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# Biochemical predictors of hepatocellular cancer development after one year in patients who achieved HCV clearance by direct-acting antiviral treatment

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**Abstract. – OBJECTIVE: The continued risk of hepatocellular cancer (HCC) following HCV clearance by direct-acting antivirals (DAAs), even if a sustained viral response (SVR) is achieved, has been previously reported. This study's objective was to identify biochemical predictors for HCC occurrence in patients who achieved HCV clearance by DAA treatment after one year.**

**PATIENTS AND METHODS: Patients who achieved SVR at week 24 with DAA between November 2015 and January 2021 and had no evidence of HCC were evaluated retrospectively. Biochemical parameters such as serum AFP (Alpha-fetoprotein), AST (Aspartate Aminotransferase), ALT (Alanine aminotransferase), albumin, PLT (platelet) count, FIB-4 and APRI indexes (non-invasive fibrosis indexes) were analyzed before starting the DAA treatment, at the end of the treatment (EOT), and 24th-week post-treatment.**

**RESULTS: Throughout a median follow-up time of 43±16.2 months, it was observed that HCC occurred in 14 (5.6%) of 248 CHC patients who reached SVR at the 24th week after DAA treatment. According to multivariate analysis, AFP levels were >7.08 ng/ml before treatment (HR, 3.8; *p*=0.050), >5.15 ng/ml at the EOT (HR, 6.8; *p*=0.019), and >5.68 ng/ml at the 24th week post-treatment (HR, 21.2; *p*=0.002); albumin levels were <3.75 g/dl before treatment (HR, 3.6; *p*=0.042), <4.05 g/dl at the EOT (HR, 6.9; *p*=0.005), and <4.15 g/dl at week 24 post-treatment (HR, 8.8; *p*=0.002); PLT counts <153.000/mm3 at the 24th week post-treatment (HR, 12.1; *p*=0.001) were determined to be independent biochemical predictors for the development of HCC after one year of ending DAA treatment.**

**CONCLUSIONS: AFP and albumin levels before treatment, at the EOT, and post-treatment at week 24, and PLT numbers at week 24 post-treatment can be used to foresee the risk of developing HCC one year after ending of DAA treatment.**

*Key Words:*
Chronic hepatitis C, Hepatocellular cancer, Direct-acting antiviral, Alpha-fetoprotein, Albumin, Platelet count.

## Introduction

Chronic hepatitis C (CHC) has significant morbidity and mortality, with an annual risk of hepatocellular cancer (HCC) of 3-7% in patients with cirrhosis[1]. However, highly potent direct-acting antivirals (DAAs) with cure rates greater than 90% have reliably induced a sustained viral response (SVR) in most patients with HCV infection[2]. Eradication of the virus with DAA has been proven to reduce the risk of hepatic decompensation and mortality in patients with CHC[3].

Studies regarding the development of HCC in patients who achieved SVR with DAA treatment are conflicting. While some studies[3-7] showed that DAA reduces the risk of HCC in CHC patients, others[8,9] argued that the risk of HCC continues in these patients even if SVR was achieved. In addition, it has been suggested that SVR achieved with DAA does not reduce the risk of HCC, especially in patients with HCV-related cirrhosis[6,10,11], and high tumor recurrence is observed in the short-term in patients who have received HCC treatment before[10]. The risk for HCC occurrence after HCV eradication appears to decrease with time after SVR[12]. Because there is a gradual increase in individuals using DAA for HCV clearance and the risk of HCC formation remains a concern even if SVR is achieved, the need to predict individuals at higher risk of HCC has emerged.

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Previous studies[13-16] have also shown that serum AFP (Alpha-fetoprotein) levels, low albumin, and platelet levels, and high FIB-4 (fibrosis) index are risk factors for developing HCC after HCV clearance by DAAs. After CHC treatment with DAA, it was seen that there was a considerable decrease in AFP levels[17]. It has also been shown that HCV RNA-positive patients have significantly higher serum AFP levels than negative groups, and this AFP level correlates with ALT[18]. Although 1-year cumulative *de novo* HCC rates can vary according to the presence of cirrhosis at baseline, it has been reported as high as 4.9%[19]. Before the treatment of DAA, HCC may have gone undiagnosed after an imaging analysis. As a result, it has been advised to establish definite markers of HCC development after a year of DAA treatment[15]. As far as we know, all the parameters mentioned above have not been enough evaluated together, and no studies have investigated the biochemical risk factors for the occurrence of HCC following one year after DAA treatment in CHC patients before starting DAA therapy, at the end of therapy (EOT), and at the 24th week post-treatment.

This study's objective was to identify biochemical predictors for HCC occurrence in patients who achieved HCV clearance by DAA treatment after one year.

## Patients and Methods

In our single-center study, we retrospectively evaluated patients who completed IFN-free DAA treatment for HCV infection between November 2015 and January 2021. Patients whose SVR could be achieved at the 24th week after DAA treatment was considered eligible to participate in the study. Those over 18 years old, those with HBV and HIV serology positivity, other chronic liver disease etiology, liver transplant patients, and participants who had been found to have HCC within the first year after completion of treatment were not eligible for the study.

SVR was described as HCV-RNA still undetectable in serum at 24 weeks from completion of therapy. It was recorded serum AFP (Alpha-fetoprotein), AST (Aspartate Aminotransferase), ALT (Alanine aminotransferase), albumin, PLT (platelet) count, APRI, and FIB-4 indexes (non-invasive fibrosis indexes) were analyzed before starting DAA treatment, at the end of the treatment (EOT), and 24th week in patients treated with DAA. The corresponding formulas were used to calculate the APRI index [(upper limit of AST/AST) x 100/PLT] and the FIB-4 index [(AST x age) / (ALT x PLT)]. The liver histology, the development of ascites on abdominal imaging, the presence of gastric varices on esophagogastroscopic examination, or any combination of these factors were used to diagnose a patient with liver cirrhosis. Liver steatosis was diagnosed by ultrasound-based techniques or liver histology.

Zonguldak Bülent Ecevit University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee accepted the present study (Protocol No.: 2022/08, Approval date: 20/04/2022). The study procedure adheres to the ethical principles established in the Helsinki Declaration of 1964.

### Statistical Analysis

The SPSS (Statistical Package for Social Sciences, IBM Corp., Armonk, NY, USA) version 22 was employed for statistical assessment. Data were presented as numbers (%) or means±standard deviations. For the comparison of categorical data, the $\chi^2$ test was used. Independent continuous variables were evaluated using non-parametric testing (Mann-Whitney U). ROC and AUC (area under the curve) values were used to analyze cut-off values for predictors of developing HCC. To identify predictors of developing HCC, an analysis of logistic regression was done. The Spearman's rank correlation test was used to check the correlations between changes in AFP level and other factors. $p$-values $\leq 0.050$ were used to define statistical significance.

## Results

Throughout a median follow-up time of 43±16.2 months, it was observed that HCC occurred in 14 (5.6%) of 248 HCV-infected patients who reached SVR at the 24th week by DAA treatment. Table I presents the main features of the patients included in the study. The female gender was dominant with 59.7%, and participants had a mean age of 68.2 (±12.5) years. Patients with HCC were 73.9 (±9.3) years old on average.

The features of patients who had no HCC (n=234) and developed HCC (n=14) after DAA treatment are shown in Table II. At baseline, the cirrhosis rates of the HCC group (100%) were significantly higher than the non-HCC group ($p$<0.001). The rate of hepatosteatosis in HCC patients was not significantly different from the non-HCC group ($p$=0.139). AFP levels measured

**Table I.** The main features of the patients included in the study.

| | | | |
|---|---|---|---|
| Age (years) | | 68.2 ± 12.5 | |
| HCV RNA (IU/ml) | | 3.929.993 ± 17.209.913 | |
| Follow-up time (months) | | 43 ± 16.2 | |
| Gender (n, %) | Male | 100 | 40.3% |
| | Female | 148 | 59.7% |
| Cirrhosis (n, %) | Yes | 83 | 33.5% |
| | No | 165 | 66.5% |
| Hepatosteatosis (n, %) | Yes | 80 | 32.3% |
| | No | 168 | 67.7% |
| Genotype (n, %) | 1 | 26 | 10.5% |
| | 1a | 15 | 6% |
| | 1b | 193 | 77.8% |
| | 2 | 2 | 0.8% |
| | 3 | 10 | 4% |
| | 4 | 2 | 0.8% |
| Treatment Regimen (n, %) | G+Pb | 38 | 15.3% |
| | L+S | 68 | 27.4% |
| | L+S+Rb | 24 | 9.7% |
| | O+Pr+Rt+D | 115 | 46.4% |
| | O+Pr+Rt+D+Rb | 3 | 1.2% |

G: Glekaprevir, Pb: Pibrentasvir, Pr: Paritaprevir, L: Ledipasvir, O: Ombitasvir, Rb: Ribavirin, Rt: Ritonavir.

**Table II.** Features of HCC and Non-HCC group.

| | HCC group (n = 14) | Non-HCC group (n = 234) | *p*-value |
|---|---|---|---|
| **Before Treatment** | | | |
| AFP (ng/ml) | 14.1 (7.11-38.0) | 4.43 (2.68-8.12) | < **0.001*** |
| AST (IU/l) | 66 (40-92) | 46 (29-75) | 0.129* |
| ALT (IU/l) | 44.5 (40-77) | 43 (26-76.5) | 0.462* |
| PLT ($\times 10^3/mm^3$) | 121 (85-156) | 174 (124-225) | **0.005*** |
| ALB (g/dl) | 3.5 (3-3.9) | 4.1 (3.6-4.3) | **0.003*** |
| FIB-4 | 5.7 (3.8-9.1) | 2.6 (1.7-5.3) | **0.002*** |
| APRI | 1.5 (1.4-2.2) | 0.8 (0.4-1.7) | **0.007*** |
| **End of Treatment** | | | |
| AFP (ng/ml) | 9.2 (7.8-11.9) | 3.2 (2.0-4.7) | < **0.001*** |
| AST (IU/l) | 31.5 (23-38) | 20 (17-25) | **0.001*** |
| ALT (IU/l) | 18.5 (17-23) | 13 (10-19) | **0.006*** |
| PLT ($\times 10^3/mm^3$) | 125.5 (95-166) | 195 (140-247) | **0.001*** |
| ALB (g/dl) | 4.0 (3.3-4.3) | 4.4 (4.1-4.6) | **0.003*** |
| FIB-4 | 4.2 (2.7-5.4) | 1.9 (1.2-3.0) | **0.002*** |
| APRI | 0.8 (0.4-1.1) | 0.3 (0.2-0.5) | < **0.001*** |
| **24$^{th}$ Week** | | | |
| AFP (ng/ml) | 8.6 (5.8-40) | 3.1 (2.2-4.8) | < **0.001*** |
| AST (IU/l) | 32 (25-51) | 20 (16-26) | < **0.001*** |
| ALT (IU/l) | 25.5 (16-35) | 14 (10-18) | **0.001*** |
| PLT ($\times 10^3/mm^3$) | 146.5 (102-177) | 206 (149-254) | **0.004*** |
| ALB (g/dl) | 4.0 (3.5-4.4) | 4.4 (4.1-4.6) | **0.036*** |
| FIB-4 | 3.8 (2.6-5.4) | 1.7 (1.2-2.8) | < **0.001*** |
| APRI | 0.8 (0.5-1.0) | 0.3 (0.2-0.4) | < **0.001*** |
| Age (years) | 75.5 (67-81) | 69 (62-78) | 0.110* |
| Age > 69.5, (n) | 8 | 116 | 0.582** |
| Male/Female, (n) | 5/9 | 95/139 | 0.717** |
| Cirrhosis, (n) | 14 | 69 | < **0.001**** |
| Hepatosteatosis, (n) | 2 | 78 | 0.139** |

HCC: Hepatocellular Cancer, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, AFP: Alpha-Fetoprotein, ALB: Albumin, PLT: Platelet count, FIB-4: Fibrosis-4 Index, APRI: AST to Platelet Ratio Index. *Mann Whitney-U Test, **Chi-Square test.

before DAA treatment at the EOT and 24 weeks post-treatment were considerably higher in the HCC group, respectively $p<0.001$, $p<0.001$, and $p<0.001$. Albumin levels measured before DAA treatment at the EOT and at week 24 post-treatment were significantly lower in the HCC group, respectively $p=0.003$, $p=0.003$, and $p=0.036$. AST and ALT levels were significantly different between the two groups at the EOT and 24 weeks post-treatment (AST, $p=0.001$, $p<0.001$; ALT, $p=0.006$, $p=0.001$, respectively). AST levels were higher in the HCC group, and ALT levels were more elevated in the non-HCC group at all times studied. PLT counts measured before DAA treatment, at the EOT, and 24 weeks post-treatment were significantly lower in the HCC group, respectively $p=0.005$, $p=0.001$, and $p=0.004$. FIB-4 indexes calculated before DAA treatment, at the EOT, and 24 weeks post-treatment were significantly higher in the HCC group, respectively $p=0.002$, $p<0.001$, and $p<0.001$. APRI indexes calculated before DAA treatment, at the EOT, and at the 24th week post-treatment were significantly higher in the HCC group, respectively $p=0.007$, $p<0.001$, and $p<0.001$.

The biochemical risk factors' cut-off values for HCC occurrence were presented in Table III based on the ROC value before DAA treatment, at the EOT, and at the 24th week post-treatment in 248 patients.

Biochemical predictors of HCC development ≥ one year after SVR achieved by DAAs are presented in Table IV. Based on the univariate analysis, AFP, albumin, PLT, FIB-4, and APRI indexes before treatment, AFP, albumin, PLT, FIB-4, and APRI indexes, AST, and ALT levels at the EOT and 24 weeks post-treatment were determined to be biochemical predictors. Multivariate analysis revealed that AFP levels were >7.08 ng/ml before treatment, >5.15 ng/ml at the EOT, and >5.68 ng/ml at the 24th week post-treatment; albumin levels were <3.75 g/dl the before treatment, <4.05 g/dl at the EOT, and <4.15 g/dl at 24 weeks post-treatment, PLT counts <153.000 /mm$^3$ at the 24th week post-treatment were found to be independent biochemical predictors for the development of HCC after one year of ending DAA treatment.

Table V show that changes in serum AFP levels were positively correlated with changes in FIB-4 index, APRI, AST, and ALT levels and were negatively correlated with albumin and PLT before treatment, at the EOT and 24th-week post-treatment.

**Table III.** The area under the receiver operating characteristic (AUC) value of biochemical factors associated with HCC development ≥ one year after DAA treatment.

| | AUC | 1-Specificity | Sensitivity | Cut-off value |
|---|---|---|---|---|
| **Before Treatment** | | | | |
| AFP (ng/ml) | 0.785 | 0.327 | 0.750 | **7.08** |
| Alb (g/dl) | 0.722 | 0.286 | 0.650 | **3.75** |
| Plt (×10$^3$/mm$^3$) | 0.715 | 0.357 | 0.710 | **133.5** |
| FIB-4 | 0.738 | 0.367 | 0.786 | **3.85** |
| APRI | 0.701 | 0.303 | 0.786 | **1.45** |
| **End of Treatment** | | | | |
| AFP (ng/ml) | 0.882 | 0.221 | 0.833 | **5.15** |
| Alb (g/dl) | 0.739 | 0.357 | 0.762 | **4.05** |
| Plt (×10$^3$/mm$^3$) | 0.772 | 0.357 | 0.739 | **148.5** |
| FIB-4 | 0.810 | 0.288 | 0.857 | **2.63** |
| APRI | 0.810 | 0.309 | 0.857 | **0.43** |
| AST (IU/l) | 0.760 | 0.322 | 0.786 | **22.5** |
| ALT (IU/l) | 0.714 | 0.337 | 0.786 | **16.5** |
| **24th Week** | | | | |
| AFP (ng/ml) | 0.904 | 0.159 | 0.833 | **5.68** |
| Alb (g/dl) | 0.669 | 0.429 | 0.744 | **4.15** |
| Plt (×10$^3$/mm$^3$) | 0.726 | 0.357 | 0.728 | **153** |
| FIB-4 | 0.803 | 0.299 | 0.857 | **2.50** |
| APRI | 0.828 | 0.185 | 0.786 | **0.57** |
| AST (IU/l) | 0.818 | 0.278 | 0.786 | **24.5** |
| ALT (IU/l) | 0.769 | 0.392 | 0.786 | **15.5** |

ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, AFP: Alpha-Fetoprotein ALB: Albumin, PLT: Platelet count, FIB-4: Fibrosis-4 Index, APRI: AST to Platelet Ratio Index.

**Table IV.** Biochemical predictors of HCC development ≥ one year after SVR achieved by DAAs.

| Variable | Univariate | | | Multivariate* | | |
|---|---|---|---|---|---|---|
| | HCC group (n = 14) | Non-HCC group (n = 234) | *p*-value | HR | 95% CI | *p*-value |
| **Before Treatment** | | | | | | |
| AFP > 7.08 ng/ml | 11 (78.6%) | 93 (39.7%) | **0.004** | 3.82 | 0.99-14.66 | **0.050** |
| Alb < 3.75 g/dl | 10 (71.4%) | 70 (29.9%) | **0.001** | 3.68 | 1.04-12.99 | **0.042** |
| Plt < 133.500/mm$^3$ | 9 (64.3%) | 63 (26.9%) | **0.003** | | | |
| FIB-4 > 3.85 | 11 (78.6%) | 89 (38%) | **0.003** | | | |
| APRI > 1.45 | 11 (78.6%) | 75 (32.1%) | < **0.001** | | | |
| **End of Treatment** | | | | | | |
| AFP > 5.15 ng/ml | 12 (85.7%) | 103 (44%) | **0.002** | 6.82 | 1.36-34.17 | **0.019** |
| Alb < 4.05 g/dl | 9 (64.3%) | 47 (20.1%) | < **0.001** | 6.93 | 1.81-26.45 | **0.005** |
| Plt < 148.500/mm$^3$ | 9 (64.3%) | 57 (24.4%) | **0.001** | | | |
| FIB-4 > 2.63 | 12 (85.7%) | 89 (38%) | < **0.001** | | | |
| APRI > 0.43 | 12 (85.7%) | 93 (39.7%) | **0.001** | | | |
| AST > 22.5 IU/l | 11 (78.6%) | 92 (39.3%) | **0.004** | | | |
| ALT > 16.5 IU/l | 11 (78.6%) | 92 (39.3%) | **0.004** | | | |
| **24$^{th}$ Week** | | | | | | |
| AFP > 5.68 ng/ml | 12 (85.7%) | 119 (50.9%) | **0.011** | 21.22 | 3.1-143.2 | **0.002** |
| Alb < 4.15 g/dl | 8 (57.1%) | 46 (19.7%) | **0.001** | 8.80 | 2.2-35.3 | **0.002** |
| Plt < 153.000/mm$^3$ | 9 (64.3%) | 50 (21.4) | < **0.001** | 12.10 | 2.9-49.7 | **0.001** |
| FIB-4 > 2.5 | 12 (85.7%) | 101 (43.2%) | **0.002** | | | |
| APRI > 0.57 | 11 (78.6%) | 79 (33.8%) | **0.001** | | | |
| AST > 24.5 IU/l | 11 (78.6%) | 98 (41.9%) | **0.007** | | | |
| ALT > 15.5 IU/l | 11 (78.6%) | 120 (51.3%) | **0.047** | | | |

HCC: Hepatocellular Cancer, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, AFP: Alpha-Fetoprotein, ALB: Albumin, PLT: Platelet count, FIB-4: Fibrosis-4 Index, APRI: AST to Platelet Ratio Index, SVR: Sustained Viral Response. Univariate analyzes were performed with the Chi-Square Test. Multivariate analyzes were performed with the Logistic Regression model *Only statistically significant values are shown in the Multivariate analysis table.

## Discussion

As observed in previous experience with IFN-based therapy, HCC is common even in CHC patients undergoing SVR. It has been shown that ALT and AFP levels, male gender, advanced age, advanced liver fibrosis, hepatosteatosis, and alcohol consumption are risk factors for the occurrence of HCC in patients who reach SVR through IFN-based therapy[20]. Compared to in-

**Table V.** Correlation between AFP and DAA treatment and other biochemical factors associated with HCC occurrence ≥ one year after SVR.

| | AFP | | | | | |
|---|---|---|---|---|---|---|
| | Before treatment | | End of treatment | | 24$^{th}$ Week | |
| | r | *p*-value | r | *p*-value | r | *p*-value |
| ALT (IU/l) | 0.274 | < 0.001 | 0.324 | < 0.001 | 0.257 | **0.002** |
| AST (IU/l) | 0.339 | < 0.001 | 0.284 | < 0.001 | 0.332 | < **0.001** |
| ALB (g/dl) | -0.242 | < 0.001 | -0.192 | 0.012 | -0.084 | 0.332 |
| PLT (×10$^3$/mm$^3$) | -0.361 | < 0.001 | -0.382 | < 0.001 | -0.331 | < **0.001** |
| FIB-4 | 0.38 | < 0.001 | 0.39 | < 0.001 | 0.362 | < **0.001** |
| APRI | 0.393 | < 0.001 | 0.429 | < 0.001 | 0.397 | < **0.00** |

r: Correlation Coefficient, HCC: Hepatocellular Cancer, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, AFP: Alpha-Fetoprotein, ALB: Albumin, PLT: Platelet count, FIB-4: Fibrosis-4 Index, APRI: AST to Platelet Ratio Index, SVR: Sustained Viral Response.

terferon-based therapy, DAA-based treatments have reduced the incidence of HCC with excellent efficacy and safety. Furthermore, HCV therapy was not adequately administered in the pre-DAA period[21]. Among those achieving SVR following DAA therapy, patients with cirrhosis at baseline were shown to have the highest risk of HCC[6,14].

According to our study, patients with HCV infection had a 5.6% probability of HCC development after one year from achieving SVR by DAA. About one-third of our participants were cirrhotic; most were older than 65. Advanced age and cirrhosis during SVR are sufficient risks to require surveillance[20]. Guidelines recommend that these patients remain on HCC surveillance[22]. In line with our study, previous investigations have shown that after DAA therapy, patients have a 3-year cumulative incidence of HCC between 2.4 percent to 6 percent[14,15,23].

All our HCC patients were found to have cirrhosis at baseline, and although we could not achieve statistical significance, HCC patients were older than the non-HCC group. We could not show a significant gender difference between the groups with and without HCC in our study, but it has been proven that the risk of HCC is higher in male patients with HCV clearance after DAA treatment[11]. Since it is well recognized that non-alcoholic hepatosteatosis considerably raises the risk of HCC, it was unexpected that baseline hepatosteatosis did not seem to change the risk of HCC. We may not have achieved statistical significance because of the low number of HCC patients.

This study revealed that AFP levels before treatment, at the EOT, and 24$^{th}$-week post-treatment; albumin levels before treatment, at the EOT, and 24$^{th}$-week post-treatment; and PLT counts at the 24$^{th}$-week post-treatment were identified as biochemical predictors for HCC occurrence ≥ one year following DAA treatment. In previous studies, pretreatment AFP≥ 10 ng/ml was found by Tanaka et al[24], and EOT AFP ≥ 9 ng/ml was found by Ogawa et al[19]. Our cut-off AFP levels were >7.08 ng/ml before treatment, >5.15 ng/ml at the EOT, and >5.68 ng/ml 24$^{th}$-week post-treatment. Although the cut-off value for the AFP level in our study was lower than in the studies mentioned above, the results were in line with other studies[15,25]. The reason for this may be the presence of microscopic HCCs that imaging tests could not detect during the first year after the completion of treatment in which patients were thought to be free of HCC. Therefore, our study evaluated patients who developed HCC ≥1 year after the ending of DAA treatment. Moreover, we also observed that the AFP values of the patients tended to decrease under the DAA treatment and increase again at the 24$^{th}$-week post-treatment. In addition, we demonstrated that the cut-off value of albumin levels <3.75 g/dl before treatment, <4.05 g/dl at the EOT, and <4.15 g/dl 24$^{th}$-week post-treatment were independent biochemical predictors for the occurrence of HCC. These results also showed that the albumin values of the patients tended to increase under DAA treatment. On the other hand, Abe et al[16] determined the cut-off value for albumin before treatment as <3.9 g/dl in patients without cirrhosis. Our cut-off value was probably lower because our study evaluated cirrhotic and non-cirrhotic patients together. We also showed that a PLT count below 153.000 / mm$^3$ at the 24$^{th}$-week post-treatment was an independent biochemical predictor for the occurrence of HCC. We also observed that the PLT counts of the patients tended to increase with DAA. In the study of Abe et al[16], a cut-off value of 82,000 /mm3 was shown to be a predictor of the occurrence of HCC in pre-treatment cirrhotic CHC patients. Consistent with our study, Ogata et al[25] identified HCC risk factors by multivariate analysis as hypoalbuminemia, thrombocytopenia, and elevated AFP levels in patients with SVR. The fact that the APRI index and the FIB-4 score could not be found as independent risk factors may probably be affected by the AST and PLT values that make up these scores in the multivariant analysis. However, in the correlation analysis, it was observed that these values were correlated with AFP.

### *Limitations*

As far as we know, this is the first study to evaluate biochemical factors associated with HCC development one year after treatment in CHC patients following DAA, before treatment, at the EOT, and 24$^{th}$-week post-treatment. The main limitations of this study are the limited number of HCCs due to its single-center design. Another limitation is that although hepatic steatosis was evaluated, comorbidities such as diabetes that could contribute to the risk of HCC were ignored.

## Conclusions

AFP levels above 7.08 ng/ml before treatment, above 5.15 ng/ml at the EOT, and above 5.68 ng/ml at the 24$^{th}$-week post-treatment; albumin lev-

els <3.75 g/dl the before treatment, <4.05 g/dl at the EOT, and <4.15 g/dl at 24th-week post-treatment, PLT counts <153.000/mm$^3$ at 24th-week post-treatment can be used to foresee the risk of developing HCC one year after ending of DAA treatment.

### Conflict of Interest
The Authors declare that they have no conflict of interests.

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### Informed Consent
Informed consent was obtained from all participants or their first-degree relatives in this study.

### Ethics Approval
Zonguldak Bülent Ecevit University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee Approved the present study (Protocol No.: 2022/08, Approved date: 20/04/2022).

### Authors' Contribution
ZGS and ID: Concept and design of study or acquisition of data or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, final approval of the version to be published.

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