UniCel DxH 800 hematology analyzer (Beckman Coulter, Brea, USA) and automated coagulation analyzer (ACL TOP 700, Instrumentation Laboratory, Bedford, USA), respectively, at the Medical Biochemistry Laboratory of Suleyman Demirel University Research and Practice Hospital. For CBC and coagulation analyses, venous blood samples taken from each patient were collected in blood tubes containing ethylenediaminetetraacetic acid (EDTA) or citrate. All assays were performed according to the manufacturers' instructions.

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to assess the normality of the data. Accordingly, Mann-Whitney U test or independent samples t-test was used to compare the differences in continuous variables between groups. The chi-square test was used for comparisons between groups in terms of categorical variables. Results are expressed as frequencies and percentages, mean ± standard deviation, or median (25th and 75th percentiles). Univariate and multivariate logistic regression analyses were also performed and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated in order to identify variables predicting viremia. Receiver operating characteristic (ROC)

Table 1. Comparison of demographic characteristics and laboratory findings in positive (viremic) and negative (non-viremic) HCV-RNA groups.

	HCV-RNA positive group (n = 94)	HCV-RNA negative group (n = 116)	p value
Age (years)	58.65 ± 20.6	56.11 ± 20.5	0.279
Gender (Male/Female)	42 (44.7) / 52 (55.3)	47 (40.5) / 69 (59.5)	0.544
ALT (IU/L)	45.51 (27.59-82.34)	16.42 (12.3-21.42)	< 0.001
AST (IU/L)	46.81 (30.73-61.68)	19.92 (15.99-24.37)	< 0.001
ALP (IU/L)	97.1 (74.13-107.05)	95.51 (78.37-104.15)	0.113
GGT (IU/L)	64.58 (36.61-89.2)	34.92 (20.7-45.1)	< 0.001
Albumin (g/dL)	4.03 ± 0.5	4.08 ± 0.38	0.076
Total bilirubin (mg/dL)	0.94 ± 0.79	0.88 ± 0.36	0.156
PT (sec)	12.66 ± 3.99	12.36 ± 2.76	0.094
INR (index)	1.08 ± 0.33	1.05 ± 0.23	0.109
aPTT (sec)	32.2 ± 5.93	32.06 ± 5.9	0.247
WBC ($\times 10^3$/μL)	7.31 ± 2.65	7.99 ± 2.8	0.079
Hb (g/dL)	13.84 ± 2.07	13.31 ± 2.08	0.068
PLT ($\times 10^3$/μL)	194.78 ± 90.42	257.15 ± 106.26	< 0.001
MPV (fL)	9.32 ± 1.14	8.63 ± 0.83	< 0.001
PCT (%)	0.17 ± 0.04	0.22 ± 0.07	< 0.001
PDW (%)	16.96 ± 0.5	16.82 ± 0.38	0.022
Anti-HCV S/CO ratio	42.60 (30.23-59.75)	3.79 (1.66-16.9)	< 0.001
HCV RIBA	94 (100) P	36 (31) P, 8 (6.9) I, 72 (62.1) N	< 0.001
HCV-RNA ($\log_{10}$ IU/ml)	5.84 ± 0.89	-	-
HCV genotype	63 genotype 1b, 3 genotype 3, 28 NA	-	-

P: positive, I: indeterminate, N: negative, NA: not available; Values are expressed as n (%), mean ± SD or median (25th-75th percentiles).

Table 2. HCV RIBA and HCV-RNA test results in different categories of anti-HCV S/CO ratios.

S/CO ratio	1-2.99 (n = 50)	3-7.99 (n = 26)	8-19.9 (n = 20)	≥ 20 (n = 114)	Total (n = 210)
RIBA positive	0	1 (3.8%)	15 (75%)	114 (100%)	130 (61.9%)
RIBA indeterminate	0	7 (26.9%)	1 (5%)	0	8 (3.8%)
RIBA negative	50 (100%)	18 (69.3%)	4 (20%)	0	72 (34.3%)
HCV-RNA positive	0	0	8 (40%)	86 (75.4%)	94 (44.8%)
HCV-RNA negative	50 (100%)	26 (100%)	12 (60%)	28 (24.6%)	116 (55.2%)

curves were drawn for significant variables, and the areas under the ROC curve (AUC) values with 95% CI were calculated. A cut-off value was determined for significant parameters and sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. A p value of < 0.05 was considered statistically significant.

Results

Out of 21035 subjects who were admitted to inpatient and outpatient departments of our hospital for general health check-up or receiving medical or surgical therapy and routinely tested for HBsAg, anti-HIV and anti-HCV assays during the study period, 297 (1.4%) patients were found to be anti-HCV positive. After applying the exclusion criteria, 210 patients were enrolled in the study. Among 210 patients, 94 (44.8%) and 116 (55.2%) were positive and negative, respectively, according to HCV-RNA PCR assay. Comparison of demographic characteristics (age and gender) and laboratory findings in the positive and

negative HCV-RNA patient groups is shown in Table 1.

There were significant differences in the levels of ALT, AST, GGT, PLT, MPV, PCT, PDW and anti-HCV between viremic and non-viremic patients (p < 0.05). Compared with the non-viremic group, the viremic patients had significantly higher ALT, AST, GGT, MPV, PDW and anti-HCV levels and significantly lower PLT and PCT levels (Table 1).

Among 94 HCV-RNA positive patients, we were able to obtain HCV genotyping results for 66 patients, retrospectively. Genotype 1b and genotype 3 were found in 63 (95.5%) and 3 (4.5%) patients, respectively (Table 1).

All viremic patients revealed positive RIBA results, while in the non-viremic group, 80 (69%) patients had negative or indeterminate RIBA results (regarded as a false positive result) and 36 (31%) patients had positive RIBA results (regarded as a true positive result without viral replication). A statistically significant difference (p < 0.001) was found in RIBA positivity between the two groups (Table 1).

Table 3. Univariate and multivariate logistic regression analyses of demographic characteristics and laboratory findings in the prediction of HCV viremia.

	Univariate OR (95% CI)	Univariate p value	Multivariate OR (95% CI)	Multivariate p value
Age	1.006 (0.993-1.020)	0.372	-	-
Gender	1.186 (0.684-2.056)	0.544	-	-
ALT	1.141 (1.096-1.188)	< 0.001	1.325 (1.135-1.545)	< 0.001
AST	1.110 (1.077-1.144)	< 0.001	0.945 (0.880-1.015)	0.119
ALP	1.005 (0.996-1.014)	0.256	-	-
GGT	1.029 (1.017-1.042)	< 0.001	1.008 (0.991-1.024)	0.355
Albumin	0.762 (0.409-1.419)	0.391	-	-
Total bilirubin	1.196 (0.731-1.957)	0.477	-	-
PT	1.027 (0.945-1.117)	0.531	-	-
INR	1.325 (0.498-3.527)	0.573	-	-
aPTT	1.004 (0.959-1.051)	0.872	-	-
WBC	0.911 (0.819-1.012)	0.083		
Hb	1.132 (0.990-1.294)	0.069	-	-
PLT	0.990 (0.986-0.995)	< 0.001	1.006 (0.993-1.019)	0.390
MPV	2.019 (1.487-2.740)	< 0.001	2.905 (0.986-8.560)	0.053
PCT	0.155 (0.077-0.315)	< 0.001	0.254 (0.028-2.341)	0.226
PDW	2.076 (1.100-3.919)	0.024	0.259 (0.025-2.653)	0.255
Anti-HCV	1.136 (1.099-1.174)	< 0.001	1.219 (1.118-1.330)	< 0.001
HCV RIBA	1.000 (0.943-1.010)	0.996	-	-

OR: odds ratio, CI: confidence interval.

Table 4. Diagnostic performance of anti-HCV and ALT cut-off values in the prediction of HCV viremia according to ROC curve analysis.

Cut-off value	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Anti-HCV, ≥ 3	100 (96.1-100)	43.1 (34.5-52.2)	58.8 (51-66.1)	100 (92.9-100)
Anti-HCV, ≥ 8	100 (96.1-100)	65.5 (56.5-73.5)	70.1 (61.9-77.2)	100 (95.2-100)
Anti-HCV, ≥ 12.34	98.9% (94.2-99.8)	71.6 (62.8-79)	73.8 (65.5-80.7)	98.8% (93.5-99.7)
Anti-HCV, ≥ 20	91.5 (84.1-95.6)	75.9 (67.3-82.7)	75.4 (66.8-82.4)	91.7 (84.4-95.7)
ALT, ≥ 17	95.7 (89.6-98.3)	54.3 (45.3-63.1)	62.9 (54.8-70.4)	94 (85.6-97.7)
ALT, ≥ 40	56.4 (46.3-66)	99.1 (95.3-99.8)	98.1 (90.2-99.7)	73.7 (66.3-80)

Values in parentheses are the limits of 95% confidence interval (CI). PPV: positive predictive value, NPV: negative predictive value.

Table 2 shows HCV RIBA and HCV-RNA test results in four different categories of anti-HCV S/CO ratios. The categories (S/CO ratios of 1-2.99, 3-7.99, 8-19.9 and ≥ 20) were determined according to previously published methods [5,6,13,14]. The S/CO ratio of anti-HCV positive samples ranged from 1.03 to 166.1. At very low anti-HCV levels (S/CO ratio < 3), both HCV RIBA and HCV-RNA tests revealed negative results. All patients with low anti-HCV levels (S/CO ratio < 8) had negative HCV-RNA result. The lowest anti-HCV S/CO ratio detected in HCV-RNA positive samples was 12.3. Among the patients with higher anti-HCV S/CO ratios (≥ 20.0), 86 (75.4%) were positive for HCV-RNA.

The results of univariate and multivariate logistic regression analyses of demographic characteristics and laboratory findings are presented in Table 3. Statistically significant variables (ALT, AST, GGT, PLT, MPV, PCT, PDW, anti-HCV) in univariate analysis were included in the multivariate analysis, and serum ALT level (OR:1.325, p < 0.001) and anti-HCV S/CO ratio (OR:1.219, p < 0.001) were found to be significant predictive factors for HCV viremia.

The most appropriate anti-HCV S/CO ratio for the prediction of HCV viremia was determined by using ROC curve analysis (AUC: 0.935, 95% CI: 0.905-0.964). The diagnostic sensitivity and specificity, PPV and NPV were calculated at S/CO ratios of 3.0, 8.0, and 20.0 (Table 4). The S/CO ratio of ≥ 12.34 was chosen as the optimal value to predict HCV viremia. This cut-off value had diagnostic sensitivity of 98.9% (CI: 94.2-99.8), diagnostic specificity of 71.6% (CI: 62.8-79), PPV of 73.8% (CI: 65.5-80.7) and NPV of 98.8% (CI: 93.5-99.7) in differentiating viremic patients from non-viremic patients (Table 4).

ROC analysis for ALT was also performed to evaluate the predictive accuracy for the diagnosis of viremia. AUC value for ALT was calculated as 0.897 (95% CI: 0.854-0.94). The cut-off value of ≥ 40 was shown to have diagnostic specificity of 99.1% and PPV of 98.1% for predicting HCV viremia (Table 4).

Discussion

The diagnosis of HCV infection usually begins with the detection of anti-HCV using immunoassay screening methods [10]. Because of the possibility of false-positive results, directly performing HCV-RNA assay is the recommended practice in anti-HCV-positive patients with clinical evidence of acute or chronic liver disease [17]. However, high costs, long and laborious procedures and the requirement for specialized equipment and qualified personnel limit the widespread usage of molecular techniques [9,16]. Furthermore, deciding on a reliable, easy-to-use and cost-effective test in order to predict true HCV infection status or HCV viremia in anti-HCV reactive patients remains a controversial issue.

Several studies have suggested that a high anti-HCV level indicates the presence of viremia [3,8,10-12,14-21]. Since anti-HCV antibodies are produced by antigenic stimulation secondary to viral replication, antibody titers will naturally increase when viral stimulation is high and continuous [11,17]. Many studies were conducted to identify an optimal S/CO value distinguishing viremic and non-viremic patients, and different S/CO ratios ranging from 2.7 to 34 were recommended [3,8,11,12,14-21]. Discrepancies in the S/CO ratios proposed as an optimal cut-off value may be due to the differences in sample size, the study population, and the kit used to detect anti-HCV or HCV-RNA viral load. Lai et al. [19] reported that an anti-HCV S/CO ratio of ≥ 20, determined with a Vitros ECi screening assay, corresponded to a diagnostic sensitivity of 95.5% and a diagnostic specificity of 58.8% in predicting viremia. In a study conducted with an Abbott Architect i2000 analyzer, it was suggested that an S/CO ratio of ≥ 10.9 could predict the presence of HCV viremia with a diagnostic sensitivity of 94.4% [11]. However, there has been very limited number of studies in which the new Roche Elecsys Anti-HCV II assay was used to determine an optimal S/CO value distinguishing viremic and non-viremic patients. Additionally, Centers for Disease Control and Prevention (CDC) recommended the predictive cut-off values for some commercially available anti-HCV

screening assays, but those values for the Roche Elecsys assays have not been provided yet [12,19].

In the current study, we analyzed anti-HCV titers by using ROC curves in order to determine a significant S/CO ratio for the prediction of HCV viremia. According to the ROC analysis, HCV viremia could be predicted with a diagnostic sensitivity of 98.9% and a diagnostic specificity of 71.6%, by using an anti-HCV S/CO ratio of ≥ 12.34. As shown in Table 4, the specificity and positive predictive value increased with the higher cut-off values. In the cases (n = 76) with low positive anti-HCV titers (S/CO ratio of < 8), the frequency of false positivity was very high and all cases had negative HCV-RNA test result (Table 2). Additionally, similar to our findings, it was reported that HCV-RNA negative results could occasionally be seen despite high anti-HCV titers (Table 2) [8,12,15,17,19,22,23]. As mentioned above, patients already diagnosed with HCV infection were excluded from this research. However, it is known that HCV-infected individuals can be asymptomatic for many years until diagnosis is made and they are most likely unaware of being infected [17]. In patients with spontaneously resolving infection, anti-HCV may decrease slightly or persist throughout life. This may be the reason why negative HCV-RNA test results can be seen despite high anti-HCV titers in our study. Long-term follow-up of such cases is crucial because a single negative HCV-RNA result may not always rule out HCV infection.

Considering these results, it may be speculated that molecular tests are unnecessary for patients with anti-HCV S/CO ratios of < 12.3 and with no clinical, biochemical and hematological evidence. Retesting of the same sample with another CLIA or testing of a new sample would be suitable and economic prior to the use of molecular assays. This comprehensive approach will help to reduce the costs particularly in settings where the availability of supplemental tests and economic resources are limited. However, it should be noted that our recommendation is appropriate only for the Roche Elecsys Anti-HCV II assay and is not applicable for other screening assays.

It was traditionally considered that serum aminotransaminases; ALT and AST are reliable markers of hepatocellular injury or necrosis. AST can also be represented in other organs and tissues, therefore, an increase in ALT serum levels is more specific for liver damage [2,24]. Additionally, other liver enzymes of ALP and GGT are more likely associated with bile duct injury or cholestasis, and a simultaneous increase in serum GGT and ALP levels occurs mainly in chronic cholestatic liver disease [25]. In this study, viremic patients had higher serum ALT, AST, GGT and ALP levels than non-viremic patients, and significant differences were detected in ALT, AST and GGT levels between the two groups. The finding of a relationship between HCV viremia and increased serum ALT levels most likely reflects hepatocellular damage due to active virus replication. These data are in agreement with the results of previous studies [8,11,14,18,26] demonstrating significantly elevated levels of ALT in viremic patients. However, even though elevated serum ALT level is usually considered as a good reflector of chronic liver disease; due to the nature of HCV infection, ALT fluctuations, even normal serum ALT levels, can be observed during the course of chronic infection [1,2,24].

Prothrombin time and serum albumin level are useful tests for evaluating liver synthetic function. Hepatic synthesis of albumin tends to decline in chronic liver disease, and an elevation in PT relies on the decreased synthesis of liver-derived coagulation factors [24]. Low levels of albumin and prolonged PT in patients with HCV viremia were reported in the study by Lee et al. [8]. Likewise, in the present study, we also found lower albumin levels and higher PT, INR and aPPT in viremic patients, compared to non-viremic patients, but the difference between the two groups was not significant.

Platelet count and platelet indices such as MPV, PCT and PDW can be considered a simple and practical way to provide valuable information during routine complete blood count without increasing the cost of diagnosis. One of the most significant effects of HCV on the hematopoietic system is decreased PLT, and due to thrombocytopenia, platelet indices may be altered in HCV-infected subjects [27,28]. In this research, we found lower PLT and PCT values and higher MPV and PDW values in viremic patients than non-viremic patients, and the differences were statistically significant. Consistent with our results, Tsai et al. [22] reported that HCV-infected patients had significantly lower PLT and PCT but significantly higher MPV and PDW when compared with the control group. The authors stated that, in other words, the HCV-infected group had larger, more irregular, and more numerous platelets compared to the controls. In many studies, it was reported that increased MPV levels were observed not only in HCV-infected subjects but also in patients with diabetes mellitus, hypertension, atherosclerosis, hypercholesterolemia, etc. [26,27,29-31]. Therefore, other clinical situations should be taken into account when high MPV results are obtained. On the other hand,

the clinical utility and validity of PCT and PDW were documented less often. PCT and PDW results in patients with suspected HCV infection should be interpreted with caution, because differences in PCT and PDW values are very small and may vary due to technical reasons.

The current study has some limitations that should be mentioned. First, due to the retrospective nature of the study, we could not collect detailed clinical data especially in patients with false positive results to clarify the specific causes of false-positivity. We also could not reach detailed clinical information about the risk factors (blood transfusions, sexual behaviors, intravenous drug use, hemodialysis, diabetes mellitus, alcohol or cigarette consumption, etc.) for HCV infection in all HCV-RNA positive cases. Second, as mentioned above, our suggestion about the optimal anti-HCV S/CO ratio is appropriate only for the Roche Elecsys Anti-HCV II assay and is not applicable for other commercial assays. Third, owing to fluctuations in viremia, ALT levels could fluctuate during the course of chronic HCV infection, and this situation should be taken into account when evaluating ALT levels and other biochemical findings in patients with suspected HCV infection. Fourth, this research is a single-center study and has relatively limited numbers of HCV-RNA positive and negative patients. Nevertheless, despite these limitations, we think that our preliminary data could offer useful insights for future investigations.

Conclusions

In conclusion, our data demonstrates that ALT, AST, GGT, PLT, MPV, PCT, PDW, and anti-HCV levels are significantly different between viremic and non-viremic patients. These relatively inexpensive and readily available laboratory parameters may predict the presence of HCV viremia. However, consistent with the results of previous studies, our multivariate regression analysis indicates that HCV viremia is strongly associated with ALT and anti-HCV levels. Additionally, an anti-HCV S/CO ratio of 12.34 may help to discriminate between viremic and non-viremic patients, when carefully evaluated together with the clinical, biochemical and hematological evidence. More studies are required to determine reliable and cost-effective test strategies in order to predict HCV viremia or true infection status in anti-HCV positive patients.

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Conflict of interests: No conflict of interests is declared.