



Letter

Serum soluble T_H cell activity markers and high-sensitivity C-reactive protein in multiple-trigger wheezers

Deviation of immune responses toward T_H2 cell reactions results in eosinophilic inflammation of the mucosa and increased responsiveness to various stimuli in the respiratory tract. Such T_H2-type inflammation has been found in endobronchial biopsy specimens in children with multitrigger wheeze and reduced lung function and might be detected as low-grade systemic inflammation. On the other hand, T_H cells other than T_H2-lymphocytes may contribute to eosinophilic inflammation of the airways.

Traditionally, in practices other than those of tertiary care, assessment of airways inflammation in wheezy preschool children has been based on clinical measures. During recent years, peripheral blood biomarkers, which reflect the activation of T_H cells, have been recognized. Serum soluble CD30 (sCD30), a marker of T_H2-cell activation, has been associated with asthma and lung function² and evaluated as a potential therapy response marker.^{2,3} The role of serum soluble CD26 (sCD26), reflecting T_H1 cell activity, seems to be more equivocal in the pathogenesis of asthma.^{4,5} To date, there are no reports on the association of these T_H cell activity markers and the presence of low-grade systemic inflammation.

In our prior study, 105 children with multiple-trigger wheeze and evidence of bronchodilator responsiveness and/or exercise-induced bronchoconstriction were randomized to receive inhaled fluticasone propionate, 100 µg twice daily, inhaled salmeterol and fluticasone propionate combination, 50/100 µg twice daily, or inhaled salmeterol, 50 µg twice daily for 8 weeks.¹ As a result of the intervention, exhaled nitric oxide fraction (FeNO) decreased and lung function (evaluated by impulse oscillometry) improved more in the fluticasone and salmeterol-fluticasone groups than in the salmeterol group.¹ Moreover, lung function improved slightly more in the salmeterol-fluticasone group when compared with the fluticasone group.¹ On this basis of this, we hypothesized that pulmonary function abnormalities observed in the cohort could be related to imbalance in activation of different T_H cell populations and might be tracked by peripheral blood biomarkers.

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Clinical Trial Registration: The original intervention study (Mäkelä MJ, Malmberg LP, Csonka P, Klemola T, Kajosaari M, Pelkonen AS. Salmeterol and fluticasone in young children with multiple-trigger wheeze. *Ann Allergy Asthma Immunol*. 2012; 109:65–70¹), on which this manuscript is based, was conducted at the time when international clinical trial registration sites were not commonly available (in 2002–2005), and the trial was registered in 2002 at the registry of Clinical Trials of Helsinki University Hospital.

Concentrations of sCD26 and sCD30 were determined from serum samples obtained at baseline and after the intervention, using the enzyme-linked immunosorbent assay method.⁶ The intra-assay precisions were 8% and 6% and the interassay precisions were 15% and 14% for serum sCD26 and sCD30, respectively.⁶ Age- and sex-adjusted reference values were applied to define abnormal serum sCD26 and sCD30 levels.⁶ Interleukins 4, 10, and 13 were measured at baseline and after the intervention using the LINCOplex assay (Linco Research Inc, St Charles, Missouri) and the Luminex R 100 TM instrument (Luminex Corp, Austin, Texas).⁷ Serum high-sensitivity C-reactive protein (hs-CRP) was measured at baseline using the immunoturbidimetric assay (CRPHS; Roche Diagnostics GmbH, Mannheim, Germany). The serum hs-CRP level was considered elevated when the concentration was at the 75 percentile or over of the age- and sex-specific percentiles.⁸

Fifteen of the original cohort of 105 children were excluded from the final analyses because of inadequate serum samples for sCD26 and sCD30 determination. In addition, 3 children were excluded from hs-CRP analyses because CRP-level was 10 mg/L or greater.⁸

The study was approved by the Research Ethics Committee of the regional university hospital. Before participation of the study, written informed consent was obtained from parents of all study children.

The statistical tests were chosen depending on whether the data were normally distributed or not. If applicable, the data were log transformed before analyses. Correlation was evaluated by Pearson or Spearman rank correlation tests or the Kendall τ statistic. To adjust for age in correlation analyses, analysis of covariance was performed. Differences between groups were analyzed by the χ^2 or Fisher exact test, t test, Mann-Whitney U test, analysis of variance, or Kruskal-Wallis test. In repeated measurements, the t test for paired samples or the Wilcoxon signed rank test was performed. To compare repeated measurements between the intervention groups, general linear model for repeated measures was applied.

At baseline, the serum sCD26 level was abnormal in 11 (12%), and the sCD30 level was abnormal in 9 children (10%). Serum sCD26 correlated positively with age ($r = 0.280$, $P = .008$) and negatively with log-transformed serum sCD30 ($r = -0.359$, $P = .001$) and hs-CRP ($\tau = -0.156$, $P = .04$). In addition, log-transformed sCD30 correlated negatively with age ($r = -0.304$, $P = .004$) and height ($r = -0.260$, $P = .01$), and positively with a z score of respiratory resistance at 5 Hz ($r = 0.230$, $P = .03$). After adjustment by age, the association between sCD26 and hs-CRP was lost ($P = .32$), but the association between log-transformed sCD30 and height-adjusted respiratory resistance at 5 Hz

Table 1Change in the levels of T_H1 and T_H2 activation markers, associated interleukins, and blood eosinophils with regard to the 8-week intervention

Variable	Fluticasone propionate (n = 31)	Salmeterol-fluticasone (n = 30)	Salmeterol (n = 29)	P value
sCD26, mean (SD), ng/mL				
At baseline	1384.2 (320.2)	1487.7 (255.2)	1433.8 (220.5)	.33
After the intervention	1384.0 (242.5)	1455.4 (236.1)	1543.0 (266.2)	.051
P value for change	1.0	.38	.03	
sCD30, geometric mean (95% CI), IU/mL				
At baseline	97.5 (87.1–109.2)	97.2 (89.1–106.0)	100.0 (89.7–111.5)	.91
After the intervention	94.0 (79.1–111.6)	86.2 (76.6–96.9)	89.5 (79.6–100.7)	.66
P value for change	.67	.03	.12	
IL-4, median (IQR), pg/mL				
At baseline	1.6 (0.1–169.8)	20.2 (0.1–248.6)	48.9 (0.1–253.7)	.40
After the intervention	14.6 (0.1–141.3)	12.3 (0.1–212.2)	82.7 (0.1–211.3)	.54
P value for change	.39	.003	.34	
IL-10, median (IQR), pg/mL				
At baseline	12.5 (6.9–24.6)	12.7 (8.1–18.4)	10.9 (8.6–23.7)	.89
After the intervention	12.5 (8.0–20.7)	11.1 (5.5–18.7)	10.5 (7.3–34.2)	.36
P value for change	.81	.04	.14	
IL-13, median (IQR), pg/mL				
At baseline	21.1 (5.2–146.3)	20.8 (4.7–163.5)	60.5 (5.3–133.6)	.67
After the intervention	21.6 (6.2–128.6)	19.6 (3.6–124.2)	67.6 (4.8–122.3)	.66
P value for change	.25	.04	.34	
Blood eosinophil count, geometric mean (95% CI), $\times 10^9/L$				
At baseline	0.40 (0.30–0.52)	0.51 (0.39–0.68)	0.33 (0.23–0.45)	.08
After the intervention	0.35 (0.25–0.48)	0.41 (0.34–0.49)	0.39 (0.28–0.57)	.71
P value for change	.21	.03	.56	
Blood eosinophil percentage, median (IQR), %				
At baseline	6 (3–10)	7 (6–11)	7 (3–10)	.22
After the intervention	6 (3–10)	5 (4–8)	6 (4–11)	.73
P value for change	.28	.01	.76	

Abbreviations: CI, confidence interval; IL, interleukin; IQR, interquartile range; sCD26, soluble CD26; sCD30, soluble CD30.

z score remained statistically significant ($P = .02$). In addition, sCD30 was higher (geometric mean, 113.70 IU/mL; 95% CI, 96.60–133.80 IU/mL) in those with elevated hs-CRP levels ($n = 15$) than in those without ($n = 72$) (geometric mean, 95.90 IU/mL; 95% CI, 90.20–102.00 IU/mL) ($P = .03$).

During the 8-week intervention, serum sCD26 increased significantly in the salmeterol group, and serum sCD30, interleukins 4, 10, and 13, and blood eosinophils decreased significantly in the salmeterol-fluticasone group but not in the fluticasone group (Table 1). The differences in repeated measurements between the intervention groups did not reach statistical significance (data not shown). The sample size was originally determined by targeting differences in FeNO¹; the results of the present study could have been strengthened if more stringent study participant selection was conducted based on sCD26 and sCD30 values of clinical significance.

Taking into account recent findings on hs-CRP and related cytokines in neonates with reduced lung function,⁹ it is plausible to surmise that similar uniform up-regulated inflammatory profile may underlie pulmonary abnormalities seen in wheezy preschool children. Although FeNO levels decreased equally in the fluticasone and salmeterol-fluticasone groups during the intervention,¹ the findings on peripheral blood inflammatory markers in the present study imply that the combination of inhaled glucocorticoid and long-acting β_2 -agonist (LABA) might have a synergistic anti-inflammatory effect. Such a synergistic effect has been proposed to be based on the increase in intracellular glucocorticoid receptor nuclear translocation.¹⁰ In contrast, in those receiving a LABA only, the increase in serum sCD26 concentration might be explained by resolution of inflammatory reactions by enhancing T_H1 activity and/or by absence of a proapoptotic effect of glucocorticoids on inflammatory cells. Whether these findings are reproducible is an issue for further studies searching for optimal therapy response markers.

In conclusion, T_H2 activation with the evidence of low-grade systemic inflammation may underlie pulmonary function abnormality in multiple-trigger wheezers. In addition, inhaled

glucocorticoids combined with LABAs might have a synergistic effect on T_H2-mediated inflammatory responses.

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