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The predictive role of AST level, prothrombin time, and platelet count in the detection of liver fibrosis in patients with chronic hepatitis C

Aim: We aimed to assess the predictive value of a combination some of basic serum biochemical markers for the diagnosis of clinically significant fibrosis in chronic hepatitis C.

Materials and Methods: Blood samples from 68 patients with chronic hepatitis C infection were analyzed for aspartate aminotransferase, prothrombin INR, and platelet count. The liver histopathology was evaluated using the Knodell protocol. The relationship between the serum biochemical markers and severity of the stages were examined.

Results: A strong association was found between the fibrosis stage and platelet count and GUCI ($P < 0.001$, $P = 0.035$ respectively) but not APRI ([AST/ULN] / Platelet count [×109 /L]) × 100) ($P = 0.052$). The relationship between the platelet count <147 × 109 /L and the age, which is greater than 50, and severe fibrosis was significant (AUC = 0.779, $P < 0.001$). In this cut off value the negative predictive value (NPV) and the positive predictive value (PPV) were 66.7% and 86.7%, respectively. An index GUCI (Göteborg University Cirrhosis Index) was calculated using the AST, platelet count, and prothrombin-INR. Using the cut of value 0.26, the sensitivity of this index was found as 58.33% and the specificity 72.7% for diagnosis of severe fibrosis (stage 3-4); NPV and PPV were 76.2% and 53.8%, respectively.

Conclusion: Peripheral platelet count and GUCI can discriminate to some degree of accuracy patients with severe fibrosis (stage 3-4) from those without severe fibrosis (stage 0-2).

Key words: Chronic hepatitis C, fibrosis, serum biochemical markers

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Kronik hepatit C'li hastalarda karaciğer fibrozisinin saptanmasında AST seviyesi, protrombin zamanı, ve trombosit sayısının belirleyici rolü

Amaç: Kronik hepatit C'de bazı temel serum biyokimyasal testlerinin şiddetli fibrozisi tanımlamada doğruluk derecelerini değelendirmeyi amaçladık.

Yöntem ve gereç: Kronik hepatit C enfeksiyonlu 68 hastadan kan örnekleri aspartat minotransferaz, protrombin zamanı ve trombosit sayısı için analiz edildi. Karaciğer histopatolojisi Knodell protokolü kullanılarak değerlendirildi. Serum biyokimyasal belirleyicileri ve stage şiddeti arasındaki ilişki araştırıldı.

Bulgular: Fibrozis derecesi ile trombosit sayısı ve GUCI arasındaki ilişki anlamlı bulundu ($P < 0,001$, $P = 0,035$, sırayla), ancak APRI ile ([AST/ULN] / Trombosit sayısı [×109 /L]) × 100) ($P = 0.052$) anlamlı bulunmadı. Trombosit sayısının <147 × 109 /L olması ve yaşın 50'nin üzerinde olması ile ciddi fibrozis arasındaki ilişki anlamlı bulundu (AUC = 0.779, $P < 0.001$). Bu eşik değerde pozitif prediktif değer ve negatif prediktif değer sırayla % 66,7 and % 86,7 olarak belirlendi. GUCI indeksi (Göteborg University Cirrhosis Index) AST, trombosit sayısı ve protrombin zamanı kullanılarak hesaplandı. Eşik değer bu indeks için 0.26 alındığında testin şiddetli fibrozisi saptamada sensitivitesi % 58,33, spesifisitesi % 72,7, negatif prediktif değeri ve pozitif prediktif değeri sırayla % 76,2 and % 53,8 bulundu.

Sonuç: Periferik trombosit sayısı ve GUCI şiddetli fibrozisli hastaları (stage 3-4), fibrozisi şiddetli olmayanlardan (stage 1-2) belirli bir doğruluk derecesinde ayırt ettirebilir.

Anahtar sözcükler: Kronik hepatit C, fibrozis, serum biyokimyasal belirleyicileri

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Introduction

One of the most important causes of chronic liver disease is hepatitis C virus. It is estimated that overall 200 million people are influenced by this virus (1,2). It leads to cirrhosis at the rate of 20% and to hepatocellular carcinoma at the rate of 5%. Although pegylated interferon and ribavirin combination therapy produces permanent virological response in over 50% of the cases, this is quite an expensive treatment with important side effects. Moreover, only a small proportion of the patients is eligible for treatment or has access to treatment (3). Therefore, establishing the indications for treatment accurately is important. Although HCV genotype is one of the most important factors determining the outcome of treatment (4), the presence of severe fibrosis at the onset of treatment is another factor with unfavourable effect on treatment. In order to establish prognosis and guide treatment, liver fibrosis should be staged accurately (5). Liver biopsy is the gold standard for staging. Unfortunately, because of the facts that this procedure is invasive, it has complications, sampling may be made inaccurately, pathological interpretations are equivocal, and patients are unwilling to undergo repeated biopsies following the course of disease restricts its use (6,7).

Therefore, many investigators have been trying to find noninvasive alternatives for the evaluation of hepatic fibrosis, one of which is transient elastography (FibroScan) (8). However, as the use of this technology is expensive and its use is limited to certain locations, investigations have focused on serum biochemical tests, attempting to find direct and indirect markers of liver fibrosis (9,10). Some of the direct markers were combined in a panel, such as FibroSpect, FibroMeter, HepaScore, and European liver fibrosis test (1). Yet, these tests are neither cheap nor available in many clinics. However, indirect markers of fibrosis, such as aspartate aminotransferase and the platelet count, are easily available. So far, many laboratory tests, scores, and indexes for indirect markers have been proposed for noninvasive estimation of hepatic fibrosis in cases with chronic HCV (11).

To this end, several indexes, such as aspartate aminotransferase:alanine aminotransferase ratio (AST/ALT), cirrhosis discriminant score (CDS), the ratio of age to platelet (A/P), platelet count per se, Pohl score, the ratio of AST to platelet (APRI), the ratio of the multiplication of AST with prothrombin-international normalized ratio (INR) to the count of platelet, as known Göteborg University Cirrhosis Index (GUCI), have been developed based upon routine laboratory tests. Therefore, they can be used easily in clinical practice. In the majority of the studies on the subject, it has been reported that these tests have acceptable accuracy in predicting severe fibrosis, bridging necrosis, and cirrhosis. However, when the these tests were evaluated in different study populations, different outcomes were found.

The aim of the present study was to establish the value of platelet count per se, APRI, and GUCI index in predicting severe fibrosis in patients with chronic hepatitis C.

Materials and methods

Sixty-eight chronic hepatitis C patients who were admitted to the infectious diseases unit in our hospital between 2005 and 2008 were included in this study. All patients were tested positive for the presence of HCV RNA using a polymerase chain reaction assay (Cobas Amplicor HCV monitor; Roche Diagnostics, Branchburg, N.J, USA), all tested positive for anti-HCV antibodies, and they all hed increased serum alanine aminotransferase (ALT) and aspartat amino transferase (AST) values. None of the patients demonstrated additional causes of chronic liver diseases confirmed by standard clinical, serological, biochemical, and radiological criteria. Additional exclusion criteria were antiviral treatment before liver biopsy, alcohol consumption in excess of 20g/d, gastrointestinal bleeding in medical history, and previous liver transplantation. To exclude the impact of hypersplenism, patients with splenomegaly on ultrasonographic evaluation and/or pancytopenia were not included in the study.

The serum levels of ALT were studied by an autoanalyser (Roche Cobas Integra 800, Roche Diagnostics GmbH, Switzerland), platelet counts were studied by a complete blood count analyzer (Sysmex 2100, Roche diagnostic GmbH, Sysmex, Switzerland), and prothrombin –INR were studied with a coagulameter (Dade Behring BCS, Siemens Healthcare Diagnostics Inc, USA). From these routine laboratory values, APRI and GUCI were calculated exactly as originally described (11, 12).

APRI = ([AST/Upper Limits of Normal (ULN)] / Platelet count [$\times 10^9$ /L]) × 100

GUCI = Normalized ASTX prothrombin-INR × 100 / Platelet count ($\times 10^9$/L)

Liver tissue studies

After obtaining informed written consents, percutaneous liver biopsy samples were obtained using tru-cut needles. For routine histological examination, all samples were fixed in 10% buffered formalin, embedded in paraffin, and stained with haematoxylin-eosin, trichrome, and Van-gieson. Liver tissues obtained from chronic viral hepatitis were scored as minimal (1–4), mild (5–8), moderate (9–12), severe (13–18), and 0–4 according to the activity and fibrotic index, respectively, using the classification of chronic viral hepatitis described by Knodell et al. (13).

Statistical methods

Statistical methods: MedCalc version 9.6.4 was used for statistical analyses. Normality assumption was checked by Shapiro Wilk test and Independent sample t test was used to test statistically significant differences between mean platelet values of stage groups. Differences between stage groups according to GUCI and APRI were evaluated using the Mann-Whitney U test. Cut off value for stage degree was investigated by ROC analysis. Moreover, to show efficiency of the cut off value for diagnosis, which classifies the degree of stage into 2 distinct groups (0-2 and 3-4), sensitivity, specificity, NPV, and PPV statistics and their confidence intervals were calculated. Mean ± SD was used for descriptive statistic of platelet and median (min-max) values were used for descriptive statistics of APRI and GUCI. The significance level was set at 0.05.

Results

Overall 68 (26 male and 42 female) chronic hepatitis C cases were included in the study. Mean age of the cases was 52.6 ± 9.647 (27-74). Forty four cases were in stage 0-2 and 24 cases in stage 3-4. Results of the univariate analysis that were used to determine the relation between stage and thrombocyte and GUCI and APRI are shown in Table 1. In patients with stage 3-4, platelet count was found to be significantly lower ($P < 0.001$) and GUCI significantly high ($P = 0.035$), while no significant difference was found between APRI and stage ($P = 0.052$). Then the cut off points that may be of use in differentiating low stages from high ones were determined for platelet and GUCI. Sensitivity, specifity, NPV, and PPV values and significance of these cut off points are presented in Table 2. When determining cut off points for platelet, age was taken into consideration and in those older than 50, a significant relation was found between the platelet value of 147×10^9/L or lower and higher stage ($AUC = 0.779$, $P < 0.001$). The relationship between platelet count, age, and the stage of fibrosis is shown in Figure 1 and ROC curve of the platelet count for severity of fibrosis is shown in Figure 2. In addition, cut off value for GUCI was established as 0.26 and values over this were found to be significantly related to stage ($AUC = 0.665$, $P = 0.031$). Box-plot chart of the relationship between GUCI and fibrosis stage is presented in Figure 3 and ROC curve of the GUCI for severity of fibrosis is presented in Figure 2.

Discussion

In the present study, statistical significance of the accuracy of APRI, GUCI and platelet count in predicting the degree of liver fibrosis in chronic

Table 1. The difference between platelet count, GUCI, APRI, and stage of fibrosis in chronic hepatitis C patients.

	Stage		P
	0-2	3-4	
Patients (n)	44	24	
Platelet count (10^9/L)	204318.18 ± 53953.37	152291.66 ± 33932.77	<0.001
GUCI	0.199 (0.066-1.178)	0.267 (0.111-3.680)	0.035
APRI	0.006 (0.0003-0.0780)	0.0081 (0.0033-0.5700)	0.052

Mean ± SD was used for descriptive statistic of platelet and median (min-max) values were used for descriptive statistics of APRI and GUCI .

Table 2. Cut-off values that were found for platelet count and GUCI, the sensitivity, specificity, AUC, PPV, NPV, and significance of these tests.

	Cut off	Sensitivity (%)	Specificity (%)	AUC	PPV (%)	NPV (%)	P
Platelet (Age >50 years)	147X10^9/L	61.90 (38.5-81.8)	88.89 (65.2-98.3)	0.779	86.7	66.7	<0.001
GUCI	0.261	58.33 (36.7-77.9)	72.73 (57.2-85.0)	0.655	53.8	76.2	0.031

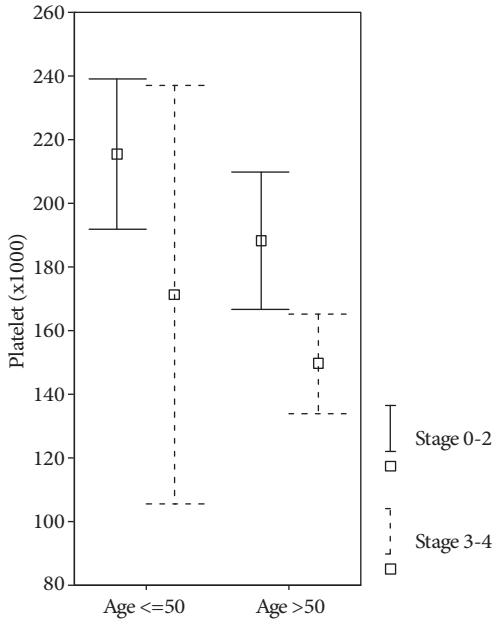

Figure 1. The difference between platelet count, age, and stage of fibrosis.

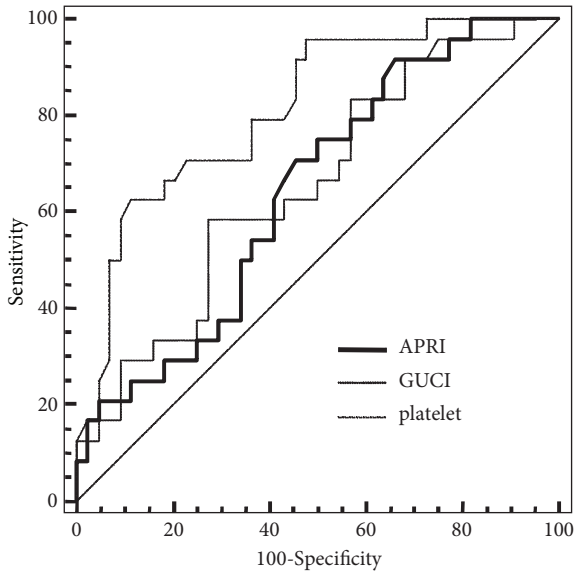

Figure 2. ROC curve of the platelet count, GUCI, and APRI for severity of fibrosis.

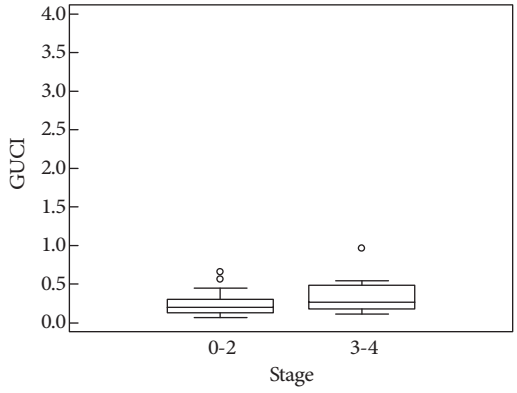

Figure 3. Box-plot graph of the difference between GUCI and fibrosis stage.

hepatitis C cases was evaluated. Platelet count per se and GUCI were found to be valuable in prediction while APRI score was not found to be significant.

Since fibrosis is the most important factor influencing mortality and morbidity in liver diseases, careful evaluation of fibrosis is necessary for early and judicious treatment. Liver biopsy, which is an invasive method, is still the gold standard in the diagnosis of liver fibrosis. However, there are many problems with the use of this method. Therefore, studies attempting to determine the severity of liver diseases have focused on noninvasive reproducible methods.

In recent studies investigating the relationship between platelet count and the severity of fibrosis in CHC, it has been reported that peripheral platelet count (PPC) reflects the severity of fibrosis in cases with chronic hepatitis C (14-17). Similarly, it was established in the present study that the decrease in the number of platelets was related to increasing degree of fibrosis.

In the study of Islam et al. (17) it was emphasized that when cut off value was taken as 190×10^9 /L for platelet value irrespective of age, majority of cases under this value had advanced fibrosis and cirrhosis. Guannani et al. (18) reported that platelet count has high diagnostic accuracy in predicting cases with cirrhosis at a cut-off point of 130×10^9 /L. In the study of Karasu et al. (16) no cut off point could be determined for platelet and only PPC values were reported to correlate negatively with age and severity of fibrosis. In the present study, unlike the others, age was taken into account when determining the relation between the platelet count and the severity of fibrosis. In those aged over 50, the relation between platelet value of 147×10^9 /L or lower and high stage was found to be significant. PPV of platelet count at this cut off value was quite good (86.7%), suggesting that the cut off value established had significant accuracy in predicting severe fibrosis.

As cases without splenomegaly were included in this study, the probability of this factor being cause of thrombopenia was ruled out from the beginning. Although thrombopenia was first correlated with an increase in the autoantibodies to thrombocytes in chronic hepatitis C cases, in a more recent study it was demonstrated that thrombocytopenia could occur in both CHB and CHC cases independent of etiology.

In another study, it was reported that in cases with chronic hepatitis C, there is difference in thrombocyte activation, which may partly account for thrombopenia (19). However, at present it is thought that the cause of thrombopenia in chronic hepatitis is the decrease in thrombopoietin and other humoral factors (they regulate megakaryocyte maturation and thrombocyte production) resulting from decreased hepatocyte mass accompanied by increased fibrosis secondary to progressive liver damage (20,21).

Even though Wai et al. (22), who investigated the relation between the severity of fibrosis and APRI score and formulated as the ratio of AST to the platelet count, reported its accuracy to be high in predicting severe fibrosis and cirrhosis. In the study of Islam et al. (17), as cited by Shahen and Myers in their systematic review (1), NPV of this test yielded better results than PPV and PPV yielded suboptimal values (PPV 59%) only in units where these cases are observed frequently. In the present study, although APRI was established to be higher in severe fibrosis, the relation was not found to be significant. This may be due to the fact that the number of our patients was low and we had a different patient population.

Blood coagulation factors apart from factor VIII are all produced by hepatocytes. Their serum half lives are much shorter than that of albumin. Owing to their rapid degradation, the measurement of coagulation factors is one of the best indicators of liver synthesis function and is beneficial for both diagnosing liver parenchymal disease and evaluating its prognosis. The most suitable measurement for this purpose is serum prothrombin time, which provides information on factors II, V, VII, and X (20). Islam et al. (17) utilised combined prothrombin time (prothrombin -INR) with APRI, and with the resulting index (GUCI) observed improvement in sensitivity, specificity, NPV, PPV, and ROC values of the test. These investigators stated that biopsy should not be carried out in those with GUCI score of <0.2 or >4. With these cut off values, patients without bridging fibrosis or having cirrhosis could be distinguished. Another study supporting the benefit of GUCI in determining the severity of fibrosis and predicting the outcome of treatment in chronic hepatitis C is that of Westin et al. (12). These investigators demonstrated that under the cut off value of 0.33, severe fibrosis and cirrhosis are not present. Similarly, in the present study it was observed that GUCI was useful in determining the severity of liver fibrosis. Consistent with the results of Islam et al., GUCI values over 0.26 were found to be significantly correlated with high stage. When this cut off value was considered, NPV was 76.2% and specificity was 72.7%, whereas PPV and sensitivity values were lower. For this reason we considered that GUCI was a good parameter for exclusion of the severe fibrosis. PPV value was found to be 53.8% and this value is a suboptimal one for PPV. It is thought that PPV value could be improved with different cut off values that could be determined by increasing the number of cases.

In conclusion, in the present study, it was established in chronic hepatitis C cases that the decrease in the number of platelets along with age was important in determining the severity of liver fibrosis. We thought that development of thrombopenia is independent of splenic enlargement or hypersplenism. In addition, it was also seen that APRI

on its own was not adequate in determining the severity of liver fibrosis and that GUCI score obtained by the addition of prothrombin time to APRI might help to determine the severity of fibrosis in untreated cases. Such scores obtained through biochemical parameters used in clinical routine practice may obviate the need for liver biopsy in CHC cases. However, such approaches are used infrequently in clinical practice. This may be related to the fact that in various studies diagnostic accuracy of the tests was not considered adequate. It is our suggestion that further studies with larger and more homogenous patient groups should be carried out for wider use of similar practices.

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