

Research Article

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Re-determining the cut-off points of FIB-4 for patients monoinfected with chronic hepatitis B virus infection

Kronik hepatit B virüsü ile monoenfekte hastalarda FIB-4 kesim noktalarının yeniden belirlenmesi

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Abstract

Objective: This study aimed to determine significant liver fibrosis and cirrhosis with different FIB-4 cut-off points, and the need for liver biopsy (LB) by optimizing the initially established cut-off points of 1.45 and 3.25.

Materials and methods: The study included 201 patients monoinfected with chronic HBV. METAVIR classification was used to determine the stage of fibrosis. ROC analysis and the Youden index were performed to define the optimum cut-off points.

Results: A FIB-4 cut-off point of 1.45 and 1.62 generated Youden indexes of 0.51 and 0.55, the accuracy of 78.6%

and 81.1% for significant liver fibrosis, respectively. The FIB-4 cut-off was set at 2.40 and 3.25 Youden indexes were 0.46 and 0.16, accuracies were 79.6% and 69.7% for significant liver fibrosis, respectively. A cut-off point of 1.45 and 1.62 for FIB-4 generated Youden indexes of 0.62 and 0.66, the accuracies of 81.6% and 84.1% for cirrhosis, while the FIB-4 cut-off point of 2.40 and 3.25 generated Youden indexes of 0.59 and 0.22, with the accuracies of 90% and 84.1% for cirrhosis, respectively.

Conclusions: The FIB-4 cut-off points of 1.62 and 2.40 have higher accuracy and may decrease the need for LB 12% more than the initially established ones in HBV monoinfected patients.

Keywords: Fibrosis; FIB-4; HBV; Liver biopsy; Cut-off point.

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Özet

Amaç: Bu çalışmada farklı FIB-4 kesim noktaları ile belirgin karaciğer fibrozisi ve sirozun tespiti ve başlangıçta belirlenen kesim noktalarının (1.45 ve 3.25) optimize edilmesi ile karaciğer biyopsisine olan gereksinimi belirlemek amaçlandı.

Gereç ve Yöntemler: Çalışma kronik HBV ile mono-enfekte 201 hasta içermektedir. Fibrozis evresini belirlemek için METAVIR sınıflandırması, optimum kesim noktalarını belirlemek için ise ROC analizi ve Youden indeksi kullanıldı.

Bulgular: Belirgin karaciğer fibrozisi için 1.45 ve 1.62 FIB-4 kesim noktalarının Youden indeksleri sırasıyla 0.51 ve 0.55 iken, doğrulukları sırasıyla %78.6 ve %81.1 idi. FIB-4 kesim noktaları 2.40 ve 3.25 olduğunda ise Youden indeksleri sırasıyla 0.46 ve 0.16 iken, doğrulukları sırasıyla %79.6 ve %69.7 idi. Ancak, siroz için 1.45 ve 1.62 FIB-4 kesim

noktaları, sırasıyla 0.62 ve 0.66 Youden indeksine, ayrıca %81.6 ve %84.1 doğruluğa sahip iken, 2.40 ve 3.25 FIB-4 kesim noktaları sırasıyla 0.59 ve 0.22 Youden endeksi ile %90 ve %84.1 doğruluğa sahiptir.

Sonuçlar: 1.62 ve 2.40 FIB-4 kesim noktaları HBV mono enfekte hastalarda daha yüksek doğruluğa sahiptir ve başlangıçta belirlenen kesim noktalarına göre karaciğer biyopsisi gereksinimini %12 oranında daha fazla azaltabilir.

Anahtar Kelimeler: Fibrozis; FIB-4; HBV; Karaciğer biyopsisi; Kesim noktası.

Introduction

Hepatitis B virus (HBV) infection is a common disease in the general population and is becoming a significant global public health problem. As patients with significant liver fibrosis are at very high risk of complications, accurate classification of the fibrosis stage, which is prognostic and provides information on the likelihood of disease progression and response to treatment, is crucial in clinical practice [1, 2]. However, the lack of accurate, reproducible and easily applied non-invasive methods for the assessment of liver fibrosis still remains as a major limitation.

Liver biopsy (LB) is currently the imperfect gold standard diagnostic tool for the staging of liver fibrosis [3]. However, it is interesting that many gastroenterologists shy away from performing LB, citing the high rate of complications, due to well-documented drawbacks, poor acceptance by the patient, high cost, limitations on the reliability of the histological information obtained, difficulties in performing repeated assessments and very occasionally, death [4, 5]. Due to the perceived risk, discomfort and patient reluctance to have LB, recent interest and many studies have led to the development of non-invasive, simple, cheap and accurate methods and indexes as alternatives to LB for the assessment of liver fibrosis and cirrhosis. FIB-4 is one of these indexes and calculated by the measurement of AST, ALT and platelet count by the combination with age. Published studies about the fibrosis index based on four factors have generally used the 1.45 and 3.25 as cut-off points to calculate accuracy, sensitivity, specificity and the predictive values to discriminate between patients with and without significant liver fibrosis and/or cirrhosis. However, the use of different cut-off points may change the diagnostic test performance characteristics. Therefore, selected cut-offs are critical to decide correctly [1, 6, 7].

In this study, a retrospective analysis was made in well-characterized chronic HBV (CHB) monoinfected carriers to determine the diagnostic performance of FIB-4 index in the prediction and exclusion of significant liver fibrosis and cirrhosis by changing the initially determined cut-off points, and to define the optimized cut-off points in CHB infection patients to thereby reduce the need for LB.

Materials and methods

Study population

Approval for the study was granted by the Local Ethics Committee of Haydarpaşa Numune Education and Research Hospital (2013/281). The study was conducted in accordance with the Helsinki II Declaration. This retrospective study included patients who underwent LB in 2010 and 2011 at the Department of Gastroenterohepatology of Haydarpaşa Numune Training Hospital. Written informed consent was obtained from all patients at the time of the LB. The confidentiality of all personal data was protected. All data collected for this study were part of a series of examinations which are routinely performed on patients chronically infected with HBV.

Of the 219 HBV carriers, 14 patients were excluded from the study due to insufficient data. Thus, the data of 205 percutaneous LB performed and serologically confirmed in chronic HBV-monoinfected patients were included in this retrospective study, but four of these 205 patients were excluded at the data analysis step due to the outlier results for FIB-4. Data were reviewed from the database of HBV-monoinfected patients attending the Department of Gastroenterology of Haydarpaşa Numune Education and Research Hospital to determine the stage of liver fibrosis by non-invasive biochemical markers and indexes. The following criteria were used to select the cases: (i) CHB defined by: HBsAg positivity for more than 6 months, detectable HBV-DNA with a level of $>10^5$ copies/mL, persistent or intermittent elevation of ALT levels and LB-proven chronic hepatitis; (ii) no previous or concomitant anti-HBV therapy; (iii) available LB results allowing a confident scoring according to the METAVIR score; (iv) laboratory test results allowing the calculation of FIB-4 obtained within 1 week from the date of LB; (v) absence of liver co-morbidity including hepatitis delta super infection, HCV co-infection, HIV co-infection, chronic alcohol consumption higher than 30 g/day for males and 20 g/day

for females, haemochromatosis, Wilson's disease, alpha-1-antitrypsin deficiency, auto-immune hepatitis, immune suppression, non-alcoholic steatohepatitis or hepatocellular carcinoma.

Laboratory parameters

AST and ALT measurements were performed using the enzymatic colorimetric method with an Olympus AU2700 autoanalyzer and commercially available reagents from Olympus Diagnostics (Beckman Coulter, USA). Platelet count was determined with ABX Pentra XL 80 (Horiba Medical, USA). HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc total, anti-HBc IgM, anti-HCV and anti-HIV were measured with the ADVIA Centaur XP Immunoassay System (Siemens, Germany). The serum HBV-DNA level was detected with a polymerase chain reaction system (ABI7300; Applied Biosystems, Foster City, CA, USA). FIB-4 was calculated according to the following formula of age (years) \times AST (U/L) / [Platelet count (10^9 /L) \times (ALT)^{1/2} (U/L)] [6].

Liver histology

Hepatic tissue was obtained using an 18-gauge automated core biopsy needle. A single senior pathologist (SO) interpreting all the biopsy specimens was blinded to the clinical history and laboratory results of the cases involved. Liver biopsies were scored using the METAVIR scoring system for fibrosis stage [8]. Fibrosis was scored on a 5-point scale: F0, no fibrosis; F1, portal fibrosis only; F2, portal fibrosis with occasional septa; F3, portal fibrosis with many septa; F4, cirrhosis. Significant liver fibrosis was defined as fibrosis stage \geq F2.

Statistical analysis

All statistical analyses were performed using SPSS for Windows v. 15 software (SPSS Inc., Chicago, IL, USA). Demographic and histological features were classified as continuous or categorical variables. Kolmogorov-Smirnov analysis was used to test for Gaussian distribution. Spearman's correlation analysis was used to show the amount of relationship between FIB-4 and fibrosis stages. Based on the ROC analysis and Youden index which defines the maximum potential effectiveness of a biomarker, the best cut-off points to predict or to exclude significant liver fibrosis and cirrhosis were selected. All

of the reported p-values were two-tailed, and a value of $p < 0.05$ was considered statistically significant.

Results

Study population and histology

The mean age was 39 ± 16 years and 128 (63%) of the patients were male. The following distributions of METAVIR fibrosis stages were observed on LB: No fibrosis in 75 of 201 (F0; 37%); portal fibrosis only in 53 of 201 (F1; 26%); portal fibrosis with occasional septa in 19 of 201 (F2; 10%); portal fibrosis with many septa in 13 of 201 (F3; 7%); and cirrhosis in 41 of 201 (F4; 20%). The overall mean FIB-4 value increased as a function of the fibrosis stage, ranging from 0.28 to 8.34.

Correlation between FIB-4 and fibrosis stage

To evaluate whether FIB-4 was suitable for the staging of liver fibrosis in chronic HBV patients, the correlation was analysed between the FIB-4 and the METAVIR fibrosis stages. A relatively high correlation was observed between FIB-4 and fibrosis stages ($r = 0.615$, $p < 0.001$).

Determination of the optimal FIB-4 cut-off points for predicting or excluding significant liver fibrosis and cirrhosis

We considered whether new cut-off points for FIB-4 index could be determined on the basis of the current data set. ROC curve analysis was performed to determine the different fibrosis levels of interest for FIB-4, then Youden index was used to determine the optimum cut-off points with the highest sensitivity and specificity. This index (sensitivity + specificity - 1) was calculated for several cut-off points and the maximum value was determined as the optimum cut-off point.

A FIB-4 cut-off point of 1.62 generated Youden index of 0.55 and accuracy of 81.1% for significant liver fibrosis. Likewise, when the FIB-4 cut-off was set at 2.40 Youden index of 0.46 and accuracy of 79.6% have been obtained for significant liver fibrosis. A cut-off point of 1.62 for FIB-4 generated Youden index of 0.66 and accuracy of 84.1% for cirrhosis, while FIB-4 of 2.40 generated Youden index of 0.59 and accuracy of 90% for cirrhosis. The sensitivity, specificity, PPV, NPV and Youden index for the other

cut-off points in predicting or excluding the significant liver fibrosis and cirrhosis in patients with CHB are summarized in Table 1.

Cross tables

The data were extracted and 2×2 tables were constructed to calculate sensitivity, specificity, PPV, NPV and Youden index for each reported FIB-4 cut-off points as shown in Table 2.

Discussion

This, to the best of our knowledge, is the first study to advocate lowering the upper cut-off point and raising the lower cut-off point of FIB-4 to reduce the need for LB in a homogenous population of CHB infection patients. When the initially determined cut-off points (1.45–3.25) were

used, the need for LB was reduced by 74%. However, when the newly proposed cut-off points (1.62–2.40) were used, the need for LB was reduced by 86% with a higher accuracy in patients monoinfected with CHB virus.

Many studies have evaluated non-invasive diagnostic models for liver fibrosis in different types of chronic liver diseases. However, most have been conducted on patients with chronic hepatitis C. Despite being much more common than other viral infections, there have been few studies evaluating the application of these non-invasive models in the prediction or exclusion of significant liver fibrosis and cirrhosis in patients with CHB [9–11]. Moreover, it is very important to estimate the liver fibrosis related to HBV infection with non-invasive methods in terms of early diagnosis and treatment besides the advantages of being painless and inexpensive. The results of this study demonstrated that the need for LB to predict and exclude significant liver fibrosis and cirrhosis may be reduced by changing the cut-off points for FIB-4 in LB proven CHB patients.

Table 1: Analytical performances of some FIB-4 cut-off points for significant liver fibrosis and cirrhosis.

Classification	Cut-offs	Accuracy (%)	Sensitivity (%)	Specificity (%)	Youden's index	PPV (%)	NPV (%)
Significant liver fibrosis	1.25	78.6	72.6	82.0	0.55	69.7	84.0
	1.45	78.6	64.4	86.7	0.51	73.4	81.0
	1.62	81.1	64.4	90.6	0.55	79.7	81.7
	2.40	79.6	47.9	97.7	0.46	92.1	75.8
	3.25	69.7	16.4	100.0	0.16	100	67.7
Cirrhosis	1.25	77.1	90.2	73.8	0.64	46.8	96.7
	1.45	81.6	80.5	81.9	0.62	53.2	94.2
	1.62	84.1	80.5	85.0	0.66	57.9	94.4
	2.40	90.0	61.0	97.5	0.59	86.2	90.8
	3.25	84.1	22.0	100.0	0.22	100	83.3

PPV, Positive predictive value; NPV, negative predictive value.

Table 2: 2×2 table for significant liver fibrosis and cirrhosis.

FIB-4 index cut-off points	Significant liver fibrosis		Cirrhosis	
	METAVIR F0-1 (n=128)	METAVIR F2-4 (n=73)	METAVIR F0-3 (n=160)	METAVIR F4 (n=41)
≤ 1.25	105	20	118	4
> 1.25	23	53	42	37
≤ 1.45	111	26	131	8
> 1.45	17	47	29	33
≤ 1.62	116	26	136	8
> 1.62	12	47	24	33
≤ 2.40	125	38	156	16
> 2.40	3	35	4	25
≤ 3.25	128	61	160	32
> 3.25	0	12	0	9

This simple, non-invasive and inexpensive test of the FIB-4 index was first used for the evaluation of the presence of liver fibrosis in HIV/HCV co-infected populations [6]. It was then validated in most studies for the prediction of significant liver fibrosis and cirrhosis for HCV mono-infected patients [12, 13]. In previously published studies on CHB, the primary focus has been on differentiating severe fibrosis, therefore, data on the utility of FIB-4 for the prediction of significant liver fibrosis where medical therapy is necessary, and cirrhosis where special attention is required for periodic screening of hepatocellular carcinoma and complications, are still lacking. We therefore validated the diagnostic accuracy of FIB-4 and suggested optimized cut-off points for predicting and excluding significant liver fibrosis and cirrhosis in patients mono-infected with CHB.

According to the meta-analysis by Chen et al. the recommended cut-off point for predicting significant liver fibrosis and cirrhosis was between 1.45 and 1.62, and between 2.9 and 3.6, respectively [14]. In the study by Kim et al. the diagnostic performance of the FIB-4 has been evaluated to predict the fibrosis stage in patients with CHB [15]. They showed that many patients with advanced liver fibrosis or cirrhosis may be misdiagnosed by the upper cut-off point of 3.25. For example, 89% (173/195) of patients with advanced fibrosis had a FIB-4 score of 3.25 or less. Moreover, 86% (30/35) of patients with advanced fibrosis or cirrhosis would have been misclassified since their scores were 3.25 or less. We therefore, considered to evaluate the cut-off points for FIB-4.

Depending on the establishment of the cut-off points, FIB-4 may have relatively better predictive values for the diagnosis of significant liver fibrosis and cirrhosis. FIB-4 cut-off points of 1.45 and 3.25 to predict significant liver fibrosis were initially determined by Sterling et al. in patients with HIV/HCV co-infection [6]. However, large proportion of patients with and/or without significant liver fibrosis or cirrhosis would be misclassified by these cut-off points due to the nature of HBV infection and the selected patient groups. For example, in the present study, patients with significant liver fibrosis had FIB-4 index over the upper cut-off point of 3.25 and 2.40 were 16% (12/73) and 48% (35/73), while patients with cirrhosis had FIB-4 index over the cut-off point of 3.25 and 2.40 were 22% (9/41) and 61% (25/41), respectively. However, 84% (61/73) and 52% (38/73) of patients with significant liver fibrosis had FIB-4 index cut-off points of 3.25 and 2.40 or less, while patients with cirrhosis had FIB-4 index below the cut-off value of 3.25 and 2.40 were 78% (32/41) and 39% (16/41), respectively. Therefore, most of the patients with significant liver fibrosis and cirrhosis may be misclassified

with the initially established upper cut-off point of 3.25, and the use of 2.40 as an upper cut-off point increases the diagnostic utility of FIB-4.

In view of the lower cut-off points, we can also conclude that many patients without significant liver fibrosis or cirrhosis may be misclassified by the initially established lower cut-off point of 1.45. In the present study patients without significant liver fibrosis had FIB-4 over the cut-off value of 1.25, 1.45 and 1.62 were 18% (23/128), 13% (17/128) and 9% (12/128), while patients without cirrhosis had FIB-4 index over the cut-off value of 1.25, 1.45 and 1.62 were 26% (42/160), 18% (29/160) and 15% (24/160), respectively. However, FIB-4 index cut-off points of 1.25, 1.45 and 1.62 correctly identified the lack of significant liver fibrosis in 82% (105/128), 87% (111/128) and 91% (116/128) of patients, while patients without cirrhosis had FIB-4 index less than the cut-off value of 1.25, 1.45 and 1.62 were 74% (118/160), 82% (131/160) and 85% (136/160), respectively. Therefore, most of the patients with lack of significant liver fibrosis and/or cirrhosis may be misclassified with the initially established lower cut-off point of 1.45, and the use of 1.62 as a lower cut-off point increases the diagnostic utility of FIB-4.

FIB-4 index has more than one cut-off point, 1.45 and 3.25, to maximize the accuracy for significant liver fibrosis and cirrhosis. However, it has gaps between these cut-off values, creating an unclassifiable zone. We therefore considered whether decreasing the upper cut-off point and increasing the lower cut-off point can make this unclassifiable zone lower, as well as the new cut-off points for FIB-4 index could be determined to predict or to exclude significant liver fibrosis and cirrhosis or not. We have based the results of ROC analysis and Youden index that takes into account both sensitivity and specificity to determine the best cut-off points by comparing the proportion of correctly classified cases. Youden index has value changing from 0 to 1, and the higher the Youden index the more accurate is the prediction at the cut-off point. By this way, we have assessed different cut-off points lower or higher than the initially established ones to minimize the unclassifiable zone with the highest Youden index values and have decided to recommend 1.62 as a lower and 2.40 as an upper cut-off point in order to reduce the need of LB, for both significant liver fibrosis and cirrhosis.

Different cut-off points were evaluated to achieve the highest Youden index and accuracy, since the primary goal of this study was to identify the lowest and the highest risk of patients who do not require LB. It was noticed that this change for FIB-4 made significant improvement in the overall diagnostic accuracy and Youden index for both significant liver fibrosis and

cirrhosis. The accuracy for the cut-off point of 1.62 was 2.5% more than each cut-off points of 1.25 and 1.45 for significant liver fibrosis. In addition, when we used the lower cut-off point <1.25 to obtain exactly 95% NPV, the diagnostic accuracy worsened as well as unclassifiable zone increased. Moreover, lower cut-off point <1.45 or 1.25 could result in large numbers of patients receiving unnecessary antiviral therapy. It was therefore concluded that the cut-off point of 1.62 that has the maximum Youden index and accuracy should be used as the lower cut-off point for FIB-4. However, a change in the upper cut-off point was also important in clinical practice. There was a 9.9% difference between the overall diagnostic accuracies and 0.30 difference between the Youden indexes for the upper cut-off points of 2.40 and 3.25, for the accurate assessment of significant liver fibrosis.

Likewise, the accuracy for the cut-off point of 1.62 was 2.5% and 7% more than for the cut-off points of 1.45 and 1.25 to exclude cirrhosis, respectively. It was therefore concluded that cut-off point of 1.62 that has the maximum Youden index and accuracy should be used as a lower cut-off point of FIB-4 for cirrhosis. A change in the upper cut-off point was also important in clinical practice. There was a 5.9% difference between the overall diagnostic accuracies and 0.37 difference between the Youden indexes for the upper cut-off points of 2.40 and 3.25, for the accurate assessment of cirrhosis.

In the meta-analysis by Yan et al. the FIB-4 cut-off point of 1.45 had the summary sensitivity and specificity values of 65.4% and 73.6%, while 3.25 had 16.2% and 95.2% for significant fibrosis, respectively [16]. The sensitivity values for both cut-off points were similar to our results, but the specificity values were 13.1% and 4.8% lower than our results for 1.45 and 3.25 cut-off points, respectively. However, we could not have done any comparison for the cirrhosis since the presented cut-off points were not including the single 1.45 and 3.25 points.

There are some limitations to this study. First, as it was a retrospective study, there may have been some potential selection bias of patients. Second, the patients in this study may be less representative of the general population compared with patients from multiple centers for the rest of the world.

Finally, it is important to establish how many patients with chronic HBV infection can be correctly diagnosed without requiring LB. When the upper cut-off point of FIB-4 is lowered from 3.25 to 2.40 and the lower FIB-4 cut-off point is increased from 1.45 to 1.62, to predict or to exclude significant liver fibrosis and cirrhosis, the biopsy requirement can be avoided in 86% of the study population which is 12% more than the result obtained

with the initially established FIB-4 cut-off points. Based on this overview, FIB-4, a combination of age, AST, ALT and platelet count with the newly proposed cut-off points could be used as an alternative to LB for the first-line assessment of liver injury in patients monoinfected with CHB to predict and/or to exclude significant liver fibrosis and/or cirrhosis. Our study has several implications for future research. For example, we believe that more studies on the diagnostic accuracy for liver fibrosis are needed in patients monoinfected with CHB. In the future, the diagnostic value in predicting the subsequent development of significant liver fibrosis and cirrhosis with sequential FIB-4 measurements during long-term follow-up needs to be further examined in a longitudinal study.

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