Diagnostic and prognostic roles of soluble CD22 in patients with Gram-negative bacterial sepsis

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BACKGROUND: Soluble CD22 (sCD22) is a fragment of CD22, a B cell-specific membrane protein that negatively regulates B-cell receptor signaling. To date, sCD22 has only been regarded as a tumor marker of B-cell malignancies. Its expression in infectious diseases has not yet been assessed.

METHODS: Serum concentrations of sCD22, procalcitonin (PCT) and interleukin-6 (IL-6) were measured by enzyme-linked immunosorbent assays in patients with intra-abdominal Gram-negative bacterial infection. Receiver operating characteristic curve analysis was performed to evaluate the diagnostic accuracy of these biomarkers in this type of infection. The correlations between biomarkers and the Acute Physiology and Chronic Health Evaluation (APACHE) II scores were also analyzed.

RESULTS: Concentrations of sCD22 were significantly elevated in patients with sepsis and the elevation is correlated with the severity of sepsis. sCD22 was also slightly elevated in patients with non-infected systemic inflammatory response syndrome or local infection. The diagnostic accuracy of sCD22 for sepsis was equivalent to that of PCT or IL-6. In addition, the correlation of sCD22 with APACHE II scores was stronger than that of PCT or IL-6.

CONCLUSIONS: Serum sCD22 is a novel inflammatory mediator released during infection. This soluble biomarker plays a potential role in the diagnosis of Gram-negative bacterial sepsis, with a diagnostic accuracy as efficient as that of PCT or IL-6. Furthermore, sCD22 is more valuable to predict the outcomes in patients with sepsis than PCT or IL-6. The present study suggested that sCD22 might be potentially useful in supplementing current criteria for sepsis.

Key Words: soluble CD22; procalcitonin; interleukin-6; sepsis; systemic inflammatory response syndrome

Introduction

Biliary infections, which are mainly induced by Gram-negative bacteria, are the leading cause of sepsis and septic shock in hepatobiliary surgery. Despite improvements in surgical methods and the introduction of novel powerful antibiotics, sepsis induced by biliary infection still has high morbidity and mortality. Various inflammatory mediators are released during infection and play key roles in the pathogenesis of sepsis. Identification of new mediators would increase the understanding of the complex pathophysiological events involved in sepsis and subsequent improvement in early diagnosis and treatment.

CD22 is a molecule belonging to the sialic acid-binding immunoglobulin-like lectin (Siglec) family. It is found on the surface of mature B cells and to a lesser extent on some immature B cells. Generally CD22 is a regulatory molecule that negatively regulates B-cell receptor signaling and plays a key part in establishing the B-cell activation threshold. Soluble CD22 (sCD22) is generated by the cleavage of the extracellular domain of CD22 on the membrane surface, and sCD22 has been regarded as a tumor marker of B-cell malignancies.
During the progression of sepsis, soluble membrane markers generated by the cleavage of membrane proteins are often released from the surface of activated immune cells.\textsuperscript{[12, 13]} Indeed, unmasking of masked CD22 on the cell surface can be observed during B-cell activation.\textsuperscript{[14,15]} We hypothesized that unmasked CD22 is susceptible to cleavage and that the serum concentration of sCD22 correlates with the level of B-cell activation.

Lipopolysaccharide (LPS), an outer-membrane component of Gram-negative bacteria, has been widely recognized as the prime stimulating factor in sepsis.\textsuperscript{[16,17]} The ability of LPS to induce B-cell activation and universal immune responses\textsuperscript{[18-20]} suggests that sCD22 levels in patients with Gram-negative bacterial sepsis, such as biliary infection, might be elevated. The aims of this study were to evaluate the role of sCD22 in Gram-negative bacterial sepsis induced by biliary infection, and to compare the diagnostic and prognostic efficiency of this soluble biomarker with that of procalcitonin (PCT) and interleukin-6 (IL-6), two laboratory markers widely used in the diagnosis and monitoring of sepsis and in the assessment of sepsis severity.\textsuperscript{[21]}

**Methods**

**Ethics statement**

The present study was approved by the Ethical Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China (20140402), and was registered with Chinese Clinical Trial Registry (ChiCTR-RCC-14004598). The study was conducted according to the principles of the Declaration of Helsinki. All subjects were required to provide written informed consent before inclusion in this study.

**Subjects**

A total of 104 serum samples were collected from patients treated at the Department of General Surgery, Wuhan General Hospital of Guangzhou Military Command, Wuhan, China from May to October 2014. The study cohort consisted of 53 patients clinically diagnosed with biliary infection (including cholangitis and cholecystitis) and 10 other intra-abdominal infections, as well as 26 non-infected patients diagnosed with systemic inflammatory response syndrome (SIRS). The criteria for SIRS included two or more of the following conditions:\textsuperscript{[22]} (i) core temperature >38 °C or <36 °C; (ii) pCO\textsubscript{2} <32 mmHg or >20 breaths/min; (iii) pulse rate >90/min; and (iv) white blood cell count >12×10\textsuperscript{9}/L, <4×10\textsuperscript{9}/L or >10% of band forms. Infections included systemic infection, which fulfilled the SIRS criteria and was diagnosed as sepsis (n=38), and local infection (n=25), defined as infection without the signs of SIRS. Sepsis with hypotension or signs of inadequate organ perfusion, such as hypoxemia, metabolic acidosis, oliguria and neurological disorders, was defined as severe sepsis (n=14).\textsuperscript{[23]} The control group consisted of 15 healthy volunteers with no history of autoimmune, inflammatory or tumorous diseases.

**Blood samples**

Blood samples were obtained from each patient at the time of admission when they were suspected to have intra-abdominal bacterial Gram-negative infection or SIRS before any treatment. Peripheral venous blood samples were collected in tubes without additive and allowed to clot at room temperature for 40 minutes. Serum was separated by centrifugation. All serum samples were stored at -80 °C until tested.

**Enzyme-linked immunosorbent assay (ELISA)**

Serum concentrations of sCD22, PCT and IL-6 were measured by ELISA. All samples were diluted 2-fold and analyzed using ELISA kits for sCD22 (Antigenix America), PCT (Abcam) or IL-6 (Biolegend) according to the manufacturers’ protocols.

**Statistical analysis**

Values are presented as means±standard deviations (SD). The comparisons among groups were assessed by one-way ANOVA with post hoc comparison. Receiver operating characteristic (ROC) curves and the areas under the curve (AUCs) were calculated to determine the predictive value of biomarkers. For ROC curve analysis, patients were regrouped into an infection group (sepsis+severe sepsis+local infection) and a non-infection group (SIRS), a systemic infection group (sepsis+severe sepsis) and a local infection group, or a sepsis group (sepsis+severe sepsis) and a SIRS group. The optimal cut-off values with the best combination of sensitivity and specificity were determined. Since the Acute Physiology and Chronic Health Evaluation (APACHE) II scores are associated with the outcomes, the correlations between biomarkers and APACHE II scores were analyzed with linear regression to evaluate the prognostic roles of biomarkers. GraphPad Prism 5.0 was used for all statistical analyses. P values <0.05 were considered statistically significant.

**Results**

**Patient characteristics**

The clinical characteristics of the patients enrolled in this prospective study are summarized in Table 1. Study
groups were not different in age ($P=0.122$) or gender distribution ($P=0.790$). The APACHE II scores were higher in patients with severe sepsis than in the other groups ($P<0.001$). The types of bacteria in 52 of 63 patients were identified and confirmed as Gram-negative bacteria by microbiological tests. No Gram-positive bacteria were identified. Other 11 patients with signs of intra-abdominal infection had no microbial data and were treated as suspected or so-called clinically diagnosed Gram-negative bacterial infection.

**Diagnostic role of sCD22, PCT and IL-6**

The levels of sCD22 (Fig. 1A), PCT (Fig. 1B) and IL-6 (Fig. 1C) rank from low to high: controls, SIRS and local infection, sepsis and severe sepsis. These levels were significantly higher in patients in different groups compared with the controls, in patients with sepsis compared with those with SIRS and local infection, and in patients

<table>
<thead>
<tr>
<th>Table 1. The clinical characteristics of the study population</th>
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<tbody>
<tr>
<td>Characteristics</td>
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<tr>
<td>Age (yr)</td>
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<td>Gender (M/F)</td>
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<td>APACHE II score</td>
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<td>Disease</td>
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<td>Cholangitis</td>
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<td>Cholecystitis</td>
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<td>Peritonitis</td>
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<td>Appendicitis</td>
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<td>Trauma of spleen</td>
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<td>Multiple trauma</td>
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<td>Type of bacteria</td>
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<tr>
<td><em>Escherichia coli</em></td>
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<td><em>Pseudomonas aeruginosa</em></td>
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<td><em>Enterococcus faecalis</em></td>
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<tr>
<td><em>Proteus mirabilis</em></td>
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<tr>
<td>Other G bacteria</td>
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<tr>
<td>No microbial data</td>
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</table>

Continuous variables and categorical variables are present as mean±standard deviation and number respectively. M: male; F: female; APACHE: Acute Physiology and Chronic Health Evaluation; NA: not applicable.
with severe sepsis compared with those with sepsis. The levels of sCD22 and IL-6 were similar in patients with SIRS and those with local infection \((P=0.05)\). PCT levels in patients with local infection were significantly higher than those in patients with SIRS \((P=0.047)\).

Diagnostic accuracy of sCD22 in comparison with PCT and IL-6

To assess the diagnostic accuracy of sCD22, PCT and IL-6, we compared their AUCs between the infection (sepsis+severe sepsis+local infection, \(n=63\)) and non-infection (SIRS, \(n=26\)) groups, the systemic infection (sepsis+severe sepsis, \(n=38\)) and local infection (\(n=25\)) groups, and the sepsis (sepsis+severe sepsis, \(n=38\)) and SIRS (\(n=26\)) groups. Comparison of the infection and non-infection groups showed that the AUCs were 0.755 for sCD22 \((P<0.001)\), 0.814 for PCT \((P<0.001)\), and 0.615 for IL-6 \((P=0.089)\) (Fig. 2A). Comparison of the systemic and local infection groups showed that the AUCs were 0.764 for sCD22 \((P<0.001)\), 0.830 for PCT \((P<0.001)\) and 0.806 for IL-6 \((P<0.001)\) (Fig. 2B). Comparison of the sepsis and SIRS groups showed that the AUCs were 0.849 for sCD22 \((P<0.001)\), 0.903 for PCT \((P<0.001)\) and 0.762 for IL-6 \((P<0.001)\) (Fig. 2C). The optimal diagnostic cut-off values of biomarkers and the sensitivity and specificity for differentiating the different entities of our enrolled patients are shown in Table 2. If the cut-off value was set to 2.2 ng/mL for sCD22, the diagnostic sensitivity for infection was 66.67% and a specificity was 67.92%; the diagnostic sensitivity for systemic infection was 78.95% when sCD22 was >2.4 ng/mL and the specificity was 64.00%; sCD22 >2.3 ng/mL could differentiate sepsis from SIRS with a sensitivity of 81.58% and a specificity of 76.92%. Both PCT and IL-6 were also efficient in differentiating sepsis from SIRS or local infection, as well as severe sepsis from sepsis. These findings showed that sCD22 concentration was valuable in diagnosing Gram-negative bacterial sepsis, when compared with PCT and IL-6 concentrations, especially for the differentiation between sepsis and SIRS.

The relationship between biomarkers and APACHE II scores

There was a positive correlation between sCD22 and APACHE II scores \((r=0.46, P<0.001, \text{Fig. 3})\). Positive correlations with APACHE II scores were also observed.

### Table 2. Diagnostic accuracy of sCD22, PCT and IL-6

<table>
<thead>
<tr>
<th></th>
<th>Infection vs non-infection</th>
<th>Systemic vs local infection</th>
<th>Sepsis vs SIRS</th>
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<tr>
<td></td>
<td>Cut-off</td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>sCD22</td>
<td>2.2 ng/mL</td>
<td>66.67%</td>
<td>67.92%</td>
</tr>
<tr>
<td>PCT</td>
<td>1.38 ng/mL</td>
<td>63.49%</td>
<td>92.31%</td>
</tr>
<tr>
<td>IL-6</td>
<td>non-significant</td>
<td>189.0 pg/mL</td>
<td>65.79%</td>
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The optimal diagnostic cut-off values of biomarkers as well as the sensitivity and specificity for distinguishing between subgroups are present.

**Fig. 2.** Receiver operating characteristic (ROC) curves of sCD22, PCT and IL-6 for distinguishing (A) infection from non-infection, (B) systemic infection from local infection, and (C) sepsis from SIRS. The diagnostic accuracy of sCD22 was as efficient as that of PCT and IL-6. A cut-off value of sCD22 >2.2 ng/mL could distinguish infection from non-infection with a sensitivity of 66.67% and a specificity of 67.92%, that of sCD22 >2.4 ng/mL could distinguish systemic from local infection with a sensitivity of 78.95% and a specificity of 64.00%, and that of sCD22 >2.3 ng/mL could distinguish sepsis from SIRS with a sensitivity of 81.58% and a specificity of 76.92%, suggesting that sCD22 concentration was efficient in diagnosing Gram-negative bacterial sepsis, when compared with PCT and IL-6 concentrations, especially for distinguishing sepsis from SIRS.
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Fig. 3. sCD22 (A), PCT (B) and IL-6 (C) were related to APACHE II scores among all the enrolled patients. sCD22 ($r^2=0.46$, $P<0.001$), PCT ($r^2=0.17$, $P<0.001$) and IL-6 ($r^2=0.13$, $P<0.001$) were positively correlated with APACHE II scores, suggesting that the correlation of sCD22 with outcome was stronger than that of PCT or IL-6.

in PCT ($r^2=0.17$, $P<0.001$) and IL-6 ($r^2=0.13$, $P<0.001$), with relatively low $r^2$. These data demonstrated the prognostic role of these three biomarkers, and the correlation of sCD22 with outcome was stronger than that of PCT or IL-6, suggesting that sCD22 was more valuable in predicting the outcomes of patients with sepsis.

Discussion

Biliary infections, such as acute cholangitis or cholecystitis, are the common conditions that induce sepsis in the field of hepatobiliary surgery. Sepsis refers to severe systemic inflammation in response to invading pathogens. An overwhelming immune response, as mediated by the release of various inflammatory mediators, can lead to shock, multiple organ damage, and even death. Although various inflammatory mediators provide a good indication of the pathological status of sepsis, high levels of inflammatory mediators in surgical patients can also be detected in patients with other conditions, such as severe trauma, invasive surgical procedures, and critical burn injury, leading to SIRS. Therefore, the identification of reliable biomarkers that enable an early diagnosis of sepsis and a prognostic prediction in surgical patients may potentially increase the success of therapeutic interventions and secondary prevention of complications. B cells are important in immune responses induced by sepsis. Apart from the traditional understanding about their roles in antibody production and antigen presentation, recent findings suggest that B cells can enhance early innate immune responses during bacterial sepsis.

However, few B-cell specific biomarkers have been identified in sepsis, despite universal B-cell activation in response to bacterial infection.

CD22 was regarded as a membrane protein on the surface of B cells. Recent findings suggest that its soluble extracellular domain, sCD22, is a tumor marker of B cell malignancies. To our knowledge, however, little is known about sCD22 concentrations in patients with nontumorous diseases. Since CD22 was shown to be involved in the processes of B-cell and even T-cell activation, we hypothesized that the level of sCD22 would correlate with disorders associated with immune activation. In this preliminary study, we attempted to measure sCD22 concentrations in patients with biliary infection and some other intra-abdominal infections, and compared its effectiveness in diagnosing sepsis and predicting outcomes with that of PCT and IL-6.

We found that sCD22 concentrations in patients with sepsis and severe sepsis were significantly higher than those in patients with SIRS and normal controls. To evaluate the diagnostic accuracy of sCD22, we divided our subjects into an infection and a non-infection group; a systemic infection and a local infection group; and a sepsis (including severe sepsis) and an SIRS group. Indeed, ROC curve analysis showed that the diagnostic accuracy of sCD22 for Gram-negative bacterial sepsis was as efficient as that of PCT and IL-6, which are regarded as the best laboratory biomarkers in the diagnosis and monitoring of sepsis. The most practical value in using sCD22 as a biomarker is to differentiate septic patients from non-infected SIRS patients. Our data showed that sCD22 had a relative high diagnostic accuracy and potential practical usefulness in clinical practice. Owing to its specific correlation with B cells, sCD22 would likely provide more specific information about the source of infection than PCT or IL-6. These findings therefore suggested that sCD22 may be potentially useful in supplementing current diagnostic criteria for sepsis.

In this present study, we also found that the serum levels of sCD22 were higher in patients with severe sepsis.
compared with those with sepsis; patients with systemic infection had higher sCD22 than those with local infection, suggesting a correlation between sCD22 concentration and infection severity. It was also showed that the correlation between sCD22 and APACHE II scores was stronger than that of PCT or IL-6. APACHE II score serves as a sensitive predictor of outcomes of patients with sepsis and other diseases in intensive care unit.\[31, 32\] Our study demonstrated that serum levels of sCD22 may predict outcomes in patients with sepsis, and its potential predicting usefulness might be more efficient than that of PCT and IL-6. Furthermore, the present study showed that sCD22 levels were not only elevated in patients with sepsis, but also in those with SIRS or local infection, suggesting that sCD22 might be a non-specific inflammatory mediator associated with immune responses rather than a specific biomarker of sepsis. The B-cell specific expression of CD22 and its unmasking subsequent to cellular activation suggest that sCD22 may be a potential marker of B-cell activation. B-cell activation is involved in many physiological and pathological processes, and is correlated with antibody production and humoral immune responses. Thus, this soluble marker may have more universal implications in the diagnosis, assessment and monitoring of diseases associated with B-cell activation, such as various infections, autoimmune diseases, graft rejections and malignancies.

Gram-negative bacteria are the major organisms observed in intra-abdominal infection and the leading cause of sepsis or septic shock. Diagnostic or prognostic biomarkers for Gram-negative bacterial sepsis would be potentially beneficial in the field of abdominal surgery. Therefore, we focused on Gram-negative bacterial infection in the present study, other pathogens, such as Gram positive bacteria, fungus, virus or parasites were excluded in this study. Definitely, sCD22 was not a biomarker specific to Gram-negative bacterial infection, and more extensive studies including other types of infection should be performed to confirm the diagnostic and prognostic value of sCD22 in infectious diseases. Methodologically, the quantitative measurement of sCD22 in this study was achieved by ELISA analysis. ELISA is a technique based on the principle of immunoassay with an enzyme as the reporter, and the corresponding kits are commercially available with reasonable cost. Other immunoassays based on the similar immune principle, such as enzyme immunoassay and radio immunoassay could also be developed for the detection of this serum biomarker. All these methods are suitable for the rapid test of sCD22 in medical laboratories.

In conclusion, this study was the first attempt to assess sCD22 in infectious diseases. We found that serum sCD22 concentrations were as efficient as PCT or IL-6 in the diagnosis of Gram-negative bacterial sepsis. In addition, sCD22 may predict outcomes in patients with sepsis, with its potential predicting effectiveness more efficient than that of PCT and IL-6. We believe that sCD22, a novel inflammatory mediator released during infection, would likely provide more specific information correlated with B cells, and may be potentially useful to supplement current diagnostic criteria for sepsis.

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Contributors: JYN and CZHK proposed the study. JYN, CX and ZHM performed the study and wrote the first draft. JWD and ZM participated in the collection of blood samples. ZY and DXX collected and analyzed the data. All authors contributed to further drafts. CZHK is the guarantor.

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Ethical approval: This study was approved by the Ethical Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China (approval number: 20140402), and was registered with Chinese Clinical Trial Registry (registration number: ChiCTR-RCC-14004598).

Competing interest: No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References
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15 Razi N, Varki A. Cryptic sialic acid binding lectins on human blood leukocytes can be unmasked by sialidase treatment or cellular activation. Glycobiology 1999;9:1225-1234.

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