Increased Serum Vascular Endothelial Growth Factor Level in Churg-Strauss Syndrome*

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**Background:** Churg-Strauss syndrome (CSS) is a rare form of systemic vasculitis occurring in patients with asthma and hypereosinophilia. For optimal treatment, prompt distinction of CSS from asthma is necessary; however, there are few serologic screening markers for this purpose. Vascular endothelial growth factor (VEGF), a vascular permeability factor, has been associated with other systemic vasculitis such as Wegener granulomatosis and giant-cell arteritis.

**Objective:** The aim of this study was to clarify the clinical value of the measurement of serum VEGF for the distinction of CSS from asthma.

**Methods:** We investigated serum VEGF levels in 18 CSS patients, 19 asthma patients, and 12 acute bronchitis patients. We also performed immunohistochemical analysis for VEGF.

**Results:** The serum VEGF levels of CSS patients were significantly higher than those of asthma patients and acute bronchitis patients. The sensitivity and specificity to distinguish CSS from asthma were 93.3% and 81.8%, respectively (cutoff, 600 pg/mL). Infiltrating eosinophils stained intensely positive for VEGF, and serum VEGF levels showed a significant correlation with peripheral eosinophil counts. Serum VEGF levels decreased significantly after therapy (p < 0.001). The infiltrating eosinophils in the CSS lesion stained positive for VEGF in the immunohistochemical analysis.

**Conclusion:** VEGF is one of the useful screening markers for the distinction of CSS from asthma. We suggest that VEGF might be associated with the pathogenesis of CSS.

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**Key words:** allergy; asthma; eosinophils; vasculitis

**Abbreviations:** CSS = Churg-Strauss syndrome; ESR = erythrocyte sedimentation rate; VEGF = vascular endothelial growth factor

Churg-Strauss syndrome (CSS) is a rare disorder that is characterized by asthma, hypereosinophilia, and evidence of vasculitis with massive infiltration of eosinophils affecting a number of organs.1

The prevalence is of the order of 1.3/100,000 in the general population, compared with 3.3/100,000 for polyarteritis nodosa and 5.3/100,000 for Wegener granulomatosis.2,3 Successful treatment of this rare syndrome needs prompt differentiation of CSS from asthma alone4,5; however, there are few serologic markers to distinguish CSS from asthma.

Vascular endothelial growth factor (VEGF), a homodimeric, heparin-binding glycoprotein of 34 to 42 kd, is one of the major mediators of angiogenesis and vascular permeability.6,7 There have been several reports describing the association of VEGF with systemic vasculitis, such as Wegener granulomatosis,8 giant-cell arteritis,9 and Kawasaki disease.10

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However, to our knowledge there are no reports describing serum VEGF levels in patients with CSS. In this study, we investigated 18 patients with CSS and found increased serum levels of VEGF compared with asthma. We suggest a possible association of VEGF with the pathogenesis of CSS.

**Materials and Methods**

**Patients**

This study was reviewed and approved by the Kagoshima University Faculty of Medicine Committee on Human Research. We investigated 18 patients with CSS who were admitted to the Division of Respiratory Medicine, Respiratory and Stress Care Center, Kagoshima University Hospital from 1995 to 2005. There were 8 men and 10 women (mean ± SD age, 58.2 ± 18.2 years). For comparison, we also investigated 19 patients with asthma (8 men and 11 women; mean age, 58.9 ± 13.3 years) and 12 patients with acute bronchitis (5 men and 7 women; mean age, 58.9 ± 13.1 years). The diagnosis of CSS was made according to the 1990 edition of CSS published by American College of Rheumatology. All patients with CSS fulfilled more than five criteria. We excluded patients with rheumatoid arthritis, diabetes mellitus, acute or chronic liver disease, and immunologic abnormalities that predispose to opportunistic infection. Peripheral eosinophil counts, platelet counts, C-reactive protein, erythrocyte sedimentation rate (ESR), smoking index (number of cigarettes smoked per day times smoking years), and PaO₂ of all patients were also determined.

**Measurement of VEGF**

In patients with CSS, asthma, and acute bronchitis, we measured serum levels of VEGF before the patients underwent therapy. In eight patients with CSS, serum VEGF levels were determined before therapy, 1 month after the start of therapy, and 6 months after the start of therapy. All participants gave written consent to participate in this study.

VEGF concentrations in sera were measured in duplicate for each sample using a commercial enzyme-linked immunosorbent assay kit (R&D Systems; Minneapolis, MN) that recognizes the soluble isoforms (VEGF₁₅₁ and VEGF₁₆₅). This assay is sensitive to 9 pg/mL (0.2 pmol/L) of VEGF and does not cross-react with platelet-derived growth factor or other homologous cytokines. Optical density at 450 nm was measured (Titertek Multiskan MC plate reader; Flow Laboratories; Helsinki, Finland), and VEGF concentration was determined by linear regression from a standard curve (GraphPad software; GraphPad; San Diego, CA) for analysis.

**Immunohistochemical Staining for VEGF**

Immunohistochemical staining for VEGF was performed using a rabbit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) employing the DAB method using the biopsy specimens of five CSS patients as described previously. Briefly, 4-μm-thick sections were dewaxed and rehydrated. For optimal antigen retrieval, sections were pressure cooked in 0.01 mol/L citrate buffer (pH 6.0) for 90 s. Endogenous peroxidase activity was blocked using a 3% hydrogen peroxide solution in methanol for 10 min. After washing, sections were incubated with primary antibody solution for 2 h at room temperature using a 1:150 concentration working dilution of the antibody. Negative control slides were incubated with rabbit polyclonal antibody (Super Sensitive Rabbit; Biogenex; San Ramon, CA). After washing, secondary biotynated anti-Ig antibody (Biogenex) was added, and the mixture was incubated for 30 min at room temperature. The sections were again washed, and streptavidin conjugated to horseradish peroxidase (Biogenex) was incubated for 30 min and then rinsed off with deionized water. Diaminobenzidine tetrahydrochloride substrate solution was then added, and the mixture was incubated for 10 min. A brown color reaction represented a positive result.

**Statistical Analysis**

We used one-way factorial analysis of variance with the Bonferroni-Dunn test and Pearson correlation coefficient; p < 0.05 was considered significant. Most values were expressed as mean ± SD.

**Results**

The serum VEGF levels in patients with CSS were significantly higher than in patients with asthma or acute bronchitis (CSS, 753.2 ± 226.8 pg/mL; asthma, 248.8 ± 188.2 pg/mL; acute bronchitis, 188.3 ± 108.4 pg/mL; Fig 1). The sensitivity of VEGF levels to distinguish CSS from asthma was 93.3%, and specificity was 81.8% (cutoff value, 600 pg/mL). The serum VEGF levels of eight patients with CSS remained high level (1,069.1 ± 225.7 pg/mL) 1 month after the start of therapy and decreased 6 months after the start of therapy (Fig 2). The serum VEGF level at 6 months after the beginning of therapy (393.1 ± 122.4 pg/mL) was significantly lower than that at the start of therapy (809.3 ± 255.1 pg/mL; Bonferroni-Dunn test with one-way factorial analysis of variance, p < 0.001). The clinical symptoms of CSS patients improved with the use of

![Figure 1. Serum VEGF levels in the three groups.](image-url)
corticosteroids. Serum VEGF levels before the start of therapy showed a significant positive correlation with peripheral eosinophil counts (Fig 3; \( r = 0.779, p < 0.0001 \)). Serum VEGF levels did not show a significant correlation with platelet counts \( (r = 0.311, p = 0.08) \), C-reactive protein level \( (r = 0.231, p = 0.12) \), ESR \( (r = 0.309, p = 0.09) \), smoking index \( (r = 0.199, p = 0.3) \), and \( \text{PaO}_2 \) level \( (r = -0.229, p = 0.11) \). There was no significant difference in smoking index and \( \text{PaO}_2 \) levels among these groups. Anti-neutrophil cytoplasmic antibody was positive in 10 CSS patients. In immunohistochemical analysis, infiltrating eosinophils in the lesions stained intensely positive for VEGF (Fig 4).

**Discussion**

Our results showed significantly higher levels of serum VEGF in CSS patients than in asthma patients. In addition, serum VEGF levels in CSS patients before the start of therapy showed a significant positive correlation with peripheral eosinophil counts \( (r = 0.779, p < 0.0001) \). Serum VEGF levels before the start of therapy showed a significant positive correlation with peripheral eosinophil counts (Fig 3; \( r = 0.779, p < 0.0001 \)). Serum VEGF levels did not show a significant correlation with platelet counts \( (r = 0.311, p = 0.08) \), C-reactive protein level \( (r = 0.231, p = 0.12) \), ESR \( (r = 0.309, p = 0.09) \), smoking index \( (r = 0.199, p = 0.3) \), and \( \text{PaO}_2 \) level \( (r = -0.229, p = 0.11) \). There was no significant difference in smoking index and \( \text{PaO}_2 \) levels among these groups. Anti-neutrophil cytoplasmic antibody was positive in 10 CSS patients. In immunohistochemical analysis, infiltrating eosinophils in the lesions stained intensely positive for VEGF (Fig 4).

**Figure 2.** Serum VEGF levels in eight patients with CSS remained high 1 month after the start of therapy and decreased 6 months after the start of therapy (*p < 0.001*). Bars indicate mean values of each group.

**Figure 3.** Serum VEGF levels of CSS patients before the start of therapy showed a significant positive correlation with peripheral eosinophil counts \( (r = 0.779, p < 0.0001) \).

**Figure 4.** Skin biopsy specimen of a patient with CSS showing expression of VEGF in tissue infiltrating eosinophils (top left, a, and top right, d; hematoxylin-eosin; center left, b, and center right, c; VEGF staining; bottom left, e, and bottom right, f; negative control; left panels, original \( \times 200 \); right panels, original \( \times 400 \)). Representative data in five different CSS patients.

**Discussion**

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Eosinophils are the main cellular source of serum VEGF in CSS. It is possible that VEGF released from eosinophils might contribute to the recruitment of inflammatory cells including T-cells and eosinophils by increasing vascular permeability in the development of CSS. Interestingly, serum VEGF levels remained high level 1 month after the beginning of therapy. In CSS, prolonged survival of eosinophils due to inhibition of CD95-mediated apoptosis by soluble CD95 seems to contribute to eosinophilia. Although the mechanisms involved in eosinophil activation in CSS have not been elucidated, data suggest a possible role of T-lymphocytes secreting eosinophil-activating cytokines. Glucocorticoid, which was used for the treatment of CSS in this study, is known to induce apoptosis of eosinophils, and eosinophil counts are very high in CSS. Therefore, we think that the massive VEGF release of the apoptotic eosinophils might explain the high VEGF levels 1 month after the start of therapy in CSS. Administration of neutralizing monoclonal antibody against VEGF, such as bevacizumab, might have therapeutic effect against CSS.

The sensitivity and specificity of VEGF levels at 600 pg/mL to distinguish CSS from asthma were 93.3% and 81.8%, respectively. Abnormal laboratory findings in patients with CSS include increased peripheral blood eosinophil counts and a raised ESR, but it is sometimes difficult to distinguish CSS from asthma using these markers for the following reasons: (1) rarely, eosinophilia is not present, and wide-ranging and rapid changes in eosinophil counts happen in CSS; (2) use of corticosteroids to treat asthma may result in failure to detect eosinophilia in patients with undiagnosed CSS; and d(3) increase in ESR occurs in other disorders such as infection. Another serologic marker, anti-neutrophil cytoplasmic antibodies, is present in 44 to 66% of CSS patients, with the most common pattern being perinuclear. In addition, anti-neutrophil cytoplasmic antibody positivity needs to be confirmed by demonstration of myeloperoxidase in serum. In our study, serum VEGF levels of CSS patients remained high for 1 month, even after the start of therapy. Therefore, we propose that measurement of serum VEGF may be a useful screening marker to distinguish CSS from asthma, as a negative result greatly reduces the likelihood of CSS. However, a positive result requires confirmation, as the specificity is relatively low. Our study is too small and short to draw a definitive conclusion. We propose that larger and longer studies addressing this point are necessary to judge its diagnostic value.

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References
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