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Original paper

Simple non-invasive markers for early diagnosis and determination of the severity of liver diseases

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Abstract

Aim of the study: The aim of the study was to evaluate the effect and severity of liver diseases of different etiologies on the values of three non-invasive fibrosis markers.

Material and methods: Serum samples from 65 patients with alcoholic cirrhosis, 31 with non-alcoholic cirrhosis and 32 with toxic hepatitis, were tested. Cirrhotic patients were classified according to the Child-Pugh scale. The age-platelet (AP) index, HUI score and Fibro Q index were calculated using the specific formulas.

Results: The values of all tested scores were significantly higher in controls than in patients with liver diseases and were significantly different between liver diseases. The patients with alcoholic and non-alcoholic cirrhosis had higher values of the AP index, HUI score and Fibro Q index than patients with toxic hepatitis. HUI and Fibro Q scores appeared to vary according to the severity of liver damage and were higher in Child-Pugh class C than in classes A and B.

Conclusions: We conclude that all tested scores based on liver function tests are good markers for non-invasive diagnosis of liver damage. Additionally, HUI and Fibro Q scores reflect the severity of liver cirrhosis.

Key words: liver cirrhosis, toxic hepatitis, non-invasive markers.

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Introduction

Liver cirrhosis is the final stage of fibrosis characterized by architectural distortion and the formation of regenerative nodules. Cirrhosis, resulting from various chronic liver diseases, is an important problem of public health in highly developed countries, being one of the most common causes of death in Europe. Literature data suggest that about 0.1% of the European population is affected by liver cirrhosis, which corresponds to 26 new cases per 100,000 population annually [1-3].

Apart from the ultrasound, computed tomography (CT), magnetic resonance imaging (MRI) and liver biopsy, the diagnosis of liver diseases can be based on the results of biochemical tests [4, 5]. Liver function tests, such as proteins, liver enzymes, bilirubin, albumin or platelet count in the blood, help to detect inflammation and liver damage. In particular, a decrease in the synthesis of anti-coagulant proteins can result in an increased level of the international normalized ratio (INR) [6]. In addition, a very common hematological abnormality in patients with liver diseases is thrombocytopenia (platelet [PLT] count $< 150 \times 10^9$/l). The drop in platelet count may occur due to decreased platelet production in the bone marrow (e.g. caused by viruses or alcoholic etiology of liver disease), increased platelet destruction through increased shear stress or decreased activity of thrombopoietin [7, 8]. The death of hepatocytes and inflammation can also result in elevated serum aminotransferases activity. Alanine ami-

notransferase (ALT) is found mainly in the cytosol of hepatocytes, and is released into the blood during liver damage [9]. By contrast, aspartate aminotransferase (AST) is present not only in the liver, but is also found in several organs including heart and muscles. As AST level itself is not a good predictor of liver damage, it is usually expressed as the AST/ALT ratio [9, 10].

The main plasma protein produced by the liver is albumin, and the gradual destruction of liver tissue over time may lead to reduced albumin synthesis. A decreased level of albumin is a prognostic factor for advanced liver diseases [11]. Another important function of the liver is bilirubin excretion into the bile. If bilirubin cannot be efficiently removed from the liver, a high level of bilirubin accumulates in the blood [12, 13].

The aim of this study was to evaluate the changes in the values describing liver functions based on three non-invasive indirect markers of liver disease – age-platelet (AP) index, HUI score and Fibro Q index – depending on the liver disease etiologies or severity. These indexes are based on the determination of PLT, bilirubin, albumin, AST, ALT and INR adjusted to age and body mass index (BMI).

Material and methods

Human subjects

The experimental group consisted of 128 patients (70 men and 58 women, age between 24 and 88 years) consecutively admitted to the Department of Infectious Diseases and Hepatology, Medical University of Bialystok. The diagnosis was based on the results of a biochemical liver panel (PLT count, mean corpuscular volume [MCV], INR, AST, ALT, γ-glutamyltransferase [GGT], albumin and bilirubin) and other clinical data (signs, symptoms, physical examination results, and abdominal ultrasound or computed tomography scan of the abdomen). To confirm the diagnosis of hepatitis C virus (HCV), an anti-HCV test was performed. According to the clinical diagnosis, the liver disease was classified as: alcoholic cirrhosis (AC) – 65 patients, non-alcoholic cirrhosis (NAC) – 31 patients and toxic hepatitis (HT) – 32 patients. Non-alcoholic cirrhosis was caused by chronic hepatitis C –15 patients, chronic hepatitis B – 1, autoimmune hepatitis – 1, primary biliary cirrhosis – 5 and by undefined factors – 9. Toxic hepatitis is an inflammation of the liver, and in our study it was caused by alcohol in 23 patients and by drugs in 9 patients. The severity of liver cirrhosis was evaluated by the Child-Pugh scale (class A – 31 subjects, class B – 37 and class C – 28). All patients were interviewed regarding their use of alcohol.

The control group (C) consisted of 30 healthy people (17 males and 13 females). All (healthy and sick) subjects gave their informed consent to participate in the study. This study was in accordance with the Helsinki Declaration and was approved by the Bioethical Committee at the Medical University of Bialystok.

Table 1. Definition of age-platelet (AP) index

Score	Age (years)	PLT (10^9/l)
0	< 30	⩾ 225
1	30-39	200-224
2	40-49	175-199
3	50-59	150-174
4	60-69	125-149
5	⩾ 70	< 125

Blood sampling

Fasting blood samples were taken by vein puncture after hospital admission and before treatment. The sera were separated by centrifugation and stored at –86°C until assayed. Besides serum, a part of each blood sample was collected into tubes containing 3.8% liquid sodium citrate and EDTA-2.

AST, ALT and bilirubin were determined on an Architect c8000 (Abbott Laboratories, Abbott Park, USA), and albumin was determined on an Image 800 analyzer (Beckman Coulter Diagnostics, USA). Prothrombin time (PT) was determined on an STA Compact Max analyzer (Diagnostica Stago, France), and PLT count was determined on a Sysmex XS-800i (Sysmex Corporation, Singapore).

Calculations

AP index was calculated based on the following formula [14]:

$$\text{AP index} = \text{age} + \text{PLT}\ (\frac{10^9}{\text{l}})$$

The AP index has a range of possible values from 0 to 10 (Table 1). The total possible score derives from two parameters: age and PLT. Different points are given and added together according to the values of these parameters.

HUI score [15] and Fibro Q index [16] were calculated using the formulas:

$$\text{HUI score} = 3.148 + 0.167 \times BMI + 0.088 \times \text{bilirubin} - 0.151 \times \text{albumin} - 0.019 \times \text{PLT}\ (\frac{10^9}{l})$$

$$\text{Fibro Q} = \frac{10 \times \text{age} \times \text{AST}\left(\frac{\text{U}}{l}\right) \times \text{INR}}{\text{PLT}\left(\frac{10^9}{l}\right) \times \text{ALT}\left(\frac{\text{U}}{l}\right)}$$

Statistical analysis

The differences between tested and control groups were evaluated using the Mann-Whitney *U* test. To test the hypothesis about the differences between liver diseases, the ANOVA rank Kruskal-Wallis test was performed. We considered *p* values less than 0.05 statistically significant. The diagnostic performance of each test was calculated as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy (ACC). The area under the receiver operating characteristic curve (AUC) was used to calculate the diagnostic performance of all algorithms.

Results

The values of scores calculated from results of non-invasive blood tests are presented in Table 2. The values of the AP index, HUI score and Fibro Q index were significantly higher in alcoholic cirrhosis (AC) and non-alcoholic cirrhosis (NAC) groups in comparison to the control group ($p < 0.001$ for all comparisons). Also, the values of AP, HUI and Fibro Q scores were higher in toxic hepatitis (HT) patients compared to the controls ($p < 0.001$, $p = 0.037$, $p = 0.018$, respectively). The analysis of variance revealed that liver diseases affects the AP, HUI and Fibro Q scores ($p < 0.001$, $p = 0.012$, $p < 0.001$, respectively). The patients with alcoholic cirrhosis had higher values of AP index, HUI score and Fibro Q index than patients with toxic hepatitis ($p < 0.001$, $p = 0.014$, $p < 0.001$, respectively). In addition, AP index, HUI score and Fibro Q index were significantly higher in non-alcoholic cirrhosis in comparison with toxic hepatitis patients ($p < 0.001$, $p = 0.045$, $p = 0.018$, respectively), but there was no significant difference between non-alcoholic cirrhosis and alcoholic cirrhosis patients ($p = 0.269$, $p = 0.912$, $p = 0.175$, respectively). ANOVA rank Kruskal-Wallis analysis showed that Child-Pugh stage does not have an impact on the AP index ($p = 0.334$), but has an impact on the HUI and Fibro Q scores ($p < 0.001$ for both). Further analysis showed that their values were higher in class C patients than in class A ($p < 0.001$ for both). Additionally, HUI score and Fibro Q index levels were higher in class C than in class B ($p < 0.001$ for both).

Diagnostic usefulness of non-invasive markers is presented in Table 3. Age-platelet index has the highest ability to detect alcoholic cirrhosis and toxic hepatitis (sensitivity of 98.2% and 100%, respectively), but the HUI score has the highest ability to exclude both of these diseases (specificity of 100%). Additionally, the HUI score exhibited the highest sensitivity (100%) and specificity (100%) in non-alcoholic cirrhosis. The highest diagnostic power (AUC) in the detection of alcoholic cirrhosis and toxic hepatitis can be attributed to the AP index (AUC ± SE; AC: 0.973 ± 0.015, HT: 0.855 ± 0.056), but the HUI score has the highest diagnostic power in non-alcoholic cirrhosis (AUC ± SE: 1.00 ± 0.00). In all of the diseases tested, the positive predictive value (PPV) of some of the indexes equals 100%. Also, PPV of the AP index in non-alcoholic cirrhosis equals 100%. These high values resulted from the lack of false-negative results.

Discussion

Prognosis in chronic liver diseases depends on the stage and severity of liver fibrosis [17]. The gold standard for the diagnosis of liver diseases is still a liver

Table 2. Results of laboratory tests in controls (C) and patients with liver diseases and according to Child-Pugh score

Group	AP index	HUI	Fibro Q
Controls (*n* = 30)	1.35 ± 1.57	2.09 ± 0.04	1.84 ± 1.03
AC (*n* = 65)	7.08 ± 1.92^{*,c}	5.18 ± 2.42^{*,c}	29.78 ± 14.40^{*,c}
NAC (*n* = 31)	7.84 ± 1.88^{*,c}	5.36 ± 1.34^{*,c}	14.17 ± 12.62^{*,c}
HT (*n* = 32)	4.48 ± 2.37^{*,a,b}	3.99 ± 2.50^{*,a,b}	8.93 ± 7.56^{*,a,b}
Child-Pugh A (*n* = 31)	6.87 ± 2.46	4.39 ± 2.55^{C}	11.18 ± 10.30^{C}
Child-Pugh B (*n* = 37)	6.92 ± 2.03	4.95 ± 1.98^{C}	21.71 ± 23.98^{C}
Child-Pugh C (*n* = 28)	7.79 ± 1.29	6.72 ± 1.50^{A,B}	47.21 ± 39.52^{A,B}

Data are mean ± standard deviation

**Significant differences in comparison to controls. Significant differences in comparison to (ANOVA rank Kruskal-Wallis): AC – a, NAC – b, HT – c, Child-Pugh A – A, Child-Pugh B – B, Child-Pugh C – C*

Table 3. Diagnostic value of age-platelet (AP), HUI and Fibro Q scores in liver diseases

Liver disease	Cut-off (from ROC)	Sensitivity [%]	Specificity [%]	ACC [%]	PPV [%]	NPV [%]	AUC ± SE
AP index							
AC	3.0	98.2	70.0	90.8	90.2	93.3	0.973 ± 0.015
NAC	5.0	93.1	100.0	95.9	100.0	90.9	0.986 ± 0.013
HT	1.0	100.0	45.0	73.8	66.7	100.0	0.855 ± 0.056
HUI score							
AC	2.6	92.9	100.0	94.7	100.0	83.3	0.929 ± 0.034
NAC	2.12	100.0	100.0	100.0	100.0	100.0	1.0 ± 0.0
HT	2.81	52.6	100.0	76.9	100.0	69.0	0.526 ± 0.115
Fibro Q index							
AC	3.06	94.4	90.0	93.2	96.2	85.7	0.965 ± 0.021
NAC	4.26	88.9	95.0	91.5	96.0	86.4	0.959 ± 0.025
HT	3.33	38.1	95.0	65.9	88.9	59.4	0.6 ± 0.092

AC – alcoholic cirrhosis, NAC – non-alcoholic cirrhosis, HT – toxic hepatitis, ACC – diagnostic accuracy, PPV – positive predictive value, NPV – negative predictive value, AUC – area under the ROC curve, SE – standard error

biopsy. The examination of liver tissue samples provides a good estimate of liver histological structure and the degree of liver fibrosis. Liver biopsy is used to obtain about 1/50 000 of the liver mass. Because it is a very small part of this organ, biopsy is associated with a high rate of sampling error [18-20]. Also, the big disadvantage of liver biopsy is its invasiveness and complications in up to 5.0% of patients (e.g. bleeding, pain, bile peritonitis, right kidney puncture and death) [21]. In addition, there are many contraindications to perform liver biopsy, such as: being an uncooperative patient, bacterial cholangitis, abnormal coagulation indices, thrombocytopenia, ascites, cystic lesions or obesity [20]. Therefore, it is necessary to find early diagnostic markers of liver fibrosis that can be measured in the blood. Circulating markers of liver disease progression are divided into class I – markers directly representing the degree of liver fibrogenesis – and class II – biomarkers which are a mathematical combination of biochemical and cytological markers [17]. In this study we aimed to determine the diagnostic value of three markers included in mathematical algorithms: the AP index, HUI score and Fibro Q index. Non-invasive fibrosis detection is important as liver damage leads to impairment of liver function.

The AP index, based on platelet count and age, is inexpensive, easy, and rapid to calculate. It was invented by Poynard and Bedossa, who wanted to find a simple diagnostic index in HCV patients classified according to the presence of necroinflammatory lesions and fibrosis of the liver [22]. Poynard and Bedossa demonstrated that platelet count and age combined in a simple algorithm correlated with the presence of fibrosis and histological activity of the disease. According to these data the value ≥ 6 of the AP index had a specificity of 93%, sensitivity of 52% and area under the ROC curve of 0.690 ± 0.085. The authors assumed that an AP score of 6 or more could circumvent the need of liver biopsy, but its negative predictive value was not high enough to prevent a liver biopsy in patients with a lower score. In other studies, the diagnostic power measured according to Ishak and Kondell scores as the area under the ROC curve (AUC) in HCV patients with ≥ F3 hepatic fibrosis reached a value between 0.57 and 0.61. At the cutoff of 4 or greater the AP index exhibited a sensitivity of 80% and specificity of 45%, but at the cutoff of 6 or greater the AP index had lower sensitivity (30%) and higher specificity (77%) for the diagnosis of histological fibrosis with scores of ≥ 3 [23]. Our study revealed that the AP index has excellent efficiency for the diagnosis of alcoholic and nonalcoholic cirrhosis (AUC: 0.973, 0.929, respectively). On the other hand, our study indicates that the AP index is unable to differentiate between the stages of liver cirrhosis according to the Child-Pugh scale. In summary, AP index calculation can be used to detect chronic hepatitis C, toxic hepatitis and cirrhosis and reflects the degree of liver cirrhosis. The AP index has very good diagnostic accuracy for predicting cirrhosis, but it is not useful to evaluate the severity of liver cirrhosis according to the Child-Pugh scale.

The next noninvasive marker is the HUI score based on the body mass index and three routine laboratory tests: albumin, bilirubin and platelet count. This predictive model was developed by Hui *et al.*, who wanted to develop a non-invasive model which could potentially decrease the need for liver biopsy in some patients with chronic hepatitis B [24]. They proved that this algorithm was accurate in predicting absence of significant fibrosis. In our study the specificity and positive predictive value were the same for alcoholic cirrhosis, non-alcoholic cirrhosis and toxic hepatitis (100%, 100%, respectively). This PPV resulted from the lack of false-negative results. There were significant differences between liver diseases. HUI scores were significantly higher in alcoholic and non-alcoholic cirrhosis than in toxic hepatitis, but there were no differences between cirrhosis of different etiologies. Similar results were obtained by Sebastiani *et al.*, who observed high PPV (> 96.3%) for HUI in advanced fibrosis with a cut-off of 0.15, but its diagnostic value was quite low and reached the value of 0.71 AUC (95% CI: 0.56-0.86). Therefore, the HUI score has a potential to be used for the detection of liver diseases, excluding significant fibrosis, evaluation of fibrosis degree and differentiation of cirrhosis of different etiologies from toxic hepatitis.

The Fibro Q score can predict alcoholic cirrhosis as well as non-alcoholic cirrhosis with 98.2% and 88.9% sensitivity, respectively, but the ability to detect toxic hepatitis is low (sensitivity: 38.1% and AUC: 0.6). We also found that Fibro Q has different values according to the severity of liver cirrhosis. The Fibro Q was approximately 5 times higher in class C patients compared with class A and 2 times higher in class C compared with class B. Our data are consistent with the study of Hsieh *et al.* They showed that Fibro Q is an easy tool to evaluate significant fibrosis in patients with chronic viral hepatitis. Among the chronic hepatitis patients who had significant fibrosis/cirrhosis, only 3.45% of patients had Fibro Q scores of 0.6 or lower. Additionally, in patients with Fibro Q scores of 1.6 or greater, 92.9% had significant fibrosis, and only 7.1% of patients without significant fibrosis were incorrectly classified. Hsieh *et al.* also compared the accuracy of Fibro Q with other non-invasive algorithms – AST-to-platelet ratio index (APRI), and AST/ALT ratio (AAR) – and demonstrated that Fibro Q is better than APRI, and equal to AAR, as a biomarker for predicting significant fibrosis [25]. In summary, our study and that of Hsieh *et al.* indicate that the Fibro Q value can quickly evaluate the progression of chronic liver diseases. It is a good marker to differentiate cirrhotic patients from those with toxic hepatitis. The data suggest that the value of this index differs depending on the intensity of the disease process and its chronicity.

Conclusions

We conclude that the values of simple algorithms are changed in liver diseases and may depend on the severity of liver cirrhosis. Our results indicate the high usefulness of these noninvasive scores for detection and exclusion of liver diseases, especially cirrhosis.

Disclosure

The authors report no conflict of interest.

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