

REGULAR ARTICLE

Association of serum-soluble CD26 and CD30 levels with asthma, lung function and bronchial hyper-responsiveness at school age

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ABSTRACT

Aim: There is a need for markers of Th1 and Th2 imbalance in diseases such as asthma. CD30 is an activation marker of Th2 cells, and importance of Th1 marker CD26 was recently found in adult asthma. We studied whether serum-soluble CD30 (sCD30) or serum-soluble CD26 (sCD26) could support early diagnosis of asthma in children at school age.

Methods: sCD26 and sCD30 were measured in 34 children with clinically confirmed asthma, 31 with possible asthma and in 147 controls. In addition, the associations of flow volume spirometry, methacholine inhalation challenge and free running test results with serum sCD26 or sCD30 were analysed.

Results: Serum sCD30 was significantly higher in children with confirmed asthma (mean 91.5 IU/mL, SD 23.0) than in the controls (78.8 IU/mL, 25.9; $p = 0.042$). No significant differences were found in serum sCD26 levels between the groups. There was a negative correlation of mean mid expiratory flow values with serum sCD26 ($r = -0.22$, $p = 0.0018$). Neither methacholine inhalation challenge nor free running test results were associated with serum sCD26 or sCD30.

Conclusion: Serum sCD30 was higher in children with asthma. However, marked overlap in serum sCD30 between asthmatic and healthy children limits the usefulness of sCD30 as a diagnostic marker of asthma.

INTRODUCTION

Bronchial asthma is a chronic inflammatory disease characterized by intermittent, reversible obstruction and hyper-responsiveness of the airways. The main feature of asthma is persistent airway inflammation that fails to resolve spontaneously (1).

Antigen-presenting cells (APCs) in the bronchial mucosae capture the inhaled allergens (animal hair, dust mites, feathers, pollutants, mould and respiratory viruses) and present them to CD4⁺ T-helper (Th) lymphocytes. The engagement of the T-cell receptor (TCR) of the naive CD4⁺ Th lymphocyte to the class II major histocompatibility complex (MHC)/peptide complex on the APC results in signal transduction from the cell membrane to the nucleus. This leads to altered gene expression and subsequent secretion of cytokines (2,3). Each naive CD4⁺ Th lymphocyte has the potential to differentiate into either of the functionally distinct Th effector subsets, Th1 or Th2. The stimulus delivered via the TCR on the naive CD4⁺ Th lymphocyte in

conjunction with activation of costimulatory pathways is essential for a naive Th cell to progress along the Th differentiation pathway. A number of factors influence the differentiation process towards the Th1 or Th2 lineage, including the type of APC, the nature of the immunogen,

Key notes

- Serum sCD30, a marker of Th2-oriented immunity, but not serum sCD26, a marker of Th1-oriented immunity, was higher in children with asthma than in controls.
- However, marked overlap in sCD30 levels between asthmatic and healthy children limits its usefulness as a diagnostic marker of asthma
- Neither sCD26 nor sCD30 differed between the children with and without bronchial hyper-responsiveness, as measured by exercise challenge and methacholine inhalation challenge tests.

cytokines (IL-12, interferons or IL-4) at the time of the antigen presentation, the dose of the antigen (duration and strength of signal) and the ligation of select costimulatory molecules (2,3). Activation of the acquired immune pathway leads to IL-5-mediated eosinophil inflammation that is characterized by elevated serum IgE concentrations and association with other allergic symptoms in the patient (1).

Th2 lymphocytes have been considered to play a major role in the pathogenesis of allergic asthma, as they secrete cytokines critical to promote IgE production and eosinophil recruitment into the airway (2,3). Mast cells carrying allergen-specific IgE from previous contact with the allergen are stimulated also by cytokines secreted by Th2 cells. Activated mast cells release inflammatory mediators, which leads to bronchospasm (1). Moreover, it has also been suggested that activation of the innate immune pathway leads to IL-8-mediated neutrophil inflammation and subsequent characteristic asthma symptoms (noneosinophilic asthma) (4).

Interestingly, Th1 type response has been proposed to protect against asthma by diminishing the activity of Th2 response (2), but in a recent study, Th1 type response was found to contribute to the pathogenesis of asthma (5). Interactions between Th1 and Th2 responses seem to be complex as Th2 type activation has been connected to down-regulation of Th1 type activation (6).

Determination of biomarkers of T-lymphocyte activation is necessary for understanding the inflammation and for follow-up of inflammatory processes (1). CD30 molecule belongs to the tumour necrosis factor receptor super-family, and its surface expression on T lymphocytes is up-regulated following T-cell activation (7). CD30 has been regarded as a marker of Th2 lymphocyte activation (7) and also as an important regulatory molecule of the balance between Th1 and Th2 lymphocytes (8).

Despite the evidence of asthma being a Th2 disease (9), the expression of Th1 activation marker CD26 was found to be elevated in asthmatic patients (10). CD26, a cell surface glycoprotein with a dipeptidyl peptidase IV activity, is expressed in activated T lymphocytes, endothelial and epithelial cells, and it may act as a costimulatory molecule of T-lymphocyte activation (11). In addition, CD26 may help activated T lymphocytes to localize to inflammatory regions (12). There are not many clinical studies of serum sCD30 and sCD26 in asthma. Serum sCD30 concentrations have been correlated with the severity of asthma in children (13), and plasma sCD26 has been elevated in adults with asthma (10).

The aim of this study was to examine the association of serum sCD26 and sCD30 concentrations with asthma in children at school age. In addition, we studied the association of lung function and bronchial hyper-responsiveness with serum sCD26 and sCD30.

METHODS

Study design

A population-based sample of 2011 children aged 7–12 years was screened by a written questionnaire for

respiratory symptoms and asthma (14). From a total of 1633 (81%) children returning the questionnaire, 186 children with respiratory symptoms and 128 randomly selected non-symptomatic control children were invited to participate in the clinical phase of the study. The clinical tests were taken when the children had no respiratory symptoms (e.g. outside of an acute infection). A total of 247 (79% of those invited) children took part in this phase of the study. More detailed description of the screening and clinical phases of the study have previously been published (14).

Serum-soluble CD26 and CD30 analyses

Venous blood samples (4 mL) were drawn into Vacutainer SST tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and allowed to clot at room temperature for 60–120 min. Samples were then centrifuged at 1000–1350 g for 10 min, and the serum was separated and aliquoted. Serum was frozen at -70°C and stored until analysed for serum sCD26 and sCD30 concentrations. Serum sCD26 and sCD30 measurements were performed by enzyme immunoassays (ELISA; Bender MedSystems, Vienna, Austria) according to the manufacturer's instructions. The detection limit for sCD26 was estimated to be 6.8 ng/mL and for sCD30 1.9 IU/mL.

Definitions of asthma

Children with a symptom history consistent with asthma and at least one objective clinical, lung function or challenge test finding: wheezing on auscultation which disappeared after inhaled β_2 agonist associated with >15% increase in peak expiratory flow (PEF); >20% variability and >15% increase in PEF on at least three occasions after inhaled β_2 agonist during a 4-week home PEF monitoring; baseline obstruction with >15% improvement in PEF or FEV₁ after inhaled β_2 agonist; pathological result in exercise challenge by free running outdoors ($\geq 10\%$ fall in FEV₁) or in methacholine inhalation challenge (provocative dose causing $\geq 20\%$ fall in FEV₁ of $\leq 400 \mu\text{g}$) were defined as having *clinically confirmed asthma* (n = 43). As steroids effectively reduce bronchial hyper-responsiveness, six asthmatic children on inhaled steroids were excluded from the analyses (14). Children with a symptom history consistent with asthma but no objective findings were defined as having *possible asthma* (n = 34). The remaining children with no symptoms suggestive of asthma or other chronic or recurrent respiratory disorder served as *control children* (n = 170).

Other explanatory variables

All children underwent lung function testing by flow volume spirometry (FVS), and two tests for bronchial hyper-responsiveness (exercise challenge by free running outdoors and methacholine inhalation challenge) (14). The parameters used in FVS (as percent predicted) were forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV₁) and maximal mid expiratory flow (MMEF). Percentage fall index for FEV₁ was used in the exercise challenge ($\Delta\text{FEV}_{1\%}$), and the provocative dose of inhaled methacholine

causing 20% fall in FEV₁ (PD20FEV₁) in the methacholine inhalation challenge.

Other explanatory variables considered were serum total IgE (IU/L), eosinophil cationic protein (ECP; $\mu\text{g/L}$) and eosinophil protein X (EPX; $\mu\text{g/L}$) (15). These laboratory parameters were treated as continuous unless stated otherwise. In addition, age (years), gender (male/female) and skin prick test reactivity (at least one positive result: yes/no) were assessed as potential confounding variables (14).

Statistical analyses

The data were analysed using STATA release 7.0 (<http://www.STATA.com>). Both serum sCD26 and sCD30 distributions, as well as results of the FVS, were normal, and no transformations were needed for these variables. Log-transformations were made for total IgE, ECP and EPX results to obtain normality. Neither exercise challenge nor methacholine inhalation challenge test results reached normality by transformations and were therefore treated as categorical. For exercise challenge test results, percentage fall for FEV₁ was categorized into three categories: <10% (normal), 10–14% (mild abnormal) and $\geq 15\%$ fall in FEV₁ (pathological). Accordingly, the methacholine inhalation challenge test results were categorized into four categories as follows: PD20FEV₁ >4900 μg (normal), 1601–4900 μg (mild abnormal), 401–1600 μg (moderate abnormal) and ≤ 400 μg (pathological).

In analysing the association of serum sCD26 and sCD30 with the explanatory and confounding variables, bivariate comparisons were initially made using Student's *t*-test (two groups) and one-way ANOVA with Bonferroni multiple comparison test (three or more groups). Correlations of the serum sCD26 and sCD30 values with continuous variables were analysed using scatter plots and Spearman's correlation coefficient. Finally, we used multiple linear regression to explain variation in serum sCD26 or sCD30 by asthma and other explanatory variables.

Approval for the clinical study was given by the ethics committee of the Kuopio University Hospital, Kuopio, Finland.

RESULTS

Among all 212 children included in this study, there were 117 boys (55.2%), and 112 children (52.8%) had at least one positive skin prick tests result against the local aeroallergens (14). The mean age of the children was 10.4 years (SD 1.66, range 7.6–13.5 years). Serum sCD26 data were available for 197 children and that of sCD30 for 202 children.

Serum sCD30 was higher in children with clinical asthma (mean 91.5 IU/mL, SD 23.0) than in children with the possible asthma (mean 85.0 IU/mL, SD 28.5) or in control children (mean 78.8 IU/mL, SD 25.9; $p = 0.042$) (Fig. 1). No significant differences were found in serum sCD26 levels between the children with clinical asthma, possible asthma or controls (mean 1540 ng/mL, SD 308; mean 1550 ng/mL, SD 270; and mean 1581 ng/mL, SD 256, respectively) (Fig. 1).

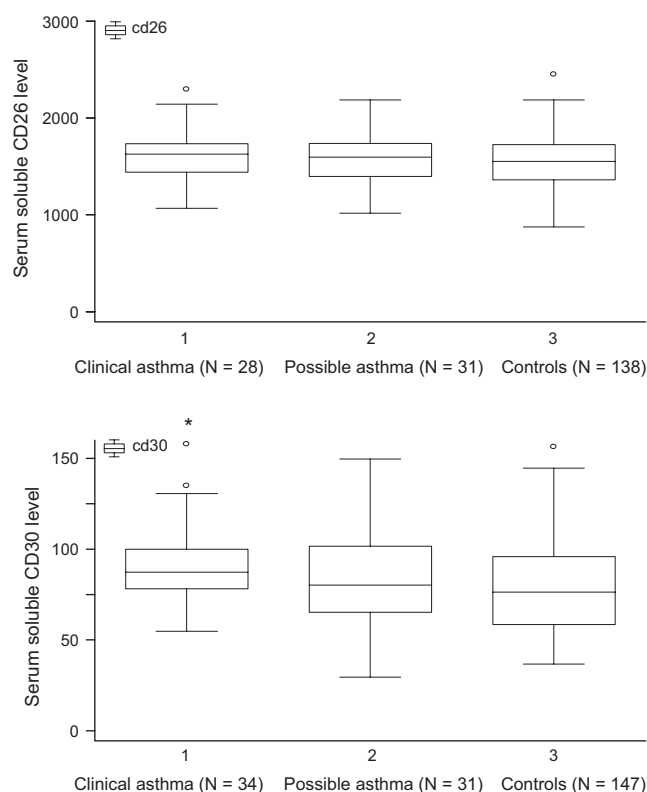


Figure 1 Box-and-whisker plots of serum sCD26 (ng/mL, the upper figure) and sCD30 (IU/mL, the lower figure) values in children with clinical asthma, possible asthma, and in the control children. Serum sCD30 was higher in clinical asthma (* $p < 0.05$) than in controls. Lines in the Box-and-whisker plots represent median (middle line in the box), 25–75 percentiles (Box extension) and upper and lower adjacent values (lines emerging from the box).

In FVS, there was a weak but statistically significant negative correlation of mean mid expiratory flow values with serum sCD26 ($r = -0.22$, $p = 0.0018$) and of borderline significance with serum sCD30 ($r = -0.14$, $p = 0.054$). Neither methacholine inhalation challenge nor exercise challenge results were associated with serum sCD26 or sCD30 levels (Table 1).

There was virtually no correlation between serum sCD26 and serum total IgE ($r = -0.11$, $p = 0.13$), serum ECP ($r = 0.049$, $p = 0.50$) or EPX ($r = 0.013$, $p = 0.86$). Similarly, no correlation was found between serum sCD30 and serum total IgE ($r = -0.0018$, $p = 0.98$), serum ECP ($r = -0.070$, $p = 0.32$) or EPX ($r = -0.0092$, $p = 0.90$).

Among all studied children, serum sCD26 was higher in boys (mean 1617 ng/mL, SD 27.1) than in girls (mean 1479 ng/mL, SD 27.0; $p < 0.001$). In contrast, no gender differences were observed in serum sCD30 (boys: mean 81.8 IU/mL, SD 2.5; girls: mean 81.3 IU/mL, SD 2.8; $p = 0.91$) (Fig. 2). The age of the children correlated neither with serum sCD26 ($r = -0.054$, $p = 0.42$) nor with serum sCD30 ($r = -0.041$, $p = 0.56$). Skin prick test reactivity was unrelated to serum sCD26 or serum sCD30 levels (not shown).

Table 1 Serum sCD26 (ng/mL) and sCD30 (IU/mL) concentrations, by categories of the free running test and methacholine inhalation challenge test results

	Serum sCD26 (ng/mL)		Serum sCD30 (IU/mL)	
	Mean (SD)	N	Mean (SD)	N
Exercise challenge by free running test result, Δ FEV ₁ * (%)				
<10	1558 (285)	178	81.7 (26.7)	183
10–14.9	1537 (252)	10	79.7 (19.6)	10
≥15	1545 (181)	9	81.6 (25.1)	9
p between the groups	NS		NS	
Methacholine inhalation challenge, PD20FEV ₁ [†] (μg)				
>4900	1551 (278)	97	82.1 (25.8)	101
1601–4900	1579 (304)	32	81.3 (28.0)	32
401–1600	1501 (263)	44	82.3 (27.2)	45
≤400	1639 (268)	22	78.8 (25.6)	22
p between the groups	NS		NS	

* Δ FEV₁: percent fall of FEV₁ in the free running test.[†]PD20FEV₁: provocative dose of inhaled methacholine causing a 20% fall in FEV₁.

In multiple linear regression, serum sCD26 and sCD30 were used as a response variable in separate models. In the final models, we included age, gender and MMEF as *a priori* selected explaining variables. MMEF was selected as it was associated with serum sCD26 and sCD30 in the bivariate analyses. Other, nonsignificant explanatory variables were

excluded in reason to get the most parsimonious model. In multiple regression models, both gender differences in serum sCD26 and the differences in serum sCD30 between the asthma groups remained statistically significant (Table 2). On the other hand, very little of the variation in serum sCD26 or sCD30 could be explained by our models. Having serum sCD30 as a response variable and including asthma grouping variable alone into the model, we were able to explain only 3.2% of the variation in serum sCD30 (R^2 0.0315). Adding age, gender and MMEF gave only minor improvement into the model fit (R^2 0.0462). Likewise, gender alone explained variation in serum sCD26 by only 6.1% (R^2 0.0611), with only minor improvement in model fit when adding asthma group, age and MMEF into the model (R^2 0.0853).

DISCUSSION

The main result in the present study was that serum sCD30, a marker of Th2-oriented immunity, but not serum sCD26, a marker of Th1-oriented immunity, was higher in children

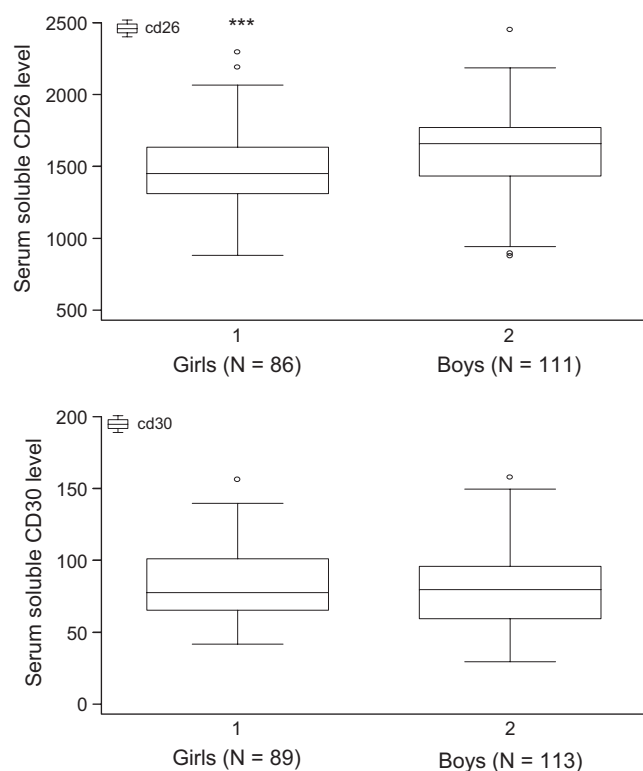


Figure 2 Box-and-whisker plots of serum sCD26 (ng/mL, the upper figure) and sCD30 (IU/mL, the lower figure) values, by gender. Serum sCD26 was lower in girls than in boys (*** $p < 0.001$). Lines in the Box-and-whisker plots represent median (middle line in the box), 25–75 percentiles (Box extension) and upper and lower adjacent values (lines emerging from the box).

Table 2 Findings of the multiple linear regression models, separately for serum sCD26 and sCD30 as response variables

Explaining variable	Serum sCD26 N = 197		Serum sCD30 N = 202	
	Adjusted coefficient (SE)	p	Adjusted coefficient (SE)	p
Diagnostic group				
Control children*	–		–	
Possible asthma	–16.0 (56.2)	0.78	4.3 (5.4)	0.43
Clinical asthma	22.4 (58.0)	0.70	11.1 (5.4)	0.043
Age (years)	–11.2 (11.8)	0.34	–1.0 (1.1)	0.36
Male gender	118.8 (41.4)	0.005	–2.9 (3.9)	0.46
Mean mid expiratory flow	–2.1 (1.11)	0.059	–0.16 (0.11)	0.13

SE = Standard error.

* Reference category.

with asthma than in controls. Neither marker differed between the children with and without bronchial hyper-responsiveness. Serum sCD26, but not serum sCD30, correlated negatively with MMEF in FVS.

Increased serum sCD30 in children with clinical asthma is in accordance with the only earlier study in children, which proposed an association between serum sCD30 and the severity of asthma in children (13). In the present study, there was a considerable overlap between asthmatics and controls, which limits the usefulness of sCD30 as a diagnostic marker of asthma. In addition, children with clinically confirmed asthma did not differ from those with possible, nonconfirmable asthma. The explanation may be that only mild to moderate asthma cases not treated by inhaled steroids were included in the analyses. Although not on maintenance medication, only a small proportion of children with clinical asthma had pathological exercise challenge and methacholine inhalation challenge test results (14).

Serum sCD26 correlated negatively with MMEF in spirometry. Although statistically significant, the correlation was weak. Reports from animal models (16,17) and peripheral blood mononuclear cell expression (18) have indicated that sCD26 probably has a functional role in the development of asthma. The pathogenesis of asthma seems to be complex; evidently, generated CD26 responses lead to activation of dendritic cells, which are capable of augmenting both Th1- and Th2-controlled immune responses (5).

Atopy was more common in the two asthma groups than in controls (14). Two previous studies have documented an association between atopy and high serum sCD30 (13,19) and one an association between atopy and sCD26 (20). Our results did not confirm this connection, as no association was found between skin prick test positivity or serum total IgE values and serum sCD30 or sCD26.

The proportion of boys was higher in the two asthma groups than in controls (14). In earlier studies, gender has been associated with neither serum sCD30 (19) nor serum sCD26 (20). In the present study, serum sCD26 was higher in boys. We found no association between serum sCD26 or sCD30 and age, although in earlier studies serum sCD30 has decreased by increasing the age (21,22).

Th2 type lymphocytes secrete cytokines, e.g. IL-4, IL-5 and IL-13 that promote B-lymphocytes to produce IgE (2). IL-13 induces also activation of eosinophils and basophils (2). An association between serum sCD26 and blood eosinophil counts has been documented in adults with allergic asthma (10), and an association between serum sCD30 and total serum IgE concentration in adults with atopic dermatitis (23), but not in children with atopic dermatitis or asthma (13). When school children and adults were included in the same study, serum sCD26 and sCD30 were associated with serum IgE in patients with atopic dermatitis (20). Concerning atopy, the present study was totally negative; no association was found between serum sCD26 or sCD30 and skin prick tests or IgE, ECP or EPX concentrations in serum.

The main result of the present study, the association of high serum sCD30 with asthma, remained the same in multiple regression analyses including the asthma grouping

(clinical, possible, no) or MMEF in spirometry as an explaining variable and age and gender as confounding variables. However, in this model, asthma grouping explained only 3.2–6.1% of the variation in serum sCD30.

Only few clinical studies on serum sCD26 and sCD30 in asthma have been conducted, and only selected attendants, patients with allergic asthma, have been enrolled (10,13). The present study was conducted in a population-based sample of children screened by questionnaires for respiratory symptoms and asthma, followed by intense clinical examination with objective measures of asthma and atopy for a subgroup of both symptomatic and nonsymptomatic children (14). Although there was a marked overlap in serum sCD30 levels between asthmatic and healthy children, we were able to show that increased serum sCD30 associated with asthma and increased serum sCD26 associated with decreased MMEF in spirometry. The clinical value of these findings remains to be resolved in further studies.

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