

Serum levels of soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) and pentraxin 3 (PTX3) as markers of infection in febrile patients with systemic lupus erythematosus

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Abstract

Objective

To investigate the role of sTREM-1 and PTX3 as markers of infection in febrile patients with SLE

Methods

In febrile (body temperature $\geq 38^{\circ}\text{C}$) patients with SLE, blood samples of day 0, 1, 2, and 14 after presentation were drawn and relevant clinical data were collected. The patients were allocated to an infection group ($n=19$) or disease flare group ($n=14$). Serum levels of sTREM-1 and PTX3 were measured by ELISA using the serum samples of SLE patients and age- and sex-matched healthy controls ($n=31$).

Results

A total of 33 febrile episodes occurred in 32 SLE patients (19 infections, 14 flares) were studied. sTREM-1 levels on day 0 were significantly higher in the infection group than in the flare group (109.9 pg/ml (median) vs. 48.0 pg/ml, $p=0.002$), but PTX3 levels were similar in these two groups. The difference of sTREM-1 levels between infection group and flare group was persistent on day 1 and 2 (day 1, $p=0.007$; day 2, $p=0.034$). The highest diagnostic value (sensitivity=1.0, specificity=0.664) of sTREM-1 was obtained at the threshold value of 53.2 pg/mL.

Conclusion

Serum sTREM-1 levels were significantly higher in the infection group than in the flare group of febrile SLE patients. Our findings suggest that serum sTREM-1 levels could be used to determine whether SLE patients have contracted an infection.

Key words

Human TREM1 protein, PTX3 protein, systemic lupus erythematosus, fever.

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Introduction

Systemic lupus erythematosus (SLE) is systemic autoimmune disease with typical autoantibodies and variable clinical manifestations. The etiology of SLE is not clear, though the dysregulation of immunity is one of the underlying mechanisms. Fever is one of the most common manifestation of disease in SLE patients with a disease lifetime prevalence of 41~86% (1). Moreover, due to the presence of an immunosuppressed state SLE patients are prone to infection and both infection and active disease are a common cause of mortality (2). Therefore, the differentiation of infection from active disease state is a crucial aspect of SLE management. Shaking chills, leukocytosis, neutrophilia, and normal anti-DNA levels are helpful for differentiating these states (1), but the determination as to whether infection or disease activity is the cause of fever is often difficult in clinical practice.

Triggering receptor expressed on myeloid cells-1 (TREM) is recently discovered protein. It is a member of the immunoglobulin superfamily and is expressed on neutrophils and on other phagocytes that are exposed to bacteria or fungi, where it participate in acute phase reaction to the bacterial components (3, 4). When infected, human tissues are infiltrated by neutrophils or monocytes which express high level of TREM-1. However, TREM-1 expression is low in tissue in noninfectious inflammatory disease. Furthermore, lipopolysaccharide administration or sepsis can provoke TREM-1 upregulation in blood, especially during bacterial infection (4, 5), and accordingly, the use of the soluble form of TREM-1 (sTREM-1) has been examined as a marker of infection in many situations. In the patients on mechanical ventilation, sTREM-1 concentrations in bronchoalveolar lavage fluid were measured to differentiate pneumonia from noninfectious pulmonary infiltrate (6), and the diagnostic value of sTREM-1 has also been investigated in patients with suspected sepsis (7).

Pentraxins are a family of multimeric pattern recognition proteins which include C-reactive protein (CRP), serum amyloid P component and pentraxin 3 (PTX3). PTX3 is induced by cytokines

and rapidly produced by several types of cells, in particular by mononuclear phagocytes, dendritic cells, fibroblasts and endothelial cells, in response to primary inflammatory signals (8). PTX3 activates the classical pathway of complement activation and facilitates pathogen recognition by macrophages and dendritic cells. Furthermore, PTX3 expression has been reported to be elevated during infection. In patients with hematologic malignancy or critical illness, serum PTX3 was elevated or correlated with disease severity (9, 10). Accordingly, in the present study, we investigated serum levels of sTREM-1 and PTX3 in febrile SLE patients in an attempt to differentiate between infection and SLE flare.

Patients and methods

Study population and serum samples

SLE patients who presented at Seoul National University Hospital or Hallym University Sacred Heart Hospital complaining of fever or who found to be febrile during admission from March, 2004 to March, 2007 were enrolled in this study. All patients were diagnosed as SLE according to 1997 American College of Rheumatology revised criteria for classification of systemic lupus erythematosus (11). A fever was defined as an axillary temperature of over 38°C. After obtaining informed consent, blood samples were collected day 0, 1, 2, and 14 post-presentation. Blood samples were centrifuged at 3000 rpm for 10 minutes, and the serum obtained was stored at -20°C. Baseline data, such as age, sex, onset of fever, leukocyte count, CRP levels, anti-dsDNA levels, and complement levels (C3, C4) were recorded at day 0. Clinical data, such as physical findings, radiograph, the results of blood or other specimen culture, and the change of Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) compared with 1 month prior to presentation were collected (12). Blood samples were also obtained from 31 healthy controls after obtaining consent.

Measurement of sTREM-1 and PTX3

Serum levels of sTREM-1 were measured by commercial ELISA kit (human

Competing interests: none declared.

Median patient age was 27.5 (range 15~70), and there were 5 men and 28 women. Median duration of fever before presentation was 6 days (range 0~29). Blood samples were drawn after antibiotic treatment for 22 episodes. Episodes were allocated to study groups as follows; infection group (including 2 combined episodes of infection and flare), 19; flare group 14. Infection types were as follows; nine episodes were symptomatic urinary tract infection (by *E. coli* in 6 episodes and cultures were negative in 3 episodes), 6 of pneumonia (by *K. pneumoniae*, *S. aureus*, *P. aeruginosa*, *Legionella*, cytomegalovirus and cytomegalovirus combined with *P. jiroveci*, respectively), 2 of tuberculosis (1 disseminated form and 1 soft tissue infection), 1 cellulitis progressed to bacteremia (by *S. aureus*), and 1 intraabdominal abscess (by *K. pneumoniae*). When the characteristics of the infection group and flare group at day 0 were compared (Table I), change of SLEDAI score compared to 1 month prior to presentation, leukocyte count, CRP levels, and complement levels (C3 and C4) were found to be significantly different.

Time courses of the levels of sTREM-1 and PTX3 in patients in the infection, flare, and normal control groups and the association with other variables

The serum levels of sTREM-1 at day 0 were elevated in infection group compared to flare group or healthy controls (infection group vs. flare group vs. controls; 109.9 pg/ml (median) vs. 48.0 pg/ml vs. 54.0 pg/ml, $p<0.001$ by Kruskal-Wallis test; infection group vs. flare group, $p=0.002$; infection group vs. controls, $p<0.001$). However, sTREM-1 levels at day 0 did not differ between flare group and controls. The difference between infection group and flare group was persistent on day 1 and 2 (day 1, $p=0.007$; day 2, $p=0.034$) as shown in Table II. Overall sTREM-1 levels from day 0 through day 2 were also significantly different in the infection group and flare group ($p=0.015$, using a repeated-measures generalized linear model). The serum levels of PTX3 at day 0 were elevated in both infection group and flare group com-

Table II. Serum levels of sTREM-1 and PTX3 in the infection and flare groups.

		Day 0	Day 1	Day 2	Day 14
sTREM-1(pg/ml)	Infection	109.9 (67.6, 183.4)	131.4 (42.8, 166.9)	89.6 (62.2, 148.7)	85.2 (38.7, 131.7)
	Flare	48.0 (4.20, 90.18)	18.5 (0, 108.4)	29.6 (0, 103.0)	20.3 (0, 88.2)
	p value ^a	0.002	0.007	0.034	0.053
PTX3 (ng/ml)	Infection	9.9 (7.67, 63.40)	9.88 (5.96, 51.39)	6.92 (5.57, 14.77)	3.41 (2.62, 6.87)
	Flare	11.5 (5.74, 21.12)	17.9 (5.37, 44.15)	12.29 (5.97, 42.48)	7.90 (5.04, 11.06)
	p value ^a	0.361	0.945	0.240	0.008

Data are expressed as medians (25th quartile, 75th quartile).

^ap-value for infection versus flare group comparison, by Mann-Whitney U-test.

pared to controls (9.9 ng/ml (median) vs. 11.5 ng/ml vs. 5.0 ng/ml, $p<0.001$ by Kruskal-Wallis test, infection group vs. controls, $p<0.001$; flare group vs. controls, $p=0.002$) whereas there were no significant difference between infection group and flare group. Moreover, the levels of PTX3 in the infection group and flare group were not different on day 1 or day 2 while the levels of PTX3 in the infection group were lower than those in the flare group on day 14 (Table II). In addition, overall PTX3 levels from day 0 through day 2 did not differ between infection group and

flare group ($p=0.815$, using a repeated-measures generalized linear model).

The sTREM-1 levels or PTX3 levels in total patients was associated with leukocyte count (sTREM-1, $r=0.398$, $p=0.022$; PTX3, $r=0.392$, $p=0.029$) whereas that was not correlated to levels of CRP (sTREM-1, $p=0.116$; PTX3, $p=0.357$). The association of number of days after the fever onset and the sTREM-1 or PTX3 level of day 0 was analyzed. There was negative correlation of sTREM-1 and fever duration in total patients (sTREM-1, $r=-0.367$, $p=0.036$; PTX3, $r=-0.111$, $p=0.551$).

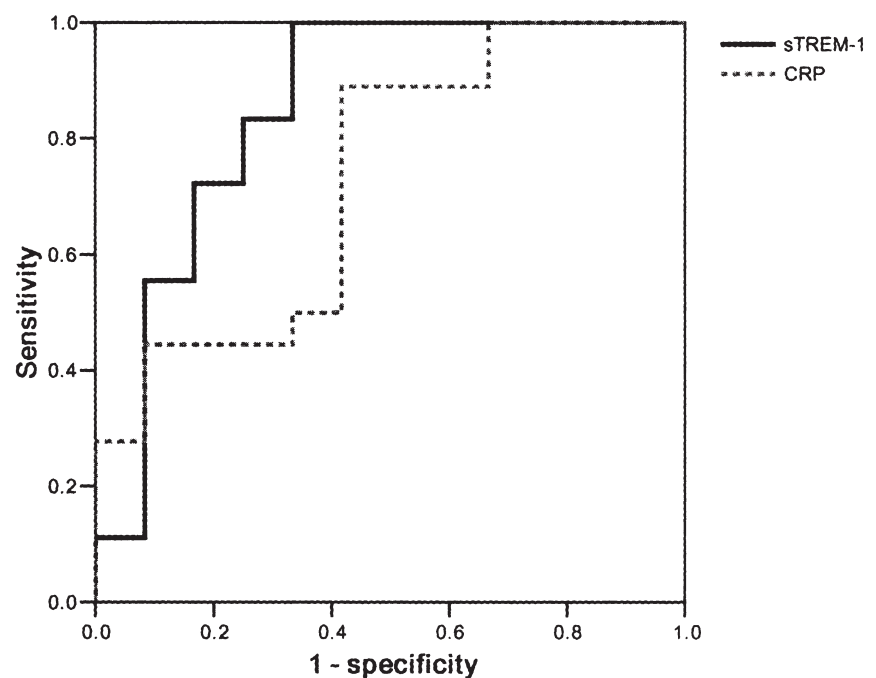


Fig. 1. Receiver operating characteristics curves of soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) and C-reactive protein (CRP) at presentation. Areas under the receiver operating characteristic curves were 0.852 and 0.731 for sTREM-1 and CRP, respectively.

However, there was no significant correlation of the number of days after the fever onset with infection group or flare group (sTREM-1, $r=-0.338$, $p=0.156$ in infection group, $r=-0.254$, $p=0.380$ in flare group; PTX3 $r=-0.132$, $p=0.614$ in infection group, $r=0.107$, $p=0.715$ in flare group). We thought the analysis with sTREM-1 on designated days after the admission to the hospital may implicate more practical situation.

Receiver operating characteristics curves for sTREM-1 and CRP

To explore the possibility of using sTREM-1 as a diagnostic marker of infection, we generated ROC curves for sTREM-1 and CRP on day 0 (Fig. 1). The area under the sTREM-1 ROC curve was 0.852 (95% confidence interval, 0.693–1.000), and that of CRP was 0.731 (95% confidence interval, 0.543–0.920). The highest value reached for sensitivity and specificity of sTREM-1 was the threshold value of 53.2 pg/mL. This value corresponded to the 25th percentile of the sTREM-1 distribution and was associated with a sensitivity of 1.0, a specificity of 0.664, and a likelihood ratio of a positive result for presence of infection of 3.0. The highest value reached for sensitivity and specificity of CRP was the threshold value of 0.525 mg/dL (the 20th percentile of the CRP distribution), with a sensitivity of 0.944, a specificity of 0.367, and a likelihood ratio for infection of 1.41.

Discussion

In this study, serum sTREM-1 levels were significantly higher in the infection group than in the flare group of febrile SLE patients whereas serum PTX3 levels were not different between infection and flare group. Moreover, the difference of serum levels of sTREM-1 was maintained for initial 3 days. The diagnostic ability of sTREM-1 might be comparable to CRP.

The diagnosis of infection in febrile SLE patients is of great importance during the management of SLE, because the symptoms and signs of SLE and infection are similar, and thus, prompt suspicion of infection and early culture studies are needed (14). To differentiate infection from SLE flare in clinical

practice, several biomolecules have been studied in febrile SLE patients. In particular, CRP has been studied extensively for detecting and differentiating infections in lupus (15–17). Active SLE in Caucasians and Orientals are usually related to only modest increases in serum CRP, while patients usually demonstrate more pronounced CRP increases with infection (15, 16). In one prospective study of SLE patients a serum concentration of serum CRP greater than 60 mg/l suggested the presence of infection, whereas a level below 30 mg/l excluded systemic infection (17). However, the use of CRP as an infection marker in SLE patients is not without controversy (18–21). Marked CRP elevations are often associated with disease activity, especially when associated with serositis (18–20), and on the other hands, even bacteremia might not induce a CRP response (21). Out of 19 infected patients in this study, 6 patients (31.6%) had CRP less than 6 mg/dl and all of them had elevated sTREM-1 levels more than 53.2 pg/ml.

Procalcitonin, another innate immune system component, has also been studied as an indicator of infection in febrile SLE patients (22, 23), but the ability of procalcitonin to screen infection has been limited, because lupus patients with a bacterial or fungal infection have higher serum procalcitonin levels than those with viral infections or controls (23, 24). Accordingly, serum procalcitonin may be incapable of differentiating infection (especially viral infections) from active disease (23).

Microheterogeneity patterns of alpha 1-acid glycoprotein or a combination of leukocyte count and alpha-2 globulin level have also been studied to differentiate infection from active disease in SLE patients with fever (25, 26). Con A-bound serum alpha 1-acid glycoprotein can be a more sensitive indicator of infection than CRP (25). Discriminant analysis showed that the cause of fever could be classified accurately in 95% of 74 febrile episodes using a combination of leukocyte count and alpha-2 globulin level (26). However, further studies on the clinical implications of these methods are required to use these extensively in variable situations.

TREM-1 is a cell-surface receptor which triggers the secretion of inflammatory cytokines, and is markedly expressed in the presence of bacteria or fungi (3, 4). On the other hand, TREM-1 expression is not up-regulated in patients with noninfectious inflammation like psoriasis, ulcerative colitis or vasculitis, which suggests that its expression is specifically associated with the presence of infection (4, 27, 28). Together with an upregulated expression of the membrane-bound TREM-1, a soluble form (sTREM-1) may be released during infection. The usefulness of sTREM-1 in diagnosing infection was shown in patients with mechanical ventilation and with sepsis (6, 7). Furthermore, sTREM-1 concentrations at admission in septic patients were significantly lower in non-survivors than in survivors, which suggests that an elevated baseline sTREM-1 level could be an independent protective prognostic factor (29).

In the present study it was found that sTREM-1 levels were higher in the infection group than in the flare group. Moreover, in immune suppressed SLE patients infections can have serious consequences, and thus, test sensitivity is probably more important than specificity. Therefore, we used low cut-off value to obtain high sensitivity (1.0) in the present study, and sTREM-1 was found to have a comparable sensitivity to CRP. In addition, sTREM-1 levels were found to be stable during the first 3 days of study despite antibiotics treatments. These findings about the stabilities of sTREM-1 levels in serum concur with those of another study in which no significant change was observed over 6-day period either in patients with ventilator-associated pneumonia or in controls (30). Although the proportion of patients administered antibiotics before study was higher in infection group than in flare group, sTREM-1 was higher in the infection group. These results suggest that sTREM-1 levels determinations might be useful for identifying the presence of infections despite initial antibiotics treatment.

Serum levels of PTX3 have also been reported to be elevated during infections (9, 10), but in the present study,

PTX3 levels were not higher in the infection group than in the flare group. Moreover, although PTX3 levels were higher in febrile SLE patients than in healthy controls, its levels in the infection and flare groups were similar, which suggests that PTX3 expression is also upregulated during some inflammatory conditions.

Limitations of our study are the small number of patients and lack of patients with serositis who have been known to show high CRP response without infection. A study of a large number of patients with diverse infections may be needed to understand the utility of sTREM-1.

In conclusion, the present study demonstrates that the serum levels of sTREM-1 in febrile SLE patients are higher in those with an infection than in those with an SLE flare. sTREM-1 level determinations may enable clinicians to better differentiate these two states in SLE patients.

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