C-reactive protein and Resistin detect bacterial Infection in Liver Cirrhosis
Hendra Koncoro,¹ Dewa Nyoman Wibawa²

ABSTRACT

Background: Bacterial infection is related with poor outcome, but often full of diagnostic difficulties in cirrhotic patients. The role of clinical parameters such as systemic inflammatory response syndrome, leukocyte count, neutrophil count, and other markers remains unclear in liver cirrhosis patients.

Aim: The aim of this study was to evaluate the usefulness of inflammatory markers and determined which markers were best for the diagnosis of infection in decompensated cirrhotic patients.

Methods: This was a diagnostic study consisted of 80 cirrhotic patients admitted to Sanglah general hospital, Denpasar from August 2014 until July 2015. The presence of infection was evaluated. Markers of infection consist of leukocyte count, neutrophil count, neutrophil to lymphocyte ratio (NLR), C-reactive protein (CRP) and resistin were measured. Accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined.

Results: Twenty patients (25%) had bacterial infections and spontaneous bacterial peritonitis (SBP) was the most common infections occurred. NLR, CRP, and resistin were higher in bacterial infections group (p < 0.05). Multiple logistic regression analyses showed that CRP and resistin were predictive factor for occurrence of bacterial infections (p < 0.05). For the diagnosis of infection, baseline CRP – using a 11.65 mg/L cut-off value - and resistin – using a 13 ng/mL cut-off value - generated area under the receiver operating characteristic (ROC) curve of 0.796 and 0.787, respectively. The sensitivity, specificity, PPV, and NPV for CRP were 90%, 73%, 52.9%, and 95.7%, respectively. For resistin, the sensitivity, specificity, PPV, and NPV were 90%, 59%, 41.9%, and 94.6% respectively.

Conclusions: The present study suggests moderate to high accuracy for CRP and resistin as a diagnostic aid for bacterial infections in liver cirrhosis.

Key words: C-reactive protein, Resistin, Bacterial infection, Liver cirrhosis

BACKGROUND

Bacterial infections are common comorbidity occurred in liver cirrhosis patients and represent one of the most reasons of progression to liver failure.¹,² Incidence and severity of infection in cirrhosis is greater than in the population without cirrhosis.¹,³ Approximately 25-35% of liver cirrhosis patients develop infections.¹ However, there is no national data in Indonesia about the prevalence of bacterial infection in liver cirrhosis. Bacterial infections need to be detected promptly due to their potencies in decompensate hepatic status which may lead to mortality.⁴ Arvaniti even found that infections can increased mortality four-fold.⁵

Bacterial infection in liver cirrhosis may be either community acquired, or hospital acquired.³ Bacterial infections that commonly occurred can be SBP, urinary tract infections, respiratory tract infections, skin and soft tissue infections, and bacteremia.⁵ In diagnosing bacterial infections in liver cirrhosis patients, culture need to be done, however, it is only positive in 50-70% patients, and this method is time-consuming.²

Infection in general is diagnosed when there is focus of infection accompanied with changes in vital signs, indicate inflammatory response syndrome. However, chronic liver disease per se is an inflammatory condition, and this disease and its treatment may induce changes in vital signs and abnormalities that make bacterial infections difficult to be
diagnosed. Hyperdynamic circulation caused tachycardia, beta-blockers treatment reduced heart rate, hepatic coma lead to tachypnea, hypersplenism decreases production of white blood cells, and hepatocellular failure decreases production of several acute phase reactants. Early diagnosis of bacterial infections is a crucial step in the management of patients with cirrhosis. Therefore, markers of infection need to be further tested to recognize their diagnostic value which can be different from non-cirrhotic population.

Several markers of bacterial infections in cirrhosis has been studied. Acute phase proteins such as CRP, procalcitonin (PCT), and lipopolysaccharide-binding protein (LBP) were significantly higher in patients with clinically overt infections. CRP and PCT are markers that had high sensitivity and specificity. Metaanalysis conducted by Lin et al (2014) had confirmed that PCT was comparable to CRP in diagnosis of systemic infection in liver cirrhosis. However, there are lots of new assays and inflammatory markers need to be studied which can detect bacterial infections in liver cirrhosis and still need to be explored in the future.

This study was aimed: (i) to obtain which infection markers may be used in detection of bacterial infection in cirrhosis and to know diagnostic value of these markers in establishing diagnosis of bacterial infection; and (ii) to define the best cut-off values for these markers to identify bacterial infections in patients with cirrhosis. These markers may be used as a screening tool in detection of bacterial infection in liver cirrhosis patients.

METHODS

This diagnostic test study was conducted in Gastroenterohepatology Division of Sanglah hospital, Denpasar, Bali, Indonesia. The study was done to recognize infection markers for detection of bacterial infection in liver cirrhosis and to know their diagnostic value. Samples were obtained by consecutive sampling from liver cirrhosis patients who were admitted to Sanglah hospital from August 2014 until July 2015.

Inclusion criteria in this study were liver cirrhosis patient aged 15-60 years old and signed informed consent. Exclusion criteria were comorbidity of chronic kidney disease, hypertension, coronary heart disease, hyperthyroid, malignancy, diabetes, and concomitant use of anti-hyperglycemic agents, insulin, and steroids.

The diagnosis of cirrhosis was based on clinical, biochemical, and ultrasonographic features. Laboratory tests and clinical data, including severity of cirrhosis graded according to the Child-Turcotte-Pugh (CTP) classification, presence of ascites, encephalopathy, blood chemistry and blood cell counts including CRP concentration, resistin concentration and ascitic fluid cell counts were captured at enrollment. Serum CRP level was measured by an immunoturbidimetric assay. Serum resistin was measured by enzyme-linked immunosorbent assay (ELISA) using Quantikine Human Resistin Immunoassay. The NLR was calculated by dividing the neutrophil count by the lymphocyte count. During hospitalization, cultures of blood, urine, ascites, and sputum were taken when an infection was suspected.

Collected data were analyzed descriptively. Normality test was done by using Kolmogorov-Smirnov test. At first, we tested several markers in detection of bacterial infection in liver cirrhosis patients such as leukocyte count, neutrophil count, NLR, CRP, and resistin by using independent t-test or Mann-Whitney. Significant markers identified through univariate analysis then tested by using multivariate analysis. Markers with p value less than 0.05 then included for analysis. Cut-off were determined by using ROC curve for markers which can differentiate bacterial infection. Diagnostic value of markers consisted of accuracy, sensitivity, specificity, PPV, NPV, positive likelihood ratios, and negative likelihood ratios were measured. Calculation of diagnostic accuracy to detect bacterial infection generated by these markers was implemented. A p value of < 0.05 was considered statistically significant. All tests were two-tailed and were performed by the SPSS software, version 17.0 (SPSS, Chicago, IL, USA).

RESULTS

Out of 92 samples obtained during data collecting, 80 liver cirrhosis patients fulfilled the inclusion criteria. In this study, 54 (67.5%) were male and 26 (32.5%) were female. Bacterial infection was found in 20 (25%) patients, with SBP as the most common findings (50%), accompanied with urinary tract infection in as much as 30% and pneumonia in the third place with amount of 10% followed by cholecystitis (5%) and cellulitis (5%). Mean age was 53.02 ± 13.64 years. Leukocyte count, neutrophil count, NLR, CRP, and resistin were not normally distributed. Complete data was shown in Table 1.

There were no significant differences in gender, age, encephalopathy, variceal bleeding, AST, ALT, total bilirubin, white blood count, and neutrophil count between the 2 groups. However, patient with infection had more ascites and higher classes of CTP than patients without infection. NLR, CRP, and resistin were significantly higher in the infection group than in the non-infection group. Distribution of each markers were explained in Fig. 1.

Multivariate analysis then conducted to 5 variables statistically significant in univariate analysis. Presence of ascites, CTP class C, NLR, CRP and resistin were variables included to differentiate both conditions. However, multivariate analysis only found two markers which adequately diagnosis bacterial infections (Table 2).

Two markers which adequately determined bacterial infections in liver cirrhosis were resistin and CRP. The performance of serum CRP and resistin for the diagnosis of infection was evaluated by ROC curve (Fig. 2). The areas under the ROC curve for CRP and resistin for diagnosing infection were 0.796 and 0.787 respectively.

Based on the ROC curve, cutoffs were chosen to predict the absence or presence of infection (Table 3). The best overall performance for CRP was observed at a cutoff of 11.65 mg/L. This value exhibited accuracy, sensitivity and specificity of
77.5%, 90% and 73% respectively. Cutoff resistin was 13 ng/mL with accuracy, sensitivity and specificity of 66.25%.

Table 1: Demographic, clinical and laboratory characteristics of patients with or without bacterial infections in liver cirrhosis

<table>
<thead>
<tr>
<th></th>
<th>Infected (n = 20)</th>
<th>Non-infected (n = 60)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/ female, n)</td>
<td>14/6</td>
<td>40/20</td>
<td>NS</td>
</tr>
<tr>
<td>Age (mean ± SD, yr)</td>
<td>50.90 ± 16.55</td>
<td>53.73 ± 12.61</td>
<td>NS</td>
</tr>
<tr>
<td>Ascites present (n [%])</td>
<td>16 (80%)</td>
<td>27 (45%)</td>
<td>0.007</td>
</tr>
<tr>
<td>Encephalopathy (n [%])</td>
<td>5 (25%)</td>
<td>10 (17%)</td>
<td>NS</td>
</tr>
<tr>
<td>Variceal bleeding (n [%])</td>
<td>4 (20%)</td>
<td>24 (40%)</td>
<td>NS</td>
</tr>
<tr>
<td>CTP (A and B/ C, n)</td>
<td>5/15</td>
<td>39/21</td>
<td>0.004</td>
</tr>
<tr>
<td>AST (U/L, median [IQR])</td>
<td>80.70 (10.50 – 2411.22)</td>
<td>58.55 (10.50 – 2411.22)</td>
<td>NS</td>
</tr>
<tr>
<td>ALT (U/L, median [IQR])</td>
<td>49.50 (20.08 – 424.30)</td>
<td>41.90 (7.50 – 974.90)</td>
<td>NS</td>
</tr>
<tr>
<td>TBIL (mg/dL, median [IQR])</td>
<td>4.63 (1.02 – 28.37)</td>
<td>1.99 (0.28 – 21.13)</td>
<td>NS</td>
</tr>
<tr>
<td>WBC (x 10^9 cells/L, median [IQR])</td>
<td>8.96 (2.78 – 25.40)</td>
<td>6.48 (0.87 – 18.86)</td>
<td>NS</td>
</tr>
<tr>
<td>Neutrophil count (x 10^9 cells/L, median [IQR])</td>
<td>5.91 (2.15 – 21.37)</td>
<td>4.64 (0.63 – 15.99)</td>
<td>NS</td>
</tr>
<tr>
<td>NLR (median [IQR])</td>
<td>5.72 (2.68 – 13.29)</td>
<td>3.49 (1.23 – 17.36)</td>
<td>0.018</td>
</tr>
<tr>
<td>CRP (mg/L, median [IQR])</td>
<td>24.60 (1.10 – 89.20)</td>
<td>7.30 (0.02 – 66.80)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Resistin (ng/mL, median [IQR])</td>
<td>31.79 (6.53 – 69.63)</td>
<td>12.34 (3.59 – 66.77)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Abbreviations: TBIL, total bilirubin; WBC, white blood cell; NLR, neutrophil lymphocyte ratio; CRP, C-reactive protein; IQR, interquartile range; NS, not significant.

Figure 1: Distribution of the serum concentration of resistin (A), C-reactive protein (CRP) (B) and neutrophil-lymphocyte ratio (NLR) (C) in liver cirrhosis patients with or without infections.
**DISCUSSION**

Liver cirrhosis is an immunocompromised condition and thus are highly susceptible to the dissemination of infections that worsen hepatic function and results in severe disease complications. In cirrhosis, there are changes in the intestinal flora and intestinal barrier, reduced reticuloendothelial function, deficiencies in C3 and C4, decreased opsonic activity of the ascetic fluid and neutrophil leukocyte dysfunction. Therefore, prompt diagnosis and early treatment of infections are vital. Obviously, it is crucial to determine a new clinical laboratory test for predicting infections and assist the physician in making treatment decisions. Although there are previous data related to inflammatory markers levels for the diagnosis of bacterial infection in patients with cirrhosis, information about the usefulness of these tests in patients admitted for complications of the liver disease is still lacking. In the present study, we assessed the clinical utility of inflammatory markers for identifying bacterial infections in liver cirrhosis.

Our data showed 20 out of 80 (25%) patients were hospitalized with bacterial infections in liver cirrhosis. This data was quite similar with prevalence worldwide, which is approximately 25-35%. Therefore, prompt diagnosis of bacterial infections need to be established on liver cirrhosis patients admitted to prevent increase of mortality. Ascites and higher degree of CTP class were greater in group with bacterial infections. These conditions were reasonable due to most bacterial infections occurred in liver cirrhosis patients were spontaneous bacterial peritonitis with ascites as its main risk factors. In our study, CTP class C was

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**Table 2:** Multivariate analysis on factors which determine bacterial infections in liver cirrhosis

<table>
<thead>
<tr>
<th></th>
<th>Infected (n = 20)</th>
<th>Non-infected (n = 60)</th>
<th>P value</th>
<th>Multivariate analysis P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascites present (n [%])</td>
<td>16 (80%)</td>
<td>27 (45%)</td>
<td>0.007</td>
<td>0.08</td>
</tr>
<tr>
<td>CTP (A and B/ C, n)</td>
<td>5/15</td>
<td>39/21</td>
<td>0.004</td>
<td>0.442</td>
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</tbody>
</table>

Abbreviations: NLR, neutrophil lymphocyte ratio; CRP, C-reactive protein; IQR, interquartile range

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**Table 3:** Diagnostic accuracy of C-reactive protein and resistin in diagnosing infection in liver cirrhosis

<table>
<thead>
<tr>
<th>Markers</th>
<th>Cutoff</th>
<th>Accuracy (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>LR+</th>
<th>LR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>11.65 mg/L</td>
<td>77.5</td>
<td>90</td>
<td>73</td>
<td>52.9</td>
<td>95.7</td>
<td>3.33</td>
<td>0.14</td>
</tr>
<tr>
<td>Resistin</td>
<td>13 ng/mL</td>
<td>66.25</td>
<td>90</td>
<td>59</td>
<td>41.9</td>
<td>94.6</td>
<td>2.20</td>
<td>0.17</td>
</tr>
</tbody>
</table>

CRP: C-reactive protein (mg/L), PPV: positive predictive value, NPV: negative predictive value
also observed as a significant risk factor for bacterial infections. The majority of bacterial infections patients (75%) belonged to Child C status. This condition may be caused by poorer liver function resulted in increased susceptibility to infection due to immune system defects, including the defective bactericidal function of immunoglobulin, decreased polymorphonuclear leukocyte activity, complement deficiency, and reduced number of Kupffer cells. Gut bacterial translocation is found to be significantly increased in Child C than in Child A and B patients.

In our study, gender, age, encephalopathy, and variceal bleeding were similar between the 2 groups. The concentrations of NLR, CRP and resistin were significantly higher in the liver cirrhosis patients with infections than in those without infections. NLR has been known nowadays as a simple marker of systemic inflammation in general population. NLR itself also has been established as indicator of infection in non-cirrhotic patients. Increase of NLR itself in bacterial infections may be explained from facts that increase of proinflammatory condition mediated by neutrophil will suppress its apoptosis to augment neutrophil capabilities on killing bacteria. At the same time, lymphocyte apoptosis also occurred in thymus and spleen. Study in Asia stated the usefulness of CRP and NLR for predicting outcome in patients with liver cirrhosis. These studies found that NLR failed in detection of infection, however this marker can predict 1 month survival in patients with liver cirrhosis. Studies conducted so far which mention NLR were limited. These studies gave information about usefulness of NLR in prediction of survival, outcome, and mortality in patients with liver cirrhosis. In this study NLR was found as one of the tools in detection of bacterial infection. In this study, cut-off was 4.14. However, when adjusted with other parameters, NLR failed to be one simple tool in diagnosing infection. Inability of NLR in prediction of liver cirrhosis when adjusted with other parameters may be explained from splenomegaly and hypersplenism condition that caused increased destruction of blood cells include white blood cells. Several research also mentioned function of NLR as prognostic factor instead of marker of bacterial infections.

In contrary to NLR, our study found that CRP and resistin may be used detection of bacterial infections. The diagnostic and prognostic value of CRP and resistin in liver diseases has been evaluated in several studies. EASL position paper in 2013 declared that CRP and PCT may be used in detecting infection in patients with cirrhosis due to its similarities with non-cirrhotic patients. Although there were decrease of CRP which is mainly produced by hepatocytes, the predictive power of CRP for detecting infection has been found to be similar in patients with and without cirrhosis. A review of literature conducted by Pieri et al in 2014 found out that CRP is still reliable in prediction of bacterial infection in cirrhosis. However, there are still many questions regarding the optimal use of CRP in this patient population. This is the rationale for assessing the predictive power of CRP in the present study. In the present study, the best cut-off value for CRP was 11.65 mg/L for the diagnosis of infections. Similarly, in general population 10 mg/L is the cut-off value that has been used for differentiating bacterial infection. By using 11.65 mg/L as cut-off level, sensitivity and specificity were 90% and 73%, respectively. On the other hand, Papp, et al. prospectively evaluated 368 patients with liver cirrhosis admitted to a hospital unit and found that 10 mg/L was the best cut-off value for CRP in diagnosing infection, with sensitivity of 84% and specificity of 91%. Based on our findings, the serum CRP cut-off values > 10 mg/L will yield lesser specificity. With 90% sensitivity CRP more than 11.65 mg/L may decrease the possibility of miss some cirrhotic patients with infections who should undergo early empirically based antibiotic treatment.

In this study, we also found that in liver cirrhosis with bacterial infections, serum resistin levels were significantly different. Serum resistin levels was biomarker for the diagnosis of bacterial infections, with cut-off of 13 ng/mL. The sensitivity and specificity were 90% and 59%, respectively. Studies of resistin as a new group of inflammatory markers was still limited in population of liver cirrhosis. So far, resistin was seen increased with increasing class of CTP, and related to inflammation and insulin resistance. Studies regarding resistin role in bacterial infections were scarce and limited only to noncirrhotic population. Study conducted by Johansson et al showed that resistin increase in patients with severe sepsis or septic shock. There are no data in the literature on resistin levels of liver cirrhosis patients with bacterial infections. Resistin is mainly synthesized by macrophages or adipocyte. Increased resistin showed that macrophages response is still adequate even in condition of immunosuppression such as liver cirrhosis. In the present study, the area under the ROC curve for resistin in the diagnosis of infection was 0.787. Until now, there are no studies evaluating the role of resistin in predicting infection. Therefore, resistin may be one of diagnostic tool in aid diagnosis of bacterial infections.

In the present study, higher levels of CRP and resistin were associated with presence of infection. As screening tools, a diagnostic test need to have a good sensitivity for screening purpose. With CRP level of 11.65 mg/L or resistin 13 ng/mL, these markers seem adequate in rule out infections.

We acknowledge some limitations to our analysis. The relatively small number of patients limiting the interpretation of the results. However, the number of patients included in most studies evaluating inflammatory markers for the diagnosis of infection in patients with hepatic cirrhosis was similar to our sample. Even so, the results presented herein require external validation by prospective randomized multicenter studies especially with regard to the chosen cut-offs of the acute-phase markers.

In conclusion, resistin and CRP showed good accuracy as screening tests in diagnosing bacterial infection in cirrhotic patients. In the future, these modalities may aid clinician in predicting which patient more likely to have infections.
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REFERENCES


