Selected Noninvasive Markers in Diagnosing Liver Diseases

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Abstract

Objective: To evaluate the effectiveness of certain noninvasive liver-damage markers in predicting liver diseases and the clinical severity of liver cirrhosis.

Methods: We tested serum specimens from 57 patients with alcoholic cirrhosis, 30 with nonalcoholic cirrhosis, and 22 with toxic hepatitis (TH). The Bonacini, King, and Göteborg University Cirrhosis Index (GUCI) scores were calculated using specific formulas.

Results: The values of the Bonacini and King scores significantly differ between liver diseases. The Bonacini score was higher in alcoholic and nonalcoholic cirrhosis than in TH, and the King score was higher in alcoholic cirrhosis than in TH. All of the tested scores appeared to vary according to the severity of liver damage and were higher in Child-Pugh class C than that in classes A and B.

Conclusions: We conclude that the Bonacini and King scores differ between liver diseases and that all the tested scores reflect the severity of liver cirrhosis.

Keywords: noninvasive markers, liver cirrhosis, toxic hepatitis, liver fibrosis, serum markers

Liver cirrhosis is the final pathological result of various chronic liver diseases and is an important public health problem in highly developed countries; for instance, it is the fourth most common cause of death in central Europe.1,2 The most common causes of liver cirrhosis are alcoholic liver disease and hepatitis C infection.3,4 The effects of irreversible liver damage are degeneration and necrosis of hepatocytes, replacement of liver parenchyma by fibrotic tissues and regenerative nodules.5 The diagnosis of liver diseases, including cirrhosis (alcoholic and nonalcoholic origin) and toxic hepatitis (TH; mostly caused by alcohol abuse) is based on the physical examination results, biochemical test results, symptoms, liver-imaging test results, and history of alcohol intake.3,6 To confirm viral-related cirrhosis, hepatitis B surface (HBs) antigen, and hepatitis C virus (HCV) antibodies are usually determined.7

Liver-function tests detect inflammation of and damage to the liver. These tests include aminotransferases, gamma-glutamyl transferase (GGT), albumin, bilirubin, and prothrombin time (PT). Liver diseases (mainly, cirrhosis) are characterized by the reduced synthesis of the procoagulant factors. The deficiency of these factors directly affects PT/international normalized ratio (INR) value. The changes in the coagulation systems are a hallmark of advanced liver disease. It has been documented that the value of INR in liver diseases is elevated.8,9 The platelets (PLTs) have a dual role in hemostasis: during primary hemostasis in PLT plug formation and during secondary hemostasis in stabilization of the PLT plug.10 The common complication in patients with chronic liver disease is thrombocytopenia (PLT count, <150 × 10^9/L). This condition

Abbreviations:

HBs, hepatitis B surface; HCV, hepatitis C virus; GGT, gamma-glutamyl transferase; PT, prothrombin time; INR, international normalized ratio; CDS, cirrhosis discriminant score; PLTs, platelets; TPO, thrombopoietin; ALT, alanine aminotransferase; AST, aspartate transaminase; BS, Bonacini score; GUCI, Göteborg University Cirrhosis Index; MCV, mean corpuscular volume; CT, computed tomography; AC, alcoholic cirrhosis; NAC, nonalcoholic cirrhosis; TH, toxic hepatitis; EDTA, ethylenediaminetetraacetic acid; CV, coefficient of variance; ANOVA, analysis of variance; PPV, positive predictive value; NPV, negative predictive value; ACC, diagnostic accuracy; ROC, receiver operating characteristic; AUC, area under the curve; C, controls; A, class A; B, class B; C, class C

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is caused by splenic and hepatic sequestration of PLTs, suppression of PLT production in the bone marrow (eg, caused by viruses or alcoholic etiology of liver disease), and decreased activity of the hematopoietic growth factor thrombopoietin (TPO).\(^{10}\) Alanine aminotransferase (ALT) is found abundantly in the cytosol of hepatocytes, and the liver has approximately 3000 times more ALT than serum. During liver injury, ALT released from damaged cells can rapidly increase ALT activity in the serum.\(^{11}\) Aspartate transaminase (AST) is present not only in the liver but also in skeletal muscles and erythrocytes, therefore, AST activity increases in the case of hepatic injury but is less specific for hepatic injury than ALT.\(^{11,12}\)

The aim of this study was 2-fold. First, we evaluated the effect of liver diseases of different etiologies. Next, we evaluated that effect and the clinical severity of cirrhosis on the values of selected noninvasive indirect markers of liver damage: Bonacini score (BS), King score, and Göteborg University Cirrhosis Index (GUCI) involving combinations of aminotransferases, INR, and PLTs.

**Materials and Methods**

**Subjects**

The tested group consisted of 109 patients (74 men and 35 women between the ages of 26 years and 88 years) consecutively admitted to the Department of Infectious Diseases and Hepatology (Medical University of Bialystok, Poland). The diagnosis was based on the biochemical liver panel results (PLT, mean corpuscular volume [MCV], INR, AST, ALT, GGT, albumin, and bilirubin) and other clinical data (signs, symptoms, physical examination results, and abdominal ultrasound or computed tomography [CT] scan of the abdomen). We classified the patients according to the clinical diagnosis of disease: alcoholic cirrhosis (AC), 57 patients; nonalcoholic cirrhosis (NAC), 30 patients; and TH), 22 patients. The cause of nonalcoholic cirrhosis was chronic hepatitis C in 13 patients, chronic hepatitis B in 1 patient, autoimmune hepatitis in 1 patient, primary biliary cirrhosis in 4 patients, and undefined factors in 11 patients. The severity of liver cirrhosis was evaluated according to the Child-Pugh score (27 patients in class A, 31 in class B, and 25 in class C). To confirm the diagnosis of HCV, anti-HCV test was performed. All patients were interviewed regarding their use of alcohol. Acute alcohol abuse was the cause of 7 cases of toxic hepatitis. The control group consisted of 20 healthy volunteers (11 men and 9 women). All subject individuals (healthy and sick) gave written informed consent to participate in our study. The study was approved by the Bioethical Committee at the Medical University in Bialystok, Poland.

**Blood Specimen Gathering**

We obtained blood specimens from fasting individuals by venipuncture after admittance and before treatment. The sera were separated by centrifugation and stored at −86°C until assayed. Besides serum, we collected a part of each blood specimen into tubes containing 3.8% liquid sodium citrate for hemostasis analyses and ethylenediaminetetraacetic acid (EDTA) for hematological analyses.

We measured ALT and AST on the ARCHITECT ci8200 (Abbott Laboratories) according to the spectrophotometric method and using Abbott reagents (for ALT: reference range, 5 to 55 U/L, measuring range, 2.0 to 942 U/L, imprecision, coefficient of variance [CV], ≤5.2%; for AST: reference range, 5 to 34 U/L, measuring range, 2.0 to 913 U/L, imprecision, CV ≤4.6%). PLT count was determined on the Sysmex XS800i (Sysmex Corporation; measuring range, 0-9999 × 10⁹/L; imprecision, CV ≤4%), and PT was determined on the STA Compact Max analyzer (Diagnostica Stago, Inc) by the viscometric method (measuring range, 8 to 120 seconds; imprecision, CV ≤1.6%).

**Calculations**

GUCI, Bonacini, and King scores were calculated based on the following formulas:

\[
\text{GUCI index} = \frac{\text{normalized AST}}{\text{INR}} \times \frac{100}{\text{PLT (10⁹/L)}}
\]

(normalized AST = AST [U/L]/upper limit of normal AST [U/L]; Upper limit of normal AST equals 50 U/L)\(^{13}\)

\[
\text{Bonacini score} = \frac{\text{PLT (× 10⁹/L)}}{\text{ALT/AST (IU/L)}} + \text{INR}^{14}
\]

The Bonacini score has a range of possible values, from 0 to 11 (Table 1).\(^{14}\) Possible total score derived from three laboratory parameters: platelets, ALT/AST ratio, and PT. Different points are given and added together according to the values of these parameters.

\[
\text{King score} = \text{age (years)} \times \frac{\text{AST (IU/L)}}{\text{INR/PLT (10⁹/L)}}^{15}
\]
Statistical Analysis

The differences between tested and control groups were evaluated by Mann-Whitney U test. To test the hypothesis about the differences between liver diseases, we performed the analysis of variance (ANOVA) rank Kruskal-Wallis test. We considered $P$ values less than .05 as 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). Accuracy is defined as the proportion of people with and without the disease who will have true results (positive and negative). We used the area under the receiver operating characteristic (ROC) curve to calculate the diagnostic performance of scores. ROC curve is the plot of sensitivity versus 1-specificity, and the area under the curve (AUC) is an effective measure of accuracy that evaluates the diagnostic ability of tests to determine the through state of subjects.

Results

The values of GUCI and King scores in AC (mean [SD]; 4.46 [6.23] and 121.88 [176.96], respectively), NAC (3.41 [7.85] and 58.61 [66.86], respectively) and TH (1.81 [1.91], 43.47 [58.10], respectively) were higher than that in the controls (0.19 [0.06], 2.76 [0.99], respectively; $P < .001$ for all comparisons). The Bonacini score was elevated only in AC (7.85 [2.19]) and NAC (7.18 [1.59]) patients compared with the control group (4.45 [0.89]) ($P < .001$ for both). There were no significant differences between TH (4.19 [1.81]) and controls. The ANOVA revealed that liver diseases affected the Bonacini and King scores ($P < .001$ and $P = .04$, respectively). Posthoc analysis showed that the values of the Bonacini and King scores were higher in the AC group compared with the TH group ($P < .001$ and $P = .03$, respectively). The value of the Bonacini score was also higher in the NAC group than in the TH group ($P < .001$) (Figure 1).

The severity of liver cirrhosis (Child-Pugh score) affected the Bonacini, GUCI, and King scores ($P < .001$ for all comparisons). Further analysis showed that these values were higher in class C (mean [SD], 9.46 [1.14], 8.13 [8.77], 191.61 [184.23]) than that in class A (6.17 [1.70], 2.99 [8.10], 47.07 [96.70]) ($P < .001$ for all comparisons) and class B (7.29 [2.15], 3.81 [6.22], 111.64 [189.97]) ($P < .001$ for all comparisons) (Figure 2).

The diagnostic usefulness of noninvasive markers is presented in Table 2. GUCI and King scores have the highest ability to detect (100% sensitivity) and exclude (100% specificity) nonalcoholic cirrhosis. Also, both of these indices have 100% ability to exclude toxic hepatitis. Besides nonalcoholic cirrhosis and toxic hepatitis, the GUCI index has 100% specificity in alcoholic cirrhosis. The Bonacini score has 100% specificity only for alcoholic cirrhosis. The highest diagnostic power (AUC) in the detection of nonalcoholic cirrhosis is held by the King score and the GUCI index (AUC [SE]; NAC, 1.0 [0] and NAC, 1.0 [1.0], respectively). Both of these indices also have high diagnostic performance in alcoholic cirrhosis and toxic hepatitis. The Bonacini score has the high diagnostic power in both types of cirrhosis but has very low diagnostic power in toxic hepatitis (AUC [SE], 0.430 [0.093]).

Discussion

Liver biopsy is still considered to be the criterion standard for the diagnosis and staging of liver diseases. However, liver biopsy is an invasive method, and its sensitivity in diagnosing of cirrhosis is not absolute; sampling error is common. This method is associated with difficulty in obtaining a liver specimen of adequate size (no less than 25 mm x 1.4 mm) to represent the whole liver. Also, liver biopsy is associated with certain complications (eg, bleeding, pain, bile peritonitis, kidney puncture, and death). If liver biopsy is associated with the potential for sampling bias, and the laboratory and clinical tests did not leave room for doubt, liver biopsy in our patients was not recommended.

Table 1. Definition of Bonacini Score

<table>
<thead>
<tr>
<th>Score</th>
<th>PLT $\times 10^9/L$</th>
<th>ALT/AST Ratio</th>
<th>INR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$&gt;340$</td>
<td>$&gt;1.7$</td>
<td>$&lt;1.1$</td>
</tr>
<tr>
<td>1</td>
<td>280-340</td>
<td>1.2-1.7</td>
<td>1.1-1.4</td>
</tr>
<tr>
<td>2</td>
<td>220-279</td>
<td>0.6-1.19</td>
<td>$&gt;1.4$</td>
</tr>
<tr>
<td>3</td>
<td>160-219</td>
<td>$&lt;0.6$</td>
<td>...</td>
</tr>
<tr>
<td>4</td>
<td>100-159</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>5</td>
<td>40-99</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>6</td>
<td>$&lt;40$</td>
<td>...</td>
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</tr>
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</table>

PLT, platelet count; ALT, alanine aminotransferase; AST, aspartate transaminase; INR, international normalized ratio.
Figure 1
The values of King, Göteborg University Cirrhosis Index (GUCI), and Bonacini scores in liver diseases. C indicates controls; AC, alcoholic cirrhosis; NAC, nonalcoholic cirrhosis; TH, toxic hepatitis.

Figure 2
The values of King, Göteborg University Cirrhosis Index (GUCI), and Bonacini scores according to the Child-Pugh score. A indicates class A; B, class B; C, class C.
In this study, we tried to examine the diagnostic value of the following simple and noninvasive fibrosis and liver disease indices, namely, INR, PLT, ALT, and AST, in special algorithm models in patients with liver alcoholic and nonalcoholic cirrhosis, as well as toxic hepatitis. The reason for this is that liver damage leads to impairment of liver functions (eg, to synthesis of proteins, such as clotting factors, which results in prolongation of prothrombin time (elevated PT and international normalized ratio [INR]).

Another common complication in patients with chronic liver disease is thrombocytopenia (PLT count, <150 × 10^9/L).

In addition, elevated serum aminotransferases activity gives evidence of inflammation and the death of hepatocytes. The reason for this is that liver damage leads to impairment of liver functions (eg, to synthesis of proteins, such as clotting factors, which results in prolongation of prothrombin time (elevated PT and international normalized ratio [INR]).

The Bonacini score, based on PLT, AST, ALT, and INR values, is inexpensive, easy, and rapid to calculate. It was invented by Bonacini and coworkers, who studied 3-parameter cirrhosis discriminant score (CDS) in 79 patients with chronic hepatitis C. They demonstrated that the value of the Bonacini score (possible total score from 0 to 11) is significantly higher in patients with advanced fibrosis or cirrhosis (F3-F4) than in patients with a histological fibrosis score of F0-F2. At the cutoff value of 8 or greater, the CDS had a sensitivity of 46% and specificity of 98% for the diagnosis of histological fibrosis scores of 3 to 4. In a study published by Colli et al, 67% of 176 total patients with chronic HCV infections were correctly classified as having high or very high risk of cirrhosis, and only 33% of cirrhotic patients required biopsy to confirm the diagnosis. The Bonacini score is an indicator that can be useful for prediction of cirrhosis (independent of etiology). We have shown that the Bonacini score has excellent efficiency for the diagnosis of alcoholic and nonalcoholic cirrhosis (AUC = 0.902 and AUC = 0.935, respectively). Our study also demonstrated an association between Bonacini score and stage of liver injury. The Bonacini score was approximately 50% higher in class C compared with class A. We observed the lowest values in toxic hepatitis.

The King score can predict alcoholic and nonalcoholic cirrhosis, as well as toxic hepatitis, with 98.2%, 100%, and 95.2% sensitivity, respectively. In the case of cirrhosis (of both etiologies), PPV was equal to 100%. This PPV resulted from the lack of false-negative results. Our data are consistent with those in a study by Cross and coworkers. The investigators proved that patients with a score of less than the aforementioned cut-off value have a low risk of cirrhosis. The results published by Bota et al were similar. The authors obtained values of 90% sensitivity, 74.1% specificity, 97.8% NPV, and 76.4% accuracy for predicting cirrhosis. They also found that the

<table>
<thead>
<tr>
<th>Liver Disease</th>
<th>Cut-off (From ROC)</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>ACC, %</th>
<th>PPV, %</th>
<th>NPV, %</th>
<th>AUC (SE)</th>
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<tbody>
<tr>
<td>Bonacini Score</td>
<td>AC 5.0</td>
<td>74.1</td>
<td>100.0</td>
<td>81.1</td>
<td>100.0</td>
<td>58.8</td>
<td>0.902 (0.035)</td>
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<tr>
<td></td>
<td>NAC 6.0</td>
<td>92.9</td>
<td>85.0</td>
<td>89.6</td>
<td>89.7</td>
<td>89.5</td>
<td>0.935 (0.038)</td>
</tr>
<tr>
<td></td>
<td>TH 6.0</td>
<td>28.6</td>
<td>85.0</td>
<td>56.1</td>
<td>66.7</td>
<td>53.1</td>
<td>0.430 (0.009)</td>
</tr>
<tr>
<td>GUCI Index</td>
<td>AC 0.309</td>
<td>89.1</td>
<td>100.0</td>
<td>92.0</td>
<td>100.0</td>
<td>76.9</td>
<td>0.952 (0.025)</td>
</tr>
<tr>
<td></td>
<td>NAC 0.316</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>1.0 (1.0)</td>
</tr>
<tr>
<td></td>
<td>TH 0.330</td>
<td>81.00</td>
<td>100.0</td>
<td>92.7</td>
<td>100.0</td>
<td>89.5</td>
<td>0.957 (0.028)</td>
</tr>
<tr>
<td>King Score</td>
<td>AC 4.582</td>
<td>98.2</td>
<td>95.0</td>
<td>97.3</td>
<td>98.2</td>
<td>95.0</td>
<td>0.982 (0.017)</td>
</tr>
<tr>
<td></td>
<td>NAC 6.682</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>1.0 (1.0)</td>
</tr>
<tr>
<td></td>
<td>TH 6.102</td>
<td>95.2</td>
<td>100.0</td>
<td>97.6</td>
<td>100.0</td>
<td>95.2</td>
<td>0.995 (0.006)</td>
</tr>
</tbody>
</table>

GUCI, Göteborg University Cirrhosis Index; ROC, receiver operating characteristic; ACC, diagnostic accuracy; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; AC, alcoholic cirrhosis; NAC, nonalcoholic cirrhosis; TH, toxic hepatitis.
King score strongly correlated with the stage of liver fibrosis. Our findings indicate stronger clinical performance characteristics (sensitivity, specificity, and accuracy) compared with the data reported by Cross et al and Bota et al.6,17 Our cut-off points were designated by the GraphROC program (GraphROC); we did not preselect patients on the grounds of biochemical tests results.

The final noninvasive marker that we examined involved combinations of AST, INR, and PLT count was the GUCI index. The cut-off points were similar for nonalcoholic and alcoholic cirrhosis and toxic hepatitis (0.316, 0.309, and 0.330, respectively). There were no significant differences in the GUCI index among liver diseases. The accuracy of the GUCI score for predicting cirrhosis and toxic hepatitis ranges from 92% to 100%. The GUCI index was also studied by Ehsan et al18 and Fouad et al13 in patients with HCV infection. With cut-off values of 1.56 or greater and 1.50 or greater, respectively, as optimal cut-off values for the diagnosis of cirrhosis, these researchers obtained values of 60% and 74% sensitivity and 88.7% and 89% specificity, respectively. Moreover, Fouad et al13 showed significant correlation between GUCI score and stage of liver fibrosis. Our study also revealed a correlation between GUCI score and severity of liver cirrhosis.

In our opinion, the imprecision of markers visible in Figures 1 and 2 did not derive from sampling errors but from the biodiversity of the study group (eg, one patient had AST activity of 30 U/L, whereas another has AST activity of 600 U/L). Despite those findings, our results indicate the high usefulness of these simple, noninvasive algorithms. However, ensuring the adequate precision of the all the individual tests is necessary for the final diagnostic evaluation of each marker. LM

Conclusion

Our results suggest that the results of simple blood tests with special scores can be helpful in identifying cirrhosis and toxic hepatitis, with greater accuracy in identifying cirrhosis than toxic hepatitis. Also, all the scoring indices precisely reflect the severity of liver cirrhosis; however, caution must be taken in the final diagnostic evaluation of each marker. LM

References