

# Soluble CD26 and CD30 Levels in Patients With Common Variable Immunodeficiency

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## ■ Abstract

**Background:** Common variable immunodeficiency (CVID) is a heterogeneous group of disorders characterized by decreased serum immunoglobulin levels and increased susceptibility to recurrent bacterial infections. There is increasing evidence that the type 1 helper T cell (T<sub>H</sub>1)/T<sub>H</sub>2 cell balance is shifted towards a T<sub>H</sub>1-type immune response in patients with CVID. This study was performed to measure levels of soluble CD26 (sCD26) and CD30 (sCD30) as plausible markers of a dysregulated immune response in a group of patients with CVID.

**Methods:** Twenty-five patients with CVID and 20 age- and sex-matched controls were enrolled in this study. A sandwich enzyme-linked immunosorbent assay was used to measure serum sCD26 and sCD30 levels.

**Results:** The mean (SD) serum sCD26 level was significantly higher in patients with CVID than in controls (88.47 [59.82] ng/mL vs 28.31 [25.61] ng/mL,  $P=0.001$ ). Serum sCD30 levels were also significantly higher in patients with CVID than in controls (196.37 [169.71] ng/mL vs 30.72 [12.98] ng/mL,  $P<0.001$ ). Analysis of serum sCD30 levels in association with different clinical variables indicated that patients with splenomegaly and malignancy had significantly higher levels than patients without these disorders. However, serum sCD30 levels did not differ with bronchiectasis or autoimmunity.

**Conclusions:** The presence of increased serum levels of sCD26 and sCD30 in patients with CVID suggests that CVID patients have a polarized immune response towards a T<sub>H</sub>1-like phenotype, whereas the association between high levels of these markers and disease severity suggests that the soluble form could be used as a prognostic tool in CVID.

**Key words:** Common variable immune deficiency. Prognosis. Soluble CD26. Soluble CD30.

## ■ Resumen

**Antecedentes:** La inmunodeficiencia común variable (IDCV) constituye un grupo heterogéneo de alteraciones caracterizado por una disminución en el nivel de inmunoglobulinas séricas y un incremento en la susceptibilidad para sufrir infecciones bacterianas recurrentes. Existen evidencias que confirman que los pacientes con IDCV muestran una alteración del balance Th1/Th2 hacia una respuesta tipo Th1. En este estudio cuantificamos los niveles de CD26 soluble (sCD26) y CD30 (sCD30) como posibles marcadores de una respuesta inmune alterada en un grupo de pacientes con IDCV.

**Métodos:** Se incluyeron 25 pacientes con IDCV y controles con edad y sexo similar, a los que se cuantificó mediante un ELISA los niveles de CD26 soluble y CD30.

**Resultados:** Los niveles medios de sCD26 en pacientes con IDCV fueron 88.47±59.82 ng/mL, significativamente más altos que en los controles 28.31±25.61 ng/mL ( $p=0.001$ ). Los niveles de sCD30 en pacientes con IDCV fueron también mayores que en los controles (196.37±169.71 ng/mL vs. 30.72±12.98 ng/mL,  $p<0.001$ ). El análisis de los niveles de sCD30 en asociación con diferentes variables clínicas indicaron que los pacientes con esplenomegalia y neoplasias eran más altos en comparación con los que no presentaban estas asociaciones. No se encontraron asociaciones de los niveles de sCD30 con la presencia de bronquiectasias o autoinmunidad.

**Conclusiones:** La elevación de sCD26 y sCD30 en pacientes con IDCV podría sugerir la hipótesis de una respuesta inmune polarizada al fenotipo Th1, y la asociación de niveles elevados de estos marcadores con la severidad de la enfermedad puede involucrar a estos marcadores como una herramienta útil en el pronóstico de la IDCV.

**Palabras clave:** Inmunodeficiencia común variable. Pronóstico. CD26 soluble. CD30 soluble.

## Introduction

Common variable immunodeficiency (CVID) is a heterogeneous group of diseases characterized by low levels of 2 or more immunoglobulin (Ig) isotypes (IgG, IgA, or IgM), impaired functional antibody responses, and increased susceptibility to recurrent bacterial infections, as well as autoimmunity, granulomatous diseases, and neoplasia [1,2]. Although CVID is the most frequent clinical primary immunodeficiency and has been studied for over 5 decades, the underlying genetic etiology remains largely unknown.

The characteristic immune defect in CVID is believed to be impaired differentiation of B cells into plasma cells, with the result that hypogammaglobulinemia is a consistent finding in patients with this syndrome. However, several studies revealed a variety of defects in the B cells and T cells of affected patients [3-10]. Dipeptidyl peptidase IV (DPPIV/CD26) is a multifunctional type II transmembrane ectopeptidase expressed in several tissues; in the hematopoietic system it is predominantly expressed by T cells [11]. It has been suggested that high levels of expression of CD26, or increased levels of its soluble form (sCD26), would indicate a type 1 helper T cell (T<sub>H</sub>1)-like immune response [12,13]. However, no data are available for CVID patients.

The surface antigen CD30, a member of the tumor necrosis factor receptor superfamily, is a type I transmembrane glycoprotein that is usually expressed in activated T and B lymphocytes and natural killer cells under normal conditions [14]. However, the immunologic function of CD30<sup>+</sup> T cells remains largely unknown. The soluble form of the molecule (sCD30) is released into the bloodstream after cellular activation, and its level has been found to be closely related to expression of CD30<sup>+</sup> T cells [15]. Increased sCD30 levels have been demonstrated in a variety of viral infections, inflammatory disorders, and selected lymphoid malignancies [14]. However, little information is available concerning sCD30 levels in patients with CVID and the possible correlation between sCD30 levels and clinical markers that indicate disease severity. We previously showed that patients with CVID had high titers of sCD30, which increased further in those with splenomegaly and malignancy [16].

In the present study, we examined serum sCD26 and sCD30 levels in patients with CVID and their association with clinical indices of disease severity. Given the highly heterogeneous clinical manifestations and immunological phenotypes in CVID, improved immunological characterization and classification of patients would help to identify the genetic defects underlying this disease.

## Materials and Methods

### *Patients and Controls*

We performed a cross-sectional study of 25 patients with CVID who were regularly evaluated and receiving monthly intravenous immunoglobulin (IVIG) substitution therapy at the Children's Medical Center Hospital, the Pediatrics Center of Excellence in Tehran, Iran. Twenty age- and sex-matched healthy volunteers were recruited as controls.

All patients were diagnosed according to the diagnostic criteria of the International Union for Immunological Societies Scientific Committee for primary immunodeficiency diseases [17]. Patients included were free of infections at the time of study and during the 6 months before sample collection. Samples were collected immediately before IVIG infusion, with 3-4 weeks between infusions. The study protocol was approved by the local institutional review board. Written informed consent was obtained from the participants.

### *Serum sCD26 and sCD30 Assays*

Serum samples were drawn from each participant and stored at -70°C until assayed. Serum sCD26 and sCD30 concentrations were measured using commercially available enzyme-linked immunosorbent assay (ELISA [Bender MedSystems]). Optical density was determined at 450 nm using an ELISA microplate reader. sCD26 and sCD30 concentrations were read from the standard curve generated using the recombinant human sCD26 and sCD30 provided with the assay kit.

### *Statistical Analysis*

The statistical analysis was performed using SPSS version 14.0 (SPSS Inc). Parametric and nonparametric variables were presented as mean (SD) and median (range), respectively. The Mann-Whitney test and Wilcoxon signed rank test were used to compare variables between groups. A *P* value <.05 was considered statistically significant.

## Results

### *Patient Characteristics*

Twenty-five patients with CVID and a median age of 20 years whose first manifestations were during childhood (43.5 [9] months) were included in this study. Mean serum IgG, IgM, and IgA levels were 165.1 mg/dL, 27.3 mg/dL, and 7.6 mg/dL, respectively. Mean percentages of CD19<sup>+</sup> B cells and CD3<sup>+</sup> T cells were 11.9% and 70.6%, respectively. Eleven patients (44%) had a CD4<sup>+</sup>/CD8<sup>+</sup> T-cell ratio of less than 1. Upper and lower respiratory tract infections (6 pneumonia, 4 otitis media, and 3 sinusitis) and diarrhea (8 cases) were the most common presentations in these patients. Other presentations included osteomyelitis, failure to thrive, cutaneous infection, and omphalitis. Seven patients developed bronchiectasis. Splenomegaly was detected in 10 patients. Autoimmune disorders and malignancies were reported in 6 and 4 cases, respectively.

### *Soluble CD26*

The mean serum sCD26 level was significantly higher in patients with CVID than in the control group (88.47 [59.82] ng/mL vs 28.31 [25.61] ng/mL; *P*=.001, chi-square test).

### *Soluble CD30*

Serum sCD30 levels in patients with CVID were

significantly higher than in the controls (196.37 [169.71] ng/mL vs 30.72 [12.98] ng/mL;  $P < .001$ ). It should be noted that whilst none of the healthy controls had a sCD30 titer  $>60$  ng/mL, only 1 CVID patient had a sCD30 titer  $<60$  ng/mL.

### Clinical Implications of sCD26 and sCD30

Association studies of different clinical phenotypes with sCD26 levels revealed that serum sCD26 level does not correlate with disease severity in patients with CVID. Seven patients developed bronchiectasis with a mean sCD26 level of 120.34 (68.87) ng/mL, which was higher than in patients without bronchiectasis (76.07 [53.34] ng/mL), although this difference was not significant. Serum sCD26 levels did not vary with splenomegaly, autoimmunity, or malignancies.

Analysis of serum sCD30 levels in association with different clinical variables indicated that patients who had splenomegaly had significantly higher levels than those who did not (288.18 [232.19] ng/mL vs 135.17 [68.41] ng/mL,  $P = .002$ ). Indeed, mean serum sCD30 level in patients with malignancy was 354.25 [347.27] ng/mL, which was significantly higher than in those without malignancy (166.30  $\pm$  102.64 ng/mL) ( $P < .001$ ). However, serum sCD30 levels did not vary with bronchiectasis or autoimmunity.

## Discussion

CVID is a heterogeneous group of disorders caused by a variety of inherited genetic defects with specific clinical manifestations and immunological abnormalities [18-21], the characterization of which would help to identify common defects at the cellular and molecular levels, and ultimately, the responsible genes.

In the present study, we found higher serum sCD26 and sCD30 concentrations in patients with CVID than in healthy controls. In addition, the results indicated that higher sCD26 and sCD30 levels correlated with indicators of disease severity, ie, bronchiectasis and splenomegaly, respectively. We recently reported the results of a study in which patients with CVID had increased serum sCD30 levels; the highest serum sCD30 levels were detected in the group of patients with splenomegaly and lymphoma [16]. The results of the present study are consistent with and extend the findings of our previous study by demonstrating an association between serum sCD26 and sCD30 levels and secondary complications in patients with CVID.

Cell surface expression of CD26 is upregulated in human activated lymphocytes by interleukin (IL) 12 and IL-2 [12, 22] and correlates with the production of interferon (IFN)  $\gamma$  [13]. These results indicate that high levels of expression of CD26 antigen correlate with a  $T_H1$ -like phenotype. Indeed, a soluble form of CD26, which lacks the cytoplasmic tail and transmembrane region, has been used in several studies as an activation marker for in vivo evaluation of  $T_H1$ -dominant diseases [23]. In contrast, decreased sCD26 levels have been demonstrated in rheumatoid arthritis [24], Crohn disease [25], and systemic lupus erythematosus [26], in which decreased serum sCD26 levels correlated with disease severity.

There is increasing evidence that the  $T_H1/T_H2$  cell balance is shifted toward a  $T_H1$ -type immune response in patients with CVID. Martinez-Pomar et al [27] reported significantly increased serum IL-12p40 subunit levels in this population. In CVID, increased production of the  $T_H1$  cytokine IFN- $\gamma$  was observed in the  $CD8^+CD28^+$  cytotoxic T-cell subset [28], and lipopolysaccharide-stimulated  $CD14^+$  monocytes expressed a higher proportion of intracellular IL-12-positive cells [29]. Moreover, the idiopathic inflammatory bowel disease observed in patients with CVID was associated with excessive  $T_H1$  cytokine production in lamina propria mononuclear cells [30]. In contrast, defective expression of the *IL2* and *IFNG* genes was observed in response to antigen, but not to anti-CD3 [31], and the lack of a significant difference in IL-2 and IFN- $\gamma$  production in vitro [32] and in the serum of patients with CVID compared to healthy controls [10] might argue against the above findings. Our results for increased sCD26 levels lend further support to a potential  $T_H1$ -biased immune response in patients with CVID. However, the possibility of characteristic immunological abnormalities in subgroups of CVID patients should not be ruled out.

CD30 antigen was once known to be an activation marker of T-cell clones that was able to produce  $T_H2$ -type cytokines; however, the results of functional studies on purified  $CD30^+$  T cells indicated that CD30 is an important costimulatory molecule for maintaining the physiological balance between  $T_H1/T_H2$  immune responses by integrating  $T_H1$ - and  $T_H2$ -specific cytokine production and Bcl-2 molecule expression [33-35]. It has been postulated that lack of CD30 signaling may enable development of  $T_H1$ - or  $T_H2$ -mediated diseases and that increased sCD30, as has been demonstrated in a variety of viral infections and inflammatory disorders [14], could blunt the CD30/CD30L interaction, thus further enhancing  $T_H1$  and  $T_H2$  immune responses. Here, we report increased sCD30 levels in patients with CVID, which are consistent with our previous findings [10], further indicating that the  $T_H1/T_H2$  imbalance is implicated in the pathogenesis of CVID.

The correlation between serum sCD26 and sCD30 levels and clinical indices of disease severity indicate that these biomarkers, in combination with phenotyping of circulating lymphocyte subsets, can be used as a prognostic tool in clinical practice. However, long-term follow-up studies are required to further support this conclusion.

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