Serum ratio of soluble triggering receptor expressed on myeloid cells-1 to creatinine is a useful marker of infectious complications in myeloperoxidase-antineutrophil cytoplasmic antibody-associated renal vasculitis

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Abstract

Background. The contribution of infections to the mortality of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis patients is important and should induce early and careful control of these events. However, the differentiation of infection from active vasculitis is

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often difficult. The usefulness of serum-soluble triggering receptor expressed on myeloid cells-1 (TREM-1) for detecting the presence of infectious complications regardless of disease activity was investigated.

Methods. Soluble TREM-1 in serum obtained from 41 patients with myeloperoxidase (MPO)-ANCA-associated vasculitis was measured by an enzyme-linked immunosorbent assay. Twenty-nine samples were from active vasculitis patients, 27 samples from inactive vasculitis patients without infection and 17 samples from inactive vasculitis patients with infectious complications. Serum-soluble TREM-1 was also measured in 10 patients with acute pyelonephritis and 30 patients with chronic kidney disease (CKD).

Results. There was a significant correlation between serum levels of soluble TREM-1 and serum creatinine levels among all patients \( r = 0.554, P < 0.0001 \). The serum-soluble TREM-1/creatinine ratio was higher in inactive vasculitis patients with infectious complications than in active vasculitis, inactive vasculitis without infection and CKD patients \( (P = 0.0005, P < 0.0001 \text{ and } P < 0.0001, \text{ respectively}) \), but not significantly different to that in acute pyelonephritis patients. On receiver-operating-characteristic curve analysis, a lower-limit value of 9.40 ng/ml for this ratio had a sensitivity of 84.6% and a specificity of 90.8% in differentiating patients with infection from those without infection.

Conclusions. The serum ratio of soluble TREM-1 to creatinine may be a useful marker for detection of infectious complications in MPO-ANCA-associated vasculitis.

Keywords: infectious complications; MPO-ANCA-associated vasculitis; triggering receptor expressed on myeloid cells-1

Introduction

The contribution of infections to the mortality of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis patients is important and should motivate early and careful control of these events. However, the differentiation of infection from active vasculitis is often difficult. Microbial evidence of infection is frequently lacking, even when the clinical signs of inflammation suggest infection. Negative blood cultures do not exclude a bacterial infection. In common laboratory examinations, leucocytosis, increased band cells in the differential cell count, and the elevation of C-reactive protein (CRP) are all helpful for the diagnosis of infection, but these parameters are also altered in patients with active ANCA-associated vasculitis.

The triggering receptor expressed on myeloid cells-1 (TREM-1) is a recently discovered member of the immunoglobulin superfamily [1]. The expression of TREM-1 is greatly upregulated in the presence of bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* and fungi such as *Aspergillus fumigatus* in cell cultures, peritoneal lavage fluid, and tissue samples from patients infected with these microorganisms [2]. TREM-1 is an important regulator in innate immunity and functions to amplify inflammation in response to infection [1–4]. Soluble TREM-1 has been investigated as a clinical marker to distinguish bacterial infection from non-infectious inflammatory conditions [5–9]. Levels of TREM-1 and soluble TREM-1 are elevated in bronchoalveolar lavage fluid or serum in pneumonia [5,6]. Plasma-soluble TREM-1 levels are also raised in critically ill patients with sepsis, especially those with severe sepsis and septic shock, compared with patients without infection [7]. In patients with pleural effusion, a high level of soluble TREM-1 is more suggestive of parapneumonic than tuberculous, neoplastic or transudative effusions [8]. On the other hand, soluble TREM-1 could also distinguish bacterial meningitis from aseptic meningitis [9].

In order to evaluate the diagnostic value of serum-soluble TREM-1, we investigated patients with ANCA-associated vasculitis at various stages of the disease.

Materials and methods

Subjects

Forty-one myeloperoxidase (MPO)-ANCA-associated renal vasculitis patients were investigated in the present study. The diagnosis of ANCA-associated renal vasculitis was based on characteristic clinical and histological features of microscopic polyangiitis as defined by the Chapel Hill Consensus Conference, clinical evidence of rapidly progressive glomerulonephritis, and a positive test for MPO-ANCA. Patients with other types of systemic small vessel vasculitis, such as Wegener’s granulomatosis, proteinase-3 ANCA-associated renal vasculitis, Henoch–Schönlein purpura, essential cryoglobulinaemic vasculitis, drug-induced vasculitis, systemic lupus erythematosus, rheumatoid arthritis or malignancy-associated vasculitis, and those with antiglomerular basement membrane disease were excluded. ANCA was examined by MPO-specific enzyme-linked immunosorbent assay (ELISA), and normal ranges were defined as values <9.0 U/ml.

Among 41 MPO-ANCA-associated renal vasculitis patients, 29 provided serum samples before the initial treatment. Twenty-two of 29 patients revealed rapidly progressive glomerulonephritis, and the other 7 patients had chance proteinuria and haematuria and were diagnosed with pauci-immune necrotizing crescentic glomerulonephritis by renal biopsy. Of all 41 MPO-ANCA-associated renal vasculitis cases, 27 provided samples during remission. Remission was defined as the absence of clinical disease activity except for pulmonary and renal disease activity based on the Birmingham Vasculitis Activity Score (BVAS). The absence of renal disease activity was defined as a stable or decreasing serum creatinine level and the absence of red cell casts. The absence of pulmonary activity was indicated by the resolution of radiological opacities, the reduction in size of existing lesions, or the absence of new pulmonary lesions. Among the 41 MPO-ANCA-associated renal vasculitis patients, 17 samples obtained during remission with infectious complications were investigated. Eleven of the 17 patients with infectious complications had pneumonia, and the other 6 had acute bronchitis.

As positive infectious controls, 10 patients with acute pyelonephritis were investigated. The diagnosis of acute pyelonephritis was based on characteristic clinical features such as fever, costovertebral tenderness, leucocyturia, bacteria in urine sediment and urinary culture examination, and elevated white blood cell count and CRP. The normal range of serum CRP is defined as a value <0.3 mg/dL. *Escherichia coli* were detected in 8 of 10 patients with acute pyelonephritis by bacteriological examination, group B *Streptococcus* was detected in 1 patient and *P. aeruginosa* was detected in 1 patient.

As kidney disease controls without infection, 30 patients with chronic kidney disease (CKD) were investigated. Fifteen of 30 CKD patients had nephrosclerosis, and the other 15 patients had chronic glomerulonephritis. Two patients with chronic glomerulonephritis were being treated with maintenance haemodialysis therapy. CKD patients with elevated levels of serum CRP were excluded.

The study protocol was accepted by the ethics committee of our institution, and written informed consent was obtained from all patients or
their immediate family members. This study also conformed to the provisions of the Declaration of Helsinki as revised in Edinburgh in 2000.

**ELISA for soluble TREM-1**

Blood samples were collected in plasma separator tubes before initial treatment, during remission status and at the time of complicating infection. Samples were separated at $1000 \times g$ for 15 min and were stored at $-80^\circ C$ for analysis.

The samples were measured by commercially available enzyme-linked immunosorbent assay kits (Quantikine®️, R & D Systems, Minneapolis, MN, USA) in duplicate. In brief, 96-well microplates were first coated with anti-TREM-1. Then, 50 μL of sample was added to each well. Plates were incubated for 2 h at room temperature and then washed, and horseradish peroxidase-conjugated polyclonal anti-TREM-1 antibody was added at 200 μL/well. The plates were incubated for 2 h at room temperature and then washed, and chromogen (tetramethylbenzidine) and hydrogen peroxide were added to each well. The plates were incubated for 30 min at room temperature, and 200 μL of 2 N sulphuric acid solution was added to each well. The plates were immediately read on a microplate reader (Sunrise Remote®️, Tecan Japan, Kanagawa, Japan) set at 450 nm for measurement and at 540 nm for wavelength correction. The inter- and intra-assay variations were <10%.

**Statistical analysis**

Variables are expressed as means ± standard deviation or as numbers with percentages of the total. Analysis of variance (ANOVA) was used to as-

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Fig. 1. Clinical parameters in MPO-ANCA-associated vasculitis patients with active vasculitis, such inactive patients without infection, such inactive patients with infectious complication, and as controls, acute pyelonephritis patients and CKD patients. Indicated clinical parameters were MPO-ANCA titres (A), serum creatinine levels (B), white blood cell count (C) and serum CRP levels (D).
To assess differences among subject groups, and post hoc comparisons were made using the Bonferroni/Dunn test. We compared categorical data between the two groups using the chi-square test with Yates’ continuity correction and Fisher’s exact test. Correlation analysis was performed using Pearson’s correlation method, and linear regression analysis was carried out. Statistical significance was defined as a P-value of <0.05. All statistical analysis was performed using PASW Statistics software, version 18 (SPSS Japan Inc, Tokyo, Japan) for Windows.

**Results**

**Clinical parameters of subjects**

There were no differences in age among patients of the five groups: active MPO-ANCA-associated vasculitis patients, inactive MPO-ANCA-associated vasculitis patients without infection, inactive MPO-ANCA-associated vasculitis patients with infectious complications, patients with acute pyelonephritis, and CKD patients. In patients with acute pyelonephritis, there were a higher number of females. The MPO-ANCA titre was significantly higher in patients with active vasculitis than in the other four groups (P < 0.0001), and MPO-ANCA was not detected in any patient with acute pyelonephritis or CKD (Figure 1A). The mean serum creatinine level in patients with active vasculitis was significantly higher than that in patients with acute pyelonephritis (P = 0.0003; Figure 1B). Compared with the white blood cell (WBC) count in CKD patients, those in patients with active vasculitis (P < 0.0001), inactive vasculitis without infection (P = 0.0041), inactive vasculitis patients with infectious complications (P < 0.0001), and acute pyelonephritis (P < 0.0001) were significantly elevated (Figure 1C). The mean serum CRP level in patients with acute pyelonephritis was significantly higher than that in patients with inactive vasculitis without infection (P < 0.0001) or CKD patients (P < 0.0001; Figure 1D). The mean serum CRP level in patients with active vasculitis was significantly higher than that in patients with inactive vasculitis without infection (P < 0.0001) or CKD patients (P < 0.0001; Figure 1D). The mean serum CRP level in inactive vasculitis patients with infectious complications was also significantly higher than

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**Fig. 2.** Serum levels of soluble TREM-1 in the five subject groups. The serum levels of soluble TREM-1 were higher in inactive vasculitis patients with infectious complications than in patients with inactive vasculitis without infection (P = 0.0003). These levels were also higher in active vasculitis patients and in inactive vasculitis patients with infectious complications than in CKD patients (P = 0.0018 and P < 0.0001, respectively).

**Fig. 3.** Relation between serum levels of soluble TREM-1 and serum CRP levels (A) or serum creatinine levels (B). There was no significant correlation between serum levels of soluble TREM-1 and serum CRP levels in all patients. However, there was a significant correlation between serum levels of soluble TREM-1 and serum creatinine levels in all patients (r = 0.554, P < 0.0001).
Serum levels of soluble TREM-1

Among patients with MPO-ANCA-associated vasculitis, the serum levels of soluble TREM-1 were higher in inactive vasculitis patients with infectious complications than in patients with acute vasculitis (P < 0.0003). However, there was no significant difference between inactive vasculitis patients with infectious complications and patients with active vasculitis. The serum levels of soluble TREM-1 were also higher in active vasculitis patients and in inactive vasculitis patients with infectious complications than in CKD patients (P = 0.0018 and P < 0.0001, respectively). There was no significant difference in soluble TREM-1 between patients with acute pyelonephritis and the other four groups (Figure 2). In most patients, in which serum soluble TREM-1 was able to be measured at multiple stages of the disease, the serum levels at the time of inactive vasculitis without infectious complications were lower than those taken during active vasculitis. However, some patients showed an increase or had nearly the same levels during inactive as they did during active vasculitis. On the other hand, the serum level at the time of inactive vasculitis with infectious complications had increased when compared with the levels at the time of inactive vasculitis without infectious complications in all of those patients.

Relation between serum levels of soluble TREM-1 and clinical parameters

There was no significant correlation between serum levels of soluble TREM-1 and white blood cell count or serum CRP in all patients (Figure 3A). However, there was a significant correlation between serum levels of soluble TREM-1 and serum creatinine levels in all patients (r = 0.554, P < 0.0001, Figure 3B).

Ratio of serum-soluble TREM-1/creatinine

Among patients with MPO-ANCA-associated vasculitis, the serum ratio of soluble TREM-1 to creatinine was higher in inactive vasculitis patients with infectious complications than in those with active vasculitis and inactive vasculitis patients without infection (P < 0.0001) or CKD patients (P < 0.0001).
without infection ($P = 0.0005$ and $P < 0.0001$, respectively). The ratio was also much higher in inactive vasculitis patients with infectious complications than in CKD patients ($P < 0.0001$), but not significantly different from that in patients with acute pyelonephritis. The ratio was higher in patients with acute pyelonephritis than in patients with active vasculitis, inactive vasculitis without infection and CKD ($P = 0.0002$, $P = 0.0001$ and $P < 0.0001$, respectively). There was no significant difference in the ratio among patients with active vasculitis, inactive vasculitis without infection and CKD (Figure 4). In all of the patients, in whom serum soluble TREM-1 was able to be measured at multiple stages of the disease, the ratios at the time of inactive vasculitis with infectious complications had increased when compared with the ratios at the time of inactive vasculitis without infectious complications.

Receiver-operating-characteristic curve analysis

The comparative receiver-operating-characteristic (ROC) curves for three measurements (WBC counts, serum CRP levels and the ratio of serum-soluble TREM-1/creatinine) for the diagnosis of infection are shown in Figure 5. Optimum diagnostic cut-off levels were identified from the ROC curves for WBC counts ($>9400$ cells/$mm^3$), serum CRP ($>0.85$ mg/dL) and the serum ratio of TREM-1/creatinine ($>9.40$ ng/mg). The area under the curve (AUC) of the serum ratio of TREM-1 to creatinine was 0.882 and was higher than that of WBC counts (0.796) or serum CRP (0.860). On the ROC curve, the serum ratio of TREM-1 to creatinine had a sensitivity of 84.6% and a specificity of 90.8% in differentiating patients with infection from those without infection. Although the sensitivity of this ratio was inferior to that of serum CRP levels (100.0%), the specificity of this ratio was far superior to that of serum CRP levels (67.1%).

Discussion

Although TREM-1 expression was not elevated in non-infectious inflammatory conditions, elevated levels of soluble TREM-1 in serum or synovial fluids of patients with several autoimmune diseases, such as inflammatory bowel syndrome [10], rheumatoid arthritis [11] and ankylosing spondylitis [12], have been reported. In ANCA-associated vasculitis, elevated serum levels of soluble TREM-1 could be detected in patients with active disease (high BVAS and increased CRP) [13]. Moreover, serum-soluble TREM-1 levels in that study were significantly higher at times of active disease as compared with remission. However, in the present study, serum-soluble TREM-1 levels at times of active disease were not significantly different to those at times of inactive disease. In the present study, serum TREM-1 levels were correlated with serum creatinine levels. Serum levels of low-molecular-weight protein are known to correlate with serum creatinine levels and logarithmic creatinine clearance [14]. Human TREM-1 is a 30-kDa monomeric transmembrane receptor [1], and an increase of serum-soluble TREM-1 in patients with renal insufficiency may be caused by low urinary excretion. In a previous study, serum-soluble TREM-1 levels were significantly higher at times of active disease as compared with remission, even though levels remained elevated in 4 of 12 patients in remission [13]. Considering that serum creatinine levels of patients with inactive vasculitis were usually better than those of patients with active vasculitis, the continued elevation of the serum-soluble TREM-1 in the previous study may have depended on the patients’ renal function. In the present study, the serum levels of soluble TREM-1 in patients with MPO-ANCA-associated vasculitis without infectious complication may have also depended upon the patients’ renal function. However, further studies are needed because there remain possibilities of altered expression of TREM-1 on monocytes or the excessive production of the soluble form of TREM-1 in renal insufficiency patients.

Plasma-soluble TREM-1 levels have been found to be more helpful in differentiating patients with sepsis from those with non-infectious diseases than either serum CRP or procalcitonin [7]. In another study, although no differences in concentrations of TNF-alpha, IL-6 or IL-8 were found between patients with sepsis, severe sepsis and septic shock on the first day of presentation of the symptoms, patients presenting with septic shock had significantly higher concentrations of soluble TREM-1 than patients with either sepsis or severe sepsis on the first day [15]. Serum procalcitonin has been investigated in ANCA-associated vasculitis to detect infectious complications [16,17]. Although CRP levels could not be used to discriminate an infection from an inflammatory response to disease activity in Wegener’s granulomatosis, procalcitonin levels of patients with bacterial infection were statistically elevated compared with patients with active vasculitis without infection and patients with inactive disease [17]. In the present study, we evaluated soluble TREM-1 instead of procalcitonin. However, serum TREM-1 levels were associated with not only the degree of inflammation but also renal function. To remove the factor of renal function, we calculated the ratio of serum-soluble TREM-1 to serum creatinine. This ratio was higher in patients with infectious complications than in those with active or inactive vasculitis; the AUC of this ratio was higher than that of WBC counts or serum CRP levels; as the lower limit, a value of 9.40 ng/mg for this ratio had a sensitivity of 84.6% and a specificity of 90.8%. Therefore, this parameter may be useful for detection of infectious complications. However, the serum ratio of soluble TREM-1 to creatinine may not be a sufficient method of correcting for the renal function. Moreover, it may not be clear-cut whether the procalcitonin level or the serum ratio of soluble TREM-1/creatinine is more useful for detection of infectious complications. Therefore, further studies are needed to establish the most suitable marker to diagnose the existence of infectious complications in vasculitis patients.

Conclusions

Serum levels of soluble TREM-1 may depend on patients’ renal function. The ratio of serum-soluble TREM-1 to cre-
atinine may be a useful marker for detection of infectious complications in patients with MPO-ANCA-associated vasculitis.

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Conflict of interest statement. None declared.

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