Original Article

Soluble Form of Triggering Receptor Expressed on Myeloid Cells-1 (sTREM-1) as a Diagnostic Marker of Serious Bacterial Infection in Febrile Infants Less Than Three Months of Age

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SUMMARY: Traditional risk factors are not adequate for predicting serious bacterial infection (SBI) in febrile infants. The purpose of this study was to evaluate the diagnostic value of the plasma level of soluble form of triggering receptor expressed on myeloid cells-1 (sTREM-1) in predicting SBI in febrile infants less than 3 months old. Forty-four febrile infants less than 3 months old with clinical suspicion of SBI were enrolled. Blood was drawn for measurement of plasma sTREM-1 levels, and microbiological cultures were obtained at the time of admission. Twenty-three infants (52.3%) had SBI and 21 infants (47.7%) had no evidence of SBI based on the results of bacterial culture. sTREM-1 levels were significantly higher in infants with SBI than in infants without SBI (mean ± SD, 324.6 ± 546.3 versus 7.7 ± 16.4, P < 0.0001 after adjusting for age). The area under the receiver-operating characteristic curve was 0.88 for the sTREM-1 level. At a cutoff level of 24.4 pg/mL, the sTREM-1 level yielded a sensitivity of 87%, a specificity of 81%, a positive likelihood ratio of 4.6, and a negative likelihood ratio of 0.2 for differentiating between presence and absence of SBI. In conclusion, sTREM-1 may become a valuable diagnostic tool in the initial evaluation of febrile young infants.

INTRODUCTION

Infants with elevated temperature have an increased risk of serious bacterial infection (SBI), including bacteremia, meningitis, urinary tract infection (UTI), and pneumonia (1,2). Definitive identification of SBI requires a positive culture of the cerebrospinal fluid (CSF), blood, or urine or an identifiable bacterial focus by physical examination or radiograph. However, because most of these infants often have no obviously abnormal physical examinations and the results of the cultures are not immediately available, clinicians must decide on appropriate patient management based on the patient’s history, physical examination, and laboratory testing (3). Traditional risk factors, such as age, sex, and temperature, symptoms and signs, or laboratory findings including leukocyte counts, and C-reactive protein (CRP) level are not adequate predictors of SBI, and well-appearing infants may be facing SBI (4,5). The use of antibiotics is required for those infants who are suspected to have SBI, but antibiotic treatment based only on clinical symptoms and signs may result in overtreatment and contribute to antibiotic resistance (6). Because it is clinically difficult to identify young infants with occult SBI, a number of diagnostic bacterial infection markers have been suggested, and the topic remains the subject of considerable debate.

The triggering receptor expressed on myeloid cells-1 (TREM-1), which belongs to the immunoglobulin superfamily, is a recently discovered cell-surface molecule that has been identified in both human and murine neutrophils and mature monocytes (7). Bouchon et al. revealed that TREM-1 mediates the acute inflammatory response to microbial products. Human tissues infected with bacteria are infiltrated with neutrophils and macrophages that express high levels of TREM-1 (8). Together with an up-regulation of TREM-1 expression on the cell surface, a soluble form of this protein (sTREM-1) has been shown to be released during human sepsis (9-11). The presence of a sTREM-1 in samples of bronchoalveolar lavage fluid from mechanically ventilated patients has been shown to be a good indicator of infectious pneumonia (10,12). In this study, we prospectively investigated the diagnostic value of an assay that measures the plasma level of sTREM-1 for predicting SBI in febrile infants less than 3 months of age, and we considered whether sTREM-1 may be used as a marker of the need for antibiotics. We also compared the plasma level of sTREM-1 and CRP in SBI and non-SBI in these young infants.

MATERIALS AND METHODS

Patients: We enrolled febrile infants who were less than 3 months old with a clinical suspicion of having SBI who were admitted to the neonatal intensive care unit or complete nursing unit of the pediatric department of Kaohsiung Medical University Hospital from October 2005 to July 2006. These febrile young infants were suspected to have SBI if they had any of the following signs and symptoms: tachypnea, dyspnea, tachycardia, bradycardia, decrease of activity, lethargy, and decrease of appetite. In all enrolled patients, a diagnostic work-up was performed to identify or rule out bacterial infection, and antibiotic therapy was prescribed at admission. Blood was drawn for measurement of complete blood counts, CRP, and plasma sTREM-1 levels.

SBI was defined as a pathogen isolated from the CSF or blood or UTI and pneumonia. Pneumonia was diagnosed as...
the presence of related clinical symptoms such as tachypnea, productive cough with consolidation, or fluid in lobar fissures/plura visible on chest X-ray. A UTI was diagnosed as pyuria in a routine urine exam and two sets of urine culture with a single pathogen growth more than 10^5 CFU/mL from a bladder catheterization or more than 10^4 CFU/mL from a bladder in a routine urine exam and two sets of urine culture with a pleura visible on chest X-ray. A UTI was diagnosed as pyuria productive cough with consolidation, or fluid in lobar fissures/the presence of related clinical symptoms such as tachypnea, positive and negative likelihood ratios were calculated for cutoff values of sTREM-1. Sensitivity, specificity, and characteristic (ROC) curves were constructed to illustrate variability of these values. sTREM-1 levels were significantly higher in infants with SBI than in the infants without SBI group.

Laboratory data: Descriptive results of continuous variables were expressed as the mean ± SD. Variables were tested for their association with the diagnosis by using the chi-square test for categorical data and the Mann-Whitney U test for numerical data. The individual values of sTREM-1 level in both groups are plotted in Fig. 1; the horizontal lines represent the means of these values. sTREM-1 levels were significantly higher in infants with SBI than in the infants without SBI group (mean ± SD, 324.6 ± 546.3 versus 7.7 ± 16.4; P = 0.0109; Table 2). Due to inequality of age (days old) in these two groups.

<table>
<thead>
<tr>
<th>Laboratory data</th>
<th>Infants with SBI</th>
<th>Infants without SBI</th>
<th>P value1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (day-old, mean ± SD)</td>
<td>51.8 ± 31.2</td>
<td>30.3 ± 30.1</td>
<td>0.0236</td>
</tr>
<tr>
<td>Sex (male:female)</td>
<td>2.3:1 (16:7)</td>
<td>2:1 (14:7)</td>
<td>0.8366</td>
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<tr>
<td>Decrease appetite (%)</td>
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</tr>
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1): Group comparison by ANCOVA with age as adjusted variable.
2): Tests were conducted in logarithm of original values.

RESULTS

Forty-four febrile infants less than 3 months old who were admitted to the pediatric department of Kaohsiung Medical University Hospital were enrolled in this study. Twenty-three infants (52.3%) had SBI and 21 infants (47.7%) had no evidence of SBI based on the results of bacterial culture. Causes of SBI included bacteremia in 2 infants, pneumonia in 2 infants, meningitis in 2 infants, and UTIs in 17 infants. *Escherichia coli* was the most commonly found causative organism of UTI (76.5%), while 3 cases were caused by *Proteus mirabilis* and 1 was caused by *Klebsiella pneumonia*.

The characteristics and clinical findings of the study subjects are shown in Table 1. There was no difference between infants with SBI and those without SBI in sex or in respiratory and gastrointestinal symptoms, but there was a significantly greater incidence of decreased appetite in the SBI group than in infants without SBI. Total leukocyte counts (after correcting by normoblast count), the percentage of segment, IT ratio, ANC, hemoglobin, platelet count, and CRP were not significantly different between the two groups (Table 2).

The individual values of sTREM-1 level in both groups are plotted in Fig. 1; the horizontal lines represent the means of these values. sTREM-1 levels were significantly higher in infants in the SBI group than in the infants without SBI group (mean ± SD, 324.6 ± 546.3 versus 7.7 ± 16.4; P = 0.0109; Table 2). Due to inequality of age (days old) in these two groups.

Table 1. Clinical presentations of febrile infants with and without serious bacterial infection (SBI)

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groups, we used ANCOVA analysis to adjust for age and log-transformation to meet normal distribution and still found a significant difference between these two groups in plasma sTREM-1 level ($P < 0.0001$; Table 2).

We analyzed the diagnostic properties of sTREM-1 using the ROC curve. The area under the ROC curve was 0.88 (95% CI, 0.78 - 0.99) for sTREM-1 level (Fig. 2). Based on the ROC analysis, cutoff values were identified for each variable that maximized both the sensitivity and specificity. At a cutoff level of 24.4 pg/mL, the sTREM-1 level yielded a sensitivity of 87% (95% CI, 78 - 97%), a specificity of 81% (95% CI, 69 - 93%), a positive likelihood ratio 4.6 (95% CI, 1.9 - 11.2), and a negative likelihood ratio of 0.2 (95% CI, 0.1 - 0.4) for differentiating between the presence and absence of SBI (Table 3). Among the 23 infants with SBI, there were 20 infants (87%) with plasma levels of sTREM-1 over the cutoff value. However, there were just 4 infants (19%) with plasma levels of sTREM-1 above the cutoff value in the 21 infants without SBI. Plasma sTREM-1 levels appeared to be helpful in predicting young febrile infants with SBI.

There was no correlation between sTREM-1 level and CRP level or total leukocyte count, ANC, IT ratio, microbial species, or any other clinical features. We further evaluated the relationship between plasma levels of sTREM-1 and the length of hospital stay. In the SBI group, the plasma levels of sTREM-1 were significantly correlated with the length of hospital stay in Pearson correlation analysis ($r = 0.419, P = 0.047$).

**DISCUSSION**

In this study, we demonstrated that plasma level of sTREM-1 had better specificity and sensitivity than CRP in predicting SBI in febrile infants less than 3 months old. These results suggested that plasma level of sTREM-1 could be considered as a valuable tool for early diagnosis of SBI in febrile infants less than 3 months old.

Very young infants have less effective defense systems, including immune systems, and therefore vulnerability to SBI is increased (2). Previous studies have shown the prevalence of SBI is higher in febrile infants less than 3 months old, ranging from 5 to 10% (2). In one large multicentric analysis, the rate of SBI was 9% (14). The dilemma in the management of febrile infants with SBI is that there is difficulty in determining which infants are suffering from SBI in the early stage and require further antibiotic treatment. Certainly, a careful history taking and physical examinations should be performed for every febrile young infant. However, even experienced clinical judgment is limited, because well-appearing infants may develop SBI after the examination has taken place (2). Bacterial culturing is a gold standard procedure to detect occult SBI, but the results are not quickly available. Efforts are therefore continuing to find a reliable marker for early identification of SBI; currently, there is no promising single clinical or biological indicator of SBI. In the clinical setting, total leukocyte is the most common test
used for screening SBI, and a clinical guideline using a cutoff value of total leukocyte more than 15,000 ($10^4$ cells/L) is currently accepted in determining the need for antibiotic treatment in febrile infants (15). However, using a level of total leukocyte more than 15,000 ($10^4$ cells/L) does not yield satisfactory sensitivity and specificity for distinguishing SBI or non-SBI groups (6,16). ANC has been considered to be better than leukocyte in detecting SBI; however, the overall data of ANC is similar to that of leukocyte (16). The immature neutrophils count in the peripheral blood smear also could not help to distinguish bacterial infections from respiratory viral infections in young febrile children (17). In this study, our results also showed that total leukocyte counts, IT ratio, and ANC were not significantly different between the SBI and non-SBI groups (Tables 2 and 3).

CRP level has been widely proposed as a useful screening test for SBI. It has been reported that CRP concentration was both more sensitive and more specific than either the total leukocyte or ANC (18,19). Several prospective studies (6, 20,21) show that CRP has better predictive value than other acute phase reactants, while one study concludes that ANC has better predictive value (22). CRP currently remains the commonest useful marker for early recognition of clinically undetectable SBI, although a single CRP value cannot be assumed to offer certainty of SBI (23). Carroll and Silverstein also claimed that knowing the CRP value should not change our current treatment of young infants with fever (24). In our study, the results revealed CRP was not significantly different between the patients of SBI and non-SBI patients. At a cutoff level of 2.3 mg/L, CRP level yielded a sensitivity of 83% (95% CI, 71 - 94%) and a specificity of 62% (95% CI, 48 - 76%) for differentiating between the presence and absence of SBI (Table 3). This cutoff value of 2.3 mg/L represents the best discrimination, as derived from the areas under the ROC curves in this study. If we use the currently acceptable cutoff value of 6 mg/L for CRP in our hospital, the sensitivity falls to 47.8% and specificity falls to 61.9%. In this study, CRP was not a satisfactory marker to identify young infants with SBI.

tREM-1 has been reported to be highly correlated with various bacterial infections. Our study demonstrated that tREM-1 is superior to other routine laboratory tests, in predicting SBI among febrile young infants (Table 3). Bouchon et al. have shown that the expression of tREM-1 was strongly up-regulated by extracellular bacteria such as Pseudomonas aeruginosa or Staphylococcus aureus in cell culture, peritoneal lavage fluid, and tissue samples from infected patients (8). In contrast, tREM-1 was hardly detectable in non-microbial inflammations, such as psoriasis, ulcerative colitis, and vasculitis caused by immune complexes. These findings indicate that this receptor is specifically involved only in cases of infectious aggression (8). Gibot et al. have shown that the level of sTREM-1 in bronchoalveolar lavage fluid of patients with suspected pulmonary infection appears to be the best predictor of pneumonia (10), as well as the best marker of adult sepsis (9). However, most of this evidence has been obtained from the data of adult patients. In our study, we showed that sTREM-1 could be detected even in young infants and neonates with SBI. With sTREM-1 level at a cutoff value of 24.4 pg/mL, the positive and negative likelihood ratios were 4.6 and 0.2, respectively, and the diagnostic odds ratio was 28.3, which is better than potentially useful diagnostic odds ratios of 20 (25). We also showed there was a relationship between sTREM-1 levels and length of hospital stay. To our best knowledge, this is the first report of a study examining the use of the plasma level of sTREM-1 to diagnose SBI in febrile infants less than 3 months old.

In conclusion, our results demonstrated that plasma sTREM-1 levels could be detected in young infants with SBI and that the sTREM-1 level may offer an additional predictor for early detection of febrile young infants with SBI. Further research is needed to validate sTREM-1 as a screening tool in this setting.

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