Turkish Journal of Internal Medicine
http://www.tjim.org
ISSN: 2687-4245
Original Article

Assessment of The Performances of Hepatitis C Virus Viral Markers, Age-Platelet Index and Aspartate aminotransferase to Alanine Aminotransferase Ratio Scores in Predicting Liver Histopathology

Ayfer BAKIR[1], Mustafa GUNEY[2], Harun ERDAL[3], Ozlem YAVUZ[4], Armagan GUNAL[5], Mustafa GULSEN[6], Mehmet Tevfik YAVUZ[2]

[1]Department of Medical Microbiology, University of Health Sciences, Gulhane Training and Research Hospital, Ankara ,Turkey
[2]Department of Medical Microbiology, University of Health Sciences, Gulhane Faculty of Medicine, Ankara ,Turkey
[3]Department of Gastroenterology, University of Health Sciences, Gulhane Training and Research Hospital, Ankara ,Turkey
[4]Department of Biochemistry, University of Health Sciences, Gulhane Faculty of Medicine, Ankara ,Turkey
[5]Department of Pathology, University of Health Sciences, Gulhane Training and Research Hospital, Ankara ,Turkey
[6]Department of Gastroenterology, University of Health Sciences, Gulhane Faculty of Medicine, Ankara ,Turkey

Abstract

Background This study aimed to reveal the utility of age-platelet (AP) index and aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio (AAR), which are non-invasive markers, in patients with chronic Hepatitis C virus (HCV) infection in prediction of fibrosis and evaluate whether viral markers could be used for that purpose or not.

Material and Methods A total of treatment-naïve 49 patients with chronic HCV who underwent liver biopsy were included in this retrospective study. Anti-HCV S/CO and HCV-RNA viral load (copy/mL) values measured simultaneously with biopsy were determined. AP index and AAR score were calculated and compared.

Results Staging of liver biopsy samples of 49 HCV patients was assessed. Comparable diagnostic accuracies of AP index and AAR in prediction of significant fibrosis (F≥2) were showed with ROC curve analysis. The areas under the ROC (AUROCs) were 0.713 and 0.506, respectively. Diagnostic accuracy of API in prediction of significant fibrosis was superior to that of AAR (p=0.03). AUROC of HCV-RNA viral load in prediction of F≥2 was 0.531. Anti-HCV S/CO value (AUROC=0.464) was not found as a suitable marker in prediction of fibrosis.

Conclusions AP index, AAR score and HCV viral load among non-invasive markers assessed in this study were useful in predicting significant fibrosis. Especially API was the most useful test in predicting significant fibrosis. AP index can be preferred in patients with near normal ALT values.

Turk J Int Med 2021;3(1): 6-12
DOI: 10.46310/tjim.825814

Keywords: *liver fibrosis, non-invasive test, AST to ALT ratio (AAR), age-platelet (AP) index, HCV viral load, anti-HCV*

Received: November 16, 2020; Accepted: December 24, 2020; Published Online: January 29, 2021

Address for Correspondence:
Ayfer Bakir, MD
Department of Medical Microbiology, University of Health Sciences, Gulhane Training and Research Hospital, Ankara ,Turkey
E-mail: dr.ayfer.bakir@gmail.com

Introduction

The stage of liver fibrosis is important for clinical management of chronic hepatitis C. Patients without fibrosis or with mild fibrosis have the chance to receive more positive results compared to the patients with severe fibrosis.[1–3] Development of cirrhosis is closely related with the stage of fibrosis. Therefore, liver biopsy is recommended before antiviral treatment.[4] The available gold standard for assessment of liver disease is degree and stage determined with liver biopsy. However, liver biopsy has limitations, risks and costs.[5] Therefore, invasive methods are needed to determine the severity of liver disease, especially the degree of fibrosis. An ideal noninvasive method to assess liver biopsy should be both reliable and based on readily available blood tests.[6]

This study aimed to reveal the utility of age-platelet (AP) index and aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio (AAR), which are non-invasive markers, in patients with chronic Hepatitis C virus (HCV) infection in prediction of fibrosis and evaluate whether viral markers could be used for that purpose or not.

Material and Methods

Study Group

The population of this retrospective, single-center and cross-sectional study consisted of 49 patients diagnosed with chronic HCV. Patients above the age of 18 who underwent liver biopsy and who were diagnosed with chronic HCV were included in the study. Patients under the age of 18, patients diagnosed with hepatocellular carcinoma, patients diagnosed with hepatitis B, hepatitis D and other hepatotropic viruses or human immunodeficiency virus infections, patients with active alcoholism history and patients diagnosed with other defined liver diseases were excluded from the study. A total of 49 patients who met the inclusion and exclusion criteria were included in the study. This study was approved by the Ethics Committee of Gulhane Training and Research Hospital at the University of Health Sciences (Reference number: 2020/03/62).

Serological and Molecular Analysis

Patients whose serum samples were sent to Microbiology Virology Laboratory between 1st of October 2016 and 31st of December 2019 for HCV-RNA test and whose results were anti-HCV reactive were evaluated in this study. Anti-HCV test was performed with chemiluminescent microparticular immunoassay (CMIA) technique in serum samples by using anti-HCV Reactive Kit (Abbott, Germany) on Architect i2000SR system (Abbott, USA). Anti-HCV test results were assessed on Sample/Cut-off (S/CO) ratio and S/CO value <1.0 was accepted as nonreactive and S/CO value ≥1 as reactive. All the samples found as an intermediate value between 0.80-0.99 were reanalyzed according to instructions of the manufacturer.

Isolation device (Magnesia 2448 Anatolia Geneworks, Turkey) and HCV-RNA isolation kit (Viral RNA Isolation kit, Anatolia Geneworks, Turkey) were used in detection of HCV-RNA. The PCR mixture prepared with Real-time PCR kit (Bosphore HCV Quantification Kit v2, Turkey) was amplified on Real-Time PCR device (Montania 4896 Anatolia Geneworks, Turkey). Patient results were retrospectively obtained from the laboratory operating system.

Non-invasive tests and formulation

Age, platelet count, and AST and ALT levels were used in calculations of AST to ALT ratio (AAR) and AP index scores of patients included in the study. Tests were conducted in the Biochemistry Laboratory of Gulhane Training and Research Hospital.

AAR: AST to ALT ratio.[7] AP Index: Age Score + Platelet Score.

Age (years) <30 = 0; 30–39 = 1; 40–49 = 2; 50–59 = 3; 60–69 = 4; ≥70 = 5.

Platelet count (10^9/L): ≥225 = 0; 200–224 = 1; 175–199 = 2; 150–174 = 3; 125–149 = 4; <125 = 5.[8]

Diagnostic accuracies of these markers in prediction of significant fibrosis were assessed with Receiver Operating Characteristic (ROC) curve analysis.

Histopathological diagnosis

Patients liver needle biopsy histopathology reports obtained from the Department of Pathology archive were reassessed. All the liver biopsy samples were stained with hematoxylin-eosin and Masson's trichrome for histological assessment. Liver biopsy samples were assessed by pathologists and scored according to Ishak

scoring system. Ishak modified-hepatitis activity index (mHAI) grading and staging system was used in detecting chronic hepatitis activity level and fibrosis level in microscopic examination performed with histochemical preparations. In this scoring system, activity level ranged from 0 to 18 and fibrosis stage from 0 to 6. Significant fibrosis was defined as stage 2 fibrosis.

Statistical Analysis

Statistical analysis was performed with SPSS 25 software program (SPSS, Inc., Chicago, IL). Continuous variables were expressed as median (interquartile range). Mann-Whitney U test was used in comparison of continuous variables between two groups. Pearson's Chi-square or Fisher's Exact tests were used in comparison of categorical variables. Spearman's correlation analysis was used to evaluate the relationship between variables. Performances of anti-HCV, HCV viral load, and API index and AAR scores, which are among viral markers, in prediction of significant F≥2 and HAI≥5 were assessed with ROC curve analysis. Statistical significance level was accepted as $p<0.05$.

Results

Data of 49 patients whose ages ranged from 20 to 82, who met the inclusion and exclusion criteria, and who were diagnosed with chronic HCV infection were analyzed. Of the patients included in the study, 34 (69%) were male. Median ages of male and female patients were 21 (interquartile range [IQR]: 20-39) and 62 (IQR: 40-72) respectively ($p<0.001$). Median age of the patients with fibrosis score of 2 and above was 61.5 and significantly high ($p=0.005$).

According to the results of liver biopsy, 21(43%) out of 49 patients had significant fibrosis (F≥2). Fibrosis score in 32% (11/34) of male patients and 67% (10/15) of female patients was 2 and above ($p=0.02$).

AAR score and AP index of all the patients were calculated. Patients with chronic HCV infection (n=49) were divided into two groups according to their liver biopsy histopathology stages of F<2 and F≥2. They were compared in terms of age, gender, serum platelet, AST, ALT, total bilirubin, albumin, anti-HCV (S/CO), HCV viral load, HAI score, AP index and AAR score *(Table 1)*. Ages of female patients with significant fibrosis were

Table 1. Basic characteristics of patients (n=49) who underwent liver biopsy due to chronic HCV

Characteristic	Fibrosis stage <2	Fibrosis stage ≥ 2	P- value
Gender n (%)			
- Female	5 (33)	10(67)	0.02
- Male	23 (68)	11(32)	
Age: years	21 (20-39)	61.5 (21-72)	0.005
- Female	39 (35-46)	68(61-76)	0.004
- Male	20(20-24)	23(20-62)	0.33
Anti-HCV (S/CO)	14.2(13.7-14.2)	14.4(13.5-15.3)	0.82
HCV viral load (copy/mL)	$91x10^4$ ($69x10^2$-$16x10^6$)	$26x10^5$ ($203x10^3$-$63x10^5$)	0.84
HAI	5 (4-6)	7 (6-9)	<0.001
AST (U/L)	35.5 (23.8-57.8)	36.0 (23.8-50.8)	0.68
ALT (U/L)	41 (32-98)	41(24-63)	0.38
Total bilirubin (mg/dL)	0.6(0.5-0.8)	0.7(0.6-0.9)	0.27
Albumin (g/dL)	4.5 (4.0-5.0)	4.0 (4.0-4.8)	0.49
Platelet count (10^9/L)	244 (203-279)	263 (193-296)	0.80
AAR	0.66 (0.56-1.10)	0.86 (0.55-1.02)	0.88
API	1 (0-2)	4 (0-6)	0.006

HAI, histological activity index; AST, aspartate aminotransferase; ALT, alanine transaminase; AAR, aspartate aminotransferase to alanine transaminase ratio; API, age-platelet index; viral, biochemical and pathological parameters were presented as median values.

more advanced (p=0.004). HAI score was higher in patients with significant fibrosis (p<0.001). AAR score and AP index were higher in patients with significant fibrosis and this difference was statistically significant for API (p=0.006) *(Table 2)*.

The relationship between AAR, AP index, anti-HCV S/CO, and HCV-RNA viral load values and fibrosis and HAI scores was assessed with Spearman's correlation analysis. A moderately significant correlation was found between AP index and fibrosis and HAI scores (Spearman's rho=0.471, p=0.001 and Spearman's rho=0.470, p=0.001 respectively) *(Table 2)*.

The areas under the ROC curve were specified in order to determine the accuracy of serum anti-HCV S/CO, HCV-RNA viral load, AAR score and AP index of patients in detecting significant fibrosis (F≥2) and HAI≥5. The presence of significant fibrosis as a result of liver biopsy was accepted as a reference and sensitivity and specificity rates of HCV-RNA viral load, AAR score, and AP index were calculated.

While the areas under the ROC curve in prediction of F≥2 for anti-HCV S/CO and HCV-RNA viral load were 0.464 and 0.531 respectively AUROCs in prediction of HAI≥5 were 0.335 and 0.382 respectively. Sensitivity and specificity rates were 60% and 59.1% at HCV-RNA viral load cut-off value of 1.65X106 in prediction of F≥2 (p=0.57) *(Figure 1)*.

While the areas under the ROC curve in prediction of F≥2 for API and ARR were 0.713 and 0.506 respectively AUROCs in diagnosis of HAI≥5 were 0.579 and 0.512 respectively. Optimal cut-off values providing total maximum sensitivity and specificity rates and predicting F≥2 and HAI≥5 were given in Table 3 and ROC curves were given in Figure 2.

Discussion

For centuries, scientists have attempted to define thIn this study, we investigated the diagnostic performances of biochemical biomarkers such as AAR and API and viral markers such as anti-HCV and viral load in order to predict the presence and absence of significant fibrosis in patients with chronic HCV. The limitation of all indices developed up to now is that they cannot differentiate the fibrosis stages alone.[9] As a result of our study, AP index was more successful in predicting significant fibrosis (F≥2) compared to the AAR score. AP index had higher sensitivity and specificity. Sensitivity and specificity rates for optimal cut-off value of 2.5 for API in diagnosing significant fibrosis were 68.4% and 78.9% respectively. While the area under the ROC curve was 0.713 for API it was 0.506 for AAR. API can also predict the degree of significant histological activity level, but we found a more important correlation with significant fibrosis stage (p=0.03 and p=0.77 respectively). AP index was the sum of age and platelet count scores.[10] It has been asserted in various studies that AP index is a good one in predicting the stage of liver fibrosis. In a study on patients with HCV by Lackner et al., the area under the ROC curve was 0.740 in prediction of significant fibrosis and predicted fibrosis.[11]

Male gender, duration of the disease and acquiring the disease above the age of 40 are factors that can affect the rate of fibrotic progression in the liver.[10,12] Sensitivity to environmental factors (especially oxidative stress) increases with aging and a decrease occurs in blood flow, mitochondrial capacity or immune capacity. Annual rate of fibrosis regression in men especially between the ages of 61 and 70 was 300 times higher

Table 2. Assessment of the correlation between fibrosis and HAI scores and AAR, API and viral markers with Spearman's rho correlation analysis

	Fibrosis score Correlation		HAI Correlation	
	Coefficient	P value	Coefficient	P value
AAR	0.174	0.303	0.160	0.343
API	0.471	0.001	0.470	0.001
Anti-HCV S/CO	0.005	0.975	-1.00	0.545
HCV-RNA viral load	0.077	0.626	0.066	0.674

HAI, histological activity index; AAR, aspartate aminotransferase to alanine transaminase ratio; API, age-platelet index

Table 3. Performances of API and AAR in diagnosis of significant fibrosis and HAI

Category	Cut-off value	Sn,%	Sp,%	AUROC	95%CI	P value
F stage ≥2						
API	2.5	68.4	78.9	0.713	0.538-0.888	0.03
AAR	0.78	57.9	63.2	0.506	0.317-0.694	0.95
HAI ≥5						
API	1.5	56.7	71.4	0.579	0.384-0.773	0.77
AAR	0.79	50	57.1	0.512	0.281-0.743	0.74

F, fibrosis; HAI, histological activity index; Sn, sensitivity; Sp, specificity; AUROC, area under the ROC curve; AAR, aspartate aminotransferase to alanine transaminase ratio; API, age-platelet index

than in men between the ages of 21 and 40.[13] Thrombopoietin (TPO) is mainly produced in liver hepatocytes and promotes the production of platelets from megakaryocytes. Serum TPO levels in patients with chronic hepatitis and liver cirrhosis are negatively correlated with progression of fibrosis in the liver.[14] Decline in hepatic TPO production, increased splenic sequestration of platelets secondary to portal hypertension, and myelosuppressive effect of HCV are among the causes of thrombocytopenia.[15,16]

No significant correlation was found between AAR and liver fibrosis stage and inflammatory activity scores in our study. Moreover, AAR had a weak diagnostic (AUROC=0.506) accuracy for significant fibrosis. Guéchot et al. revealed that ROC curve analysis and AST to ALT ratio did not differentiate significant fibrosis (F≥2) (AUROC=0.531) in 590 treatment-naïve patients with chronic HCV and that they had only a very weak diagnostic accuracy for fibrosis (F≥3) (AUROC=0.584) or cirrhosis (F4) (AUROC=0.626), which is similar to the results of our study. They also found that AST to ALT ratio significantly increased with histological stage of liver fibrosis and that there was a significant correlation (r=0.129, p<0.0035) between METAVIR fibrosis stage and AST to ALT ratio.[17] No significant correlation was found between AAR and fibrosis stage in this study. It was reported that although liver fibrosis developed very slowly in most of HCVs which continuously had normal or near normal ALT levels within years a progress in liver fibrosis occurred in about 40% of asymptomatic patients with HCV infection and cirrhosis developed in a few patients with near normal ALT level.[18] Elevation of AST to ALT ratio in cirrhotic patients can be explained by the increase in serum AST levels due to reduction in AST clearance. Moreover, AST release from mitochondria and cytoplasm can increase as a result of mitochondrial damage in advanced liver disease.[19]

In this study, no significant difference was found between HCV viral load and anti-HCV S/CO levels and groups of F<2 and F≥2. In addition, no correlation was found between increasing fibrosis stage and histological activity

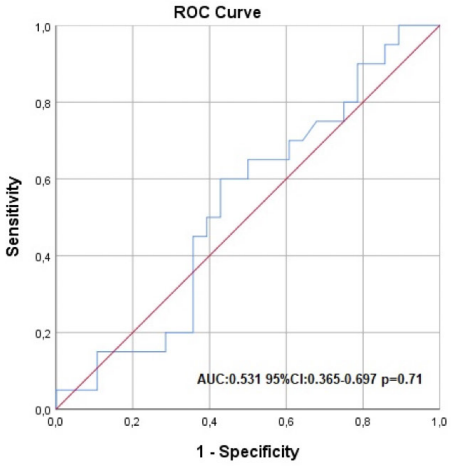

Figure 1. Receiver-operating characteristic (ROC) curve of HCV-RNA viral load for predicting the results of F ≥ 2 in 49 patients diagnosed with chronic hepatitis C.

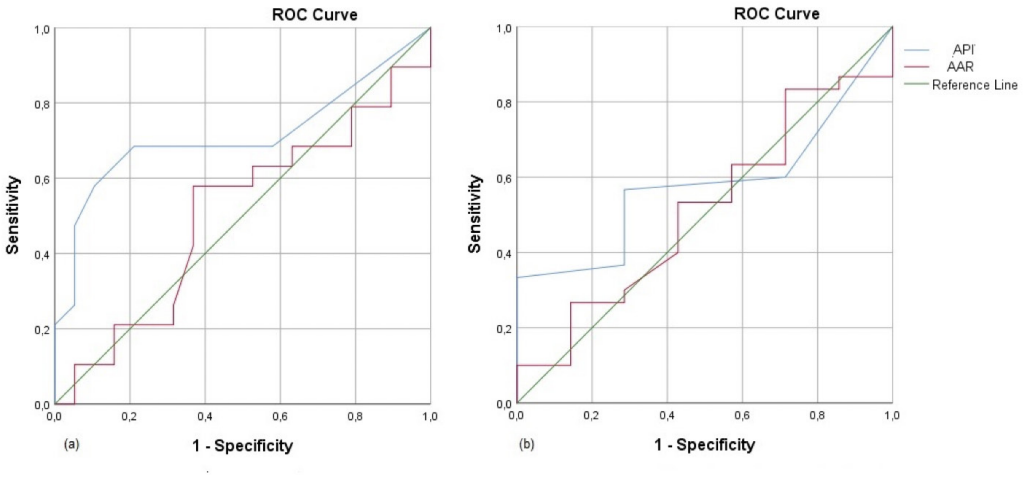

Figure 2. Receiver-operating characteristic (ROC) curve of API and AAR for predicting the results of (a) F≥2 and (b) HAI≥5 in 49 patients diagnosed with chronic hepatitis C.

scores. Similarly, there are studies revealing no correlation between histological result and HCV-RNA levels.[20–22] However, it was asserted in some study reports that viral titer may affect the severity of liver damage and that high viremia titer was associated with severe liver damage.[20–23] These different results can be associated with the truth that serum HCV-RNA viral load shows a fluctuation and is an unstable parameter.[24] Moreover, HCV is known to increase in both liver and areas outside of liver. Therefore, high HCV in circulation does not always mean an active viral replication in the liver or indicate a more severe liver disease.[25,26] The most important limitation of this study is that the number of patients included in the study is limited.

In conclusion, AP index, AAR score and HCV viral load, which are non-invasive markers, assessed in this study were useful in predicting significant fibrosis in chronic HCV. Especially API was the most useful test in predicting significant fibrosis. AP index can be preferred in patients with near normal ALT values.

Conflict of interest

The authors declared that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Bonino F, Oliveri F, Colombatto P, Coco B, Mura D, Realdi G, Brunetto MR. Treatment of patients with chronic hepatitis C and cirrhosis. J Hepatol. 1999;31 Suppl 1:197-200. doi: 10.1016/s0168-8278(99)80401-2.
2. Kaserer K, Fiedler R, Steindl P, Müller CH, Wrba F, Ferenci P. Liver biopsy is a useful predictor of response to interferon therapy in chronic hepatitis C. Histopathology. 1998 May;32(5):454-61. doi: 10.1046/j.1365-2559.1998.00413.x.
3. Davis GL, Lau JY. Factors predictive of a beneficial response to therapy of hepatitis C. Hepatology. 1997 Sep;26(3 Suppl 1):122S-127S. doi: 10.1002/hep.510260721.
4. Wong JB, Bennett WG, Koff RS, Pauker SG. Pretreatment evaluation of chronic hepatitis C: risks, benefits, and costs. JAMA. 1998 Dec 23-30;280(24):2088-93. doi: 10.1001/jama.280.24.2088.
5. Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl J Med. 2001 Feb 15;344(7):495-500. doi: 10.1056/NEJM200102153440706.
6. Cheung RC, Currie S, Shen H, Bini EJ, Ho SB, Anand BS, Hu KQ, Wright TL, Morgan TR; VA HCV-001 Study Group. Can we predict the degree of fibrosis in chronic hepatitis C patients using routine blood tests in our daily practice? J Clin Gastroenterol. 2008 Aug;42(7):827-34. doi: 10.1097/MCG.0b013e318046ea9a.
7. Bonacini M, Hadi G, Govindarajan S, Lindsay KL. Utility of a discriminant score for diagnosing advanced fibrosis or cirrhosis in patients with chronic hepatitis C virus infection. Am J Gastroenterol. 1997 Aug;92(8):1302-4. PMID: 9260794.
8. Hsieh YY, Tung SY, Lee K, Wu CS, Wei KL, Shen CH, Chang TS, Lin YH. Routine blood tests to predict liver fibrosis in chronic hepatitis C. World J Gastroenterol. 2012 Feb 28;18(8):746-53. doi: 10.3748/wjg.v18.i8.746.
9. European Association for Study of Liver; Asociacion Latinoamericana para el Estudio del Higado. EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. J Hepatol. 2015 Jul;63(1):237-64. doi: 10.1016/j.jhep.2015.04.006.
10. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet. 1997 Mar 22;349(9055):825-32. doi: 10.1016/s0140-6736(96)07642-8.
11. Lackner C, Struber G, Liegl B, Leibl S, Ofner P, Bankuti C, Bauer B, Stauber RE. Comparison and validation of simple noninvasive tests for prediction of fibrosis in

chronic hepatitis C. Hepatology. 2005 Jun;41(6):1376-82. doi: 10.1002/hep.20717.
12. Marcellin P, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. Hepatology. 2002 Nov;36(5 Suppl 1):S47-56. doi: 10.1053/jhep.2002.36993.
13. Poynard T, Bedossa P. Age and platelet count: a simple index for predicting the presence of histological lesions in patients with antibodies to hepatitis C virus. METAVIR and CLINIVIR Cooperative Study Groups. J Viral Hepat. 1997 May;4(3):199-208. doi: 10.1046/j.1365-2893.1997.00141.x.
14. Massard J, Ratziu V, Thabut D, Moussalli J, Lebray P, Benhamou Y, Poynard T. Natural history and predictors of disease severity in chronic hepatitis C. J Hepatol. 2006;44(1 Suppl):S19-24. doi: 10.1016/j.jhep.2005.11.009.
15. Peck-Radosavljevic M. Hypersplenism. Eur J Gastroenterol Hepatol. 2001 Apr;13(4):317-23. doi: 10.1097/00042737-200104000-00004.
16. Dai CY, Ho CK, Huang JF, Hsieh MY, Hou NJ, Lin ZY, Chen SC, Hsieh MY, Wang LY, Chang WY, Yu ML, Chuang WL. Hepatitis C virus viremia and low platelet count: a study in a hepatitis B & C endemic area in Taiwan. J Hepatol. 2010 Feb;52(2):160-6. doi: 10.1016/j.jhep.2009.11.017.
17. Guéchot J, Boisson RC, Zarski JP, Sturm N, Calès P, Lasnier E; ANRS HCEP 23 Fibrostar Group. AST/ALT ratio is not an index of liver fibrosis in chronic hepatitis C when aminotransferase activities are determinate according to the international recommendations. Clin Res Hepatol Gastroenterol. 2013 Nov;37(5):467-72. doi: 10.1016/j.clinre.2013.07.003.
18. Fabris C, Smirne C, Toniutto P, Colletta C, Rapetti R, Minisini R, Falleti E, Pirisi M. Assessment of liver fibrosis progression in patients with chronic hepatitis C and normal alanine aminotransferase values: the role of AST to the platelet ratio index. Clin Biochem. 2006 Apr;39(4):339-43. doi: 10.1016/j.clinbiochem.2006.01.011.
19. Okuda M, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, Weinman SA. Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. Gastroenterology. 2002 Feb;122(2):366-75. doi: 10.1053/gast.2002.30983.
20. Fouad SA, Esmat S, Omran D, Rashid L, Kobaisi MH. Noninvasive assessment of hepatic fibrosis in Egyptian patients with chronic hepatitis C virus infection. World J Gastroenterol. 2012 Jun 21;18(23):2988-94. doi: 10.3748/wjg.v18.i23.2988.
21. Lee YS, Yoon SK, Chung ES, Bae SH, Choi JY, Han JY, Chung KW, Sun HS, Kim BS, Kim BK. The relationship of histologic activity to serum ALT, HCV genotype and HCV RNA titers in chronic hepatitis C. J Korean Med Sci. 2001 Oct;16(5):585-91. doi: 10.3346/jkms.2001.16.5.585.
22. Saleem N, Mubarik A, Qureshi AH, Siddiq M, Ahmad M, Afzal S, Hussain AB, Hashmi SN. Is there a correlation between degree of viremia and liver histology in chronic hepatitis C? J Pak Med Assoc. 2004 Sep;54(9):476-9. PMID: 15518372.
23. Anand BS, Velez M. Assessment of correlation between serum titers of hepatitis C virus and severity of liver disease. World J Gastroenterol. 2004 Aug 15;10(16):2409-11. doi: 10.3748/wjg.v10.i16.2409.
24. Zeuzem S, Schmidt JM, Lee JH, Rüster B, Roth WK. Effect of interferon alfa on the dynamics of hepatitis C virus turnover in vivo. Hepatology. 1996 Feb;23(2):366-71. doi: 10.1002/hep.510230225.
25. Müller HM, Pfaff E, Goeser T, Kallinowski B, Solbach C, Theilmann L. Peripheral blood leukocytes serve as a possible extrahepatic site for hepatitis C virus replication. J Gen Virol. 1993 Apr;74 (Pt 4):669-76. doi: 10.1099/0022-1317-74-4-669.
26. Ballardini G, Manzin A, Giostra F, Francesconi R, Groff P, Grassi A, Solforosi L, Ghetti S, Zauli D, Clementi M, Bianchi FB. Quantitative liver parameters of HCV infection: relation to HCV genotypes, viremia and response to interferon treatment. J Hepatol. 1997 Apr;26(4):779-86. doi: 10.1016/s0168-8278(97)80242-5.