RESEARCH ARTICLE

Diagnostic value of ROC curve evaluation of serum markers in acute cholecystitis with bacterial infection

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Abstract

Objective: To explore correlation of serum markers human neutrophil lipocalin and C-reactive protein with acute cholecystitis associated with bacterial infection, and to evaluate the diagnostic value of the markers.

Method: The cross-sectional study was conducted from January 2018 to April 2020 at the Beijing Luhe Hospital, Capital Medical University, Beijing, China, and comprised acute cholecystitis patients who were divided into bacterial infection group A and non-bacterial infection group B. Serum human neutrophil lipocalin and C-reactive protein were measured for both the groups. Receiver operating characteristic curve was used to evaluate the diagnostic value of the two markers in acute cholecystitis associated with bacterial infection. Data was analysed using SPSS 25.

Results: Of the 145 patients, 65(45%) were in group A; 36(55.38%) males and 29(44.62%) females with a mean age of 45.79±2.50 years. In group B there were 80(55%) subjects; 45(56.25%) males and 35(43.75%) females with a mean age of 46.16±2.52 years ($p>0.05$). In group A, there were 60(92.31%) cases of acute calculous cholecystitis, and 5(7.69%) had acute acalculous cholecystitis compared to 73(91.25%) and 7(8.75%), respectively, in group B ($p>0.05$). Serum human neutrophil lipocalin and C-reactive protein levels in group A were higher than group B ($p<0.001$). Serum human neutrophil lipocalin showed a high positive correlation with C-reactive protein in group A ($r=0.800$, $p<0.001$), and a moderate positive correlation in group B ($r=0.683$, $p<0.001$). Area under the curve of serum human neutrophil lipocalin associated with C-reactive protein was 0.901 (95% confidence interval: 0.850-0.953), which was higher than that of serum human neutrophil lipocalin and C-reactive protein alone, with sensitivity 95.40% and specificity 80%.

Conclusion: The combined use of serum human neutrophil lipocalin and C-reactive protein may be used as an effective indicator for early diagnosis, identification and monitoring of acute cholecystitis with bacterial infection.

Keywords: Acute cholecystitis, Bacterial infection, C-reactive protein, Human neutrophil lipocalin, Receiver operating characteristic. (JPMA 72: 1133; 2022)

DOI: https://doi.org/10.47391/JPMA.3422

Introduction

Acute cholecystitis is one of the common acute abdominal diseases. It is an acute inflammation of gallbladder. The main clinical symptoms include sudden right upper abdominal cramps, accompanied by nausea, vomiting and fever.[1] When associated with bacterial infection, acute cholecystitis patients in a serious condition may develop gallbladder gangrene, perforation, septic shock and even death if not diagnosed and treated in time.[2,3]

Blood culture and bile bacterial culture tests are currently the gold standard for clinical diagnosis of acute cholecystitis associated with bacterial infection,[4] but the detection takes too long to provide clinical emergency services. Meanwhile, these test methods, subject to many external influence factors, suffer high pollution rates and low positive rates.

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In recent years, some new serum markers are found easier to be applied in clinical practice, and their sensitivity and specificity are also relatively high. Human neutrophil lipocalin (HNL) is an essential component of the secondary granules of human neutrophils.[5] When the body is in a normal physiological state, its serum HNL content is low and relatively stable. In case of infection, neutrophils in the peripheral blood are further activated, and HNL is released to the outside of cells, causing a significant increase in serum HNL level. A study showed HNL could be used as a biomarker for the diagnosis of acute infection.[6] Fjaertoft et al. found that the serum HNL of children with acute bacterial infection was significantly raised compared to acute viral infection and HNL was considered an effective tool for identifying acute bacterial or viral infections.[7] Therefore, clinical HNL detection can be used for the diagnosis and differential diagnosis of bacterial and viral infections in patients.

C-reactive protein (CRP), a sensitive indicator of bacterial infection, is a non-specific acute-phase protein produced

by liver.[8] The CRP content is very low in healthy individuals. Serum CRP level in patients with acute bacterial infection increases significantly, but, as the condition improves, the CRP level decreases. The determination of CRP is relatively simple, easy and low-cost. A meta-analysis showed that CRP detection has a higher accuracy in the diagnosis of bacterial infection in patients with cirrhosis.[9] Potempa et al. found that serum CRP level significantly increased in patients with coronavirus disease-2019 (COVID-19), and CRP can be used to assess the severity of disease in patients with COVID-19.[10] Wasunna et al. found that serum CRP can quickly diagnose or exclude early bacterial infection in newborns.[11] Therefore, serum CRP plays an important role in diagnosing bacterial infections.

At present, the correlation of serum HNL and CRP with acute cholecystitis associated with bacterial infection is not clear.

The current study was planned to detect the serum HNL and CRP levels of patients diagnosed with acute cholecystitis, to explore the correlation between the two and acute cholecystitis with bacterial infection, and to evaluate the diagnostic value of the two markers in acute cholecystitis associated with bacterial infection.

Patients and Methods

The cross-sectional study was conducted from January 2018 to April 2020 at the Beijing Luhe Hospital, Capital Medical University, Beijing, China. After approval by the institutional ethics review committee, the sample size was calculated using G-Power calculator.[12]

The sample was raised by randomly selecting patients with acute cholecystitis. Those included were patients meeting the clinical diagnostic criteria for acute cholecystitis,[13] not on drugs affecting the immune function or antibacterial drugs for the preceding 3 months, those who came to hospital after the onset of illness without having received any prior treatment, and those with a one-month history of inflammation.

Combined bacterial infection was diagnosed if the patient's blood culture was positive after admission, or the culture of gallbladder bile, taken from laparoscopic or open cholecystectomy, was positive, or the culture of biliary drainage, taken from percutaneous transhepatic gallbladder drainage, was positive.[14]

Patients with severe dysfunction of other organs, those having acute pancreatitis, bile duct stones, acute cholangitis, malignant tumours, chronic cholecystitis, viral infection, infections of other sites of the body, and other conditions of raised CRP were excluded. Written informed consent was obtained from each patient.

The patients were divided into bacterial infection group A and non-bacterial infection group B.

Venous blood was collected for HNL and CRP detection before the subjects received antimicrobial therapy. Serum HNL was measured using enzyme-linked immunosorbent assay (ELISA). Serum CRP was measured on a fully automatic biochemical analyser using rate nephelometry assay.

Data was analysed using SPSS 25. Normal distribution of numerical variables in the groups was assessed using the Kolmogorov-Smirnov test. Numerical variables conforming to normal distribution, such as HNL and CRP etc., were expressed as mean and standard deviation. Independent sample t test was used to compare normally-distributed numerical variables. Categorical variables were expressed as frequencies and percentages, and chi-square test was used for inter-group comparisons. Pearson correlation test was used for normally distributed data. Receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of the two markers in acute cholecystitis associated with bacterial infection. The level of statistical significance was set at $p<0.05$.

Results

Of the 145 patients, 65(45%) were in group A; 36(55.38%)

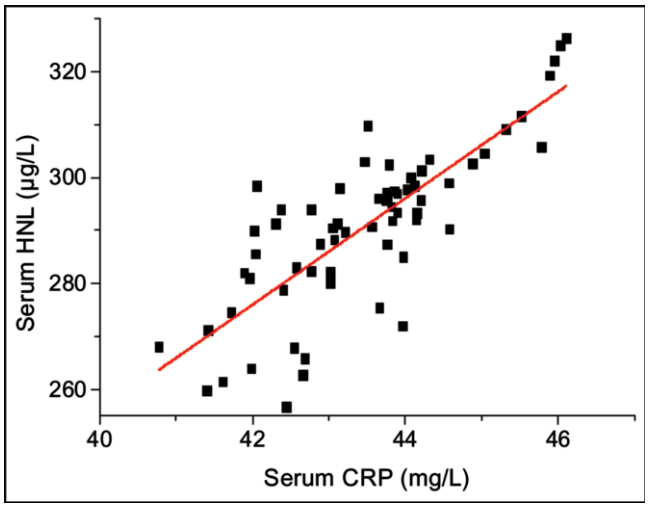

Figure-1: Serum Human neutrophil lipocalin (HNL) showed a high positive correlation with C-reactive protein (CRP) in the bacterial infection group (r=0.800, p<0.001).

Table: Comparison of serum HNL and CRP levels between bacterial infection group and non-bacterial infection groups.

Parameter	Bacterial infection group (n=65)	Non-bacterial infection group (n=80)	P value
Serum HNL(µg/L)	290.85±15.42	115.52±5.63	?0.001
Serum CRP(mg/L)	43.48±1.23	18.09±0.68	?0.001

HNL: Human neutrophil lipocalin, CRP: C-reactive protein.

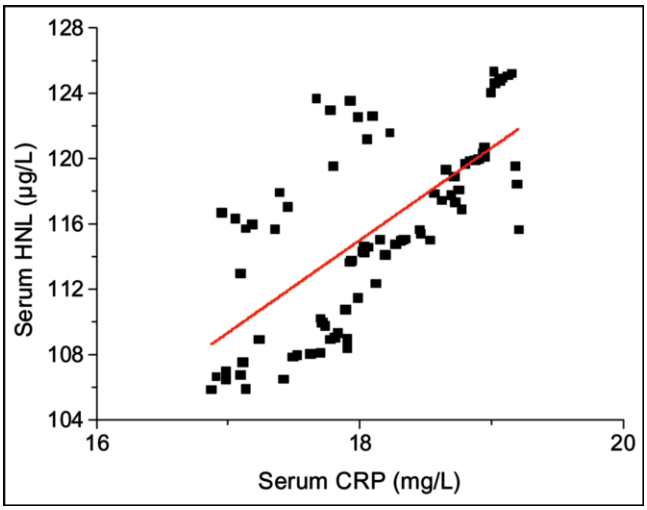

Figure-2: Serum Human neutrophil lipocalin (HNL) showed a moderate positive correlation with C-reactive protein (CRP) in the non-bacterial infection group (r=0.683, p<0.001).

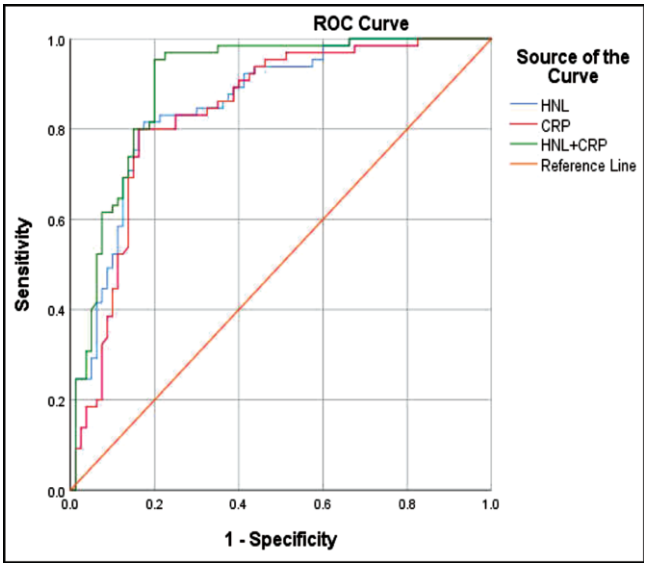

Figure-3: Receiver operating characteristic (ROC) curve of serum Human neutrophil lipocalin (HNL) and C-reactive protein (CRP) in the diagnosis of acute cholecystitis with bacterial infection.

males and 29(44.62%) females with a mean age of 45.79±2.50 years. In group B there were 80(55%) subjects; 45(56.25%) males and 35(43.75%) females with a mean age of 46.16±2.52 years (p>0.05). In group A, there were 60(92.31%) cases of acute calculous cholecystitis, and 5(7.69%) had acute acalculous cholecystitis compared to 73(91.25%) and 7(8.75%), respectively, in group B (p>0.05).

Serum HNL and CRP levels in group A were higher than group B (p<0.001) (Table-1). Serum HNL showed a high positive correlation with CRP in group A (r=0.800, p<0.001) (Figure-1), and a moderate positive correlation in group B (r=0.683, p<0.001) (Figure-2).

ROC curve analysis showed that the area under the curve (AUC) of serum HNL in group A was 0.856 (95% confidence interval [CI]: 0.794-0.917), with the diagnostic sensitivity 81.50% and specificity 82.50%, while AUC of serum CRP was 0.754 (95% CI: 0.634-0.875), with sensitivity 80% and specificity 83.80%. The AUC of serum HNL combined with CRP was 0.901 (95% CI: 0.850-0.953), which was higher than that of serum HNL and CRP alone, with sensitivity 95.40% and specificity 80% (Figure-3).

Discussions

Acute cholecystitis is easily misdiagnosed because its early symptoms are not typical. It is also clinically difficult to assess its progression in patients. Acute cholecystitis with bacterial infection is a sign of aggravation. Therefore, early detection and intervention of bacterial infection in such patients are of great significance.

HNL is the product of neutrophil degranulation. Under normal physiological conditions, HNL is lowly expressed in kidney, lung, stomach, colon and other tissues. HNL detection can help diagnose infectious diseases.[15] It has been shown that HNL can be used to diagnose respiratory bacterial infections.[16] The current study showed that serum HNL had a high diagnostic value in acute cholecystitis associated with bacterial infection.

Inflammatory markers can help diagnose pathological jaundice due to bacterial infection.[17] Blood CRP can be used as a parameter for ongoing inflammation.[18] A study revealed that CRP >40mg/l had an infectious focus suggestive of bacterial infection.[19]

In the current study, serum CRP levels had a high value in the auxiliary diagnosis of acute cholecystitis and bacterial infection.

Also, serum HNL showed a high positive correlation with CRP in the bacterial infection group and a moderate positive correlation in the non-bacterial infection group. Xu et al. also found that serum HNL levels were positively correlated with CRP levels in patients with bacterial infection.[20]

The current study also found that the combined use of serum HNL and CRP was highly effective in diagnosing acute cholecystitis with bacterial infection. The reason may be that the diagnosis of serum HNL with serum CRP can better reflect the level of inflammation and bacterial infection in the body. When the body is stimulated by inflammation and bacterial infection, neutrophils release a large amount of HNL.[6] CRP is a kind of acute reactive

protein generated by liver. Since it is involved in eliminating microorganisms directly, it is regarded as a sensitive index for bacterial infections.[21] The rise of CRP is related to the presence of inflammation and infection.[22]

Conclusions

The combined use of serum HNL and CRP was an effective indicator for early diagnosis, identification and monitoring of acute cholecystitis with bacterial infection.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

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