

## SHORT PAPER

# Soluble CD163 Levels and CD163+CD14+ Monocyte/Macrophage Counts in Patients with Asthma

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### ABSTRACT

**Background:** CD163-expressing macrophages are involved in the inflammatory response in asthma. **Objective:** To assess sputum and serum soluble CD163 (sCD163) and cytokine levels in patients with asthma. Further discussed was the difference between sCD163 and other classic inflammatory mediators. **Methods:** Sputum was successfully induced in asthma patients (n=85) and healthy controls (n=21). Interleukin (IL)-4, IL-5, IL-1 $\beta$ , IL-8, IL-9, IL-6, and sCD163 levels in sputum were measured. CD163+ monocytes in blood were evaluated using flow cytometry. **Results:** Sputum sCD163 level significantly increased in asthma (median: 22.4 pg/ml; IQR, 11.52-42.91), unlike healthy controls (10.54 pg/ml; 9.85-23.5; P<0.001). Sputum sCD163 (P=0.020) and serum sCD163 (P=0.032) levels were significantly higher in patients with severe asthma compared to those with mild/moderate asthma. Percentage of CD163+ monocytes in patients with asthma was significantly lower than the controls (P<0.001). **Conclusion:** Increased sCD163 levels in sputum are associated with the impairment of lung function.

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**Keywords: Asthma, CD163, Macrophages, Monocytes**

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## INTRODUCTION

CD163 is a type I transmembrane protein belonging to group B of the cysteine-rich scavenger receptor family (1). Expression of CD163 is constitutive and/or induced on circulating monocytes and most tissue macrophages (2). The extracellular region of CD163 is stimulated by inflammatory mediators, such as glucocorticoids, IL-6, and oxidative stress, and released in plasma as soluble CD163 (sCD163) (3,4). The marker of macrophages with an M2 phenotype is sCD163 (5). The level of inflammatory factors and sCD163 in sputum and blood of asthma patients and their association with disease severity is yet to be known. It was hypothesized that the levels of inflammatory mediators in sputum vary according to airway inflammation, as evidenced by the presence of different inflammatory cells, and that blood monocyte count in patients with asthma is different from healthy controls.

## MATERIALS AND METHODS

**Study Population.** Enrolled in the study were patients with asthma (18–75 years old) attending the Department of Respiratory Medicine of the Second Affiliated Hospital of Jilin University and the Department of Respiratory Medicine of Changchun Center Hospital between December 2013 and October 2014. Twenty-eight healthy individuals were recruited as controls. Asthma patients were stratified as severe asthma or mild/moderate asthma according to previously published criteria (6). This study was approved by the local Ethics Committee, and informed consent was obtained from all study participants.

**Assessments.** Demographic and clinical characteristics of all participants were recorded. Each asthma patient underwent a 6 min walk test (6MWT), as previously described (7). Spirometry was performed using a Medgraphics spirometer (Minato Autospiro AS-600 Minato Medical Science, Osaka Japan or KoKo K31300 PDS) according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines (8). The fraction of exhaled nitric oxide (FeNO) was measured using a NIOX analyzer (Aerocrine, New York, NY, USA) according to ATS/ERS guidelines (9). The skin prick test was performed on the skin over the palmaris longus muscle of the left forearm to assess atopy to common aeroallergens. The skin prick test was considered positive if a positive reaction was observed in any allergen site. Sputum was induced with physiological saline or hypertonic saline (4.5% NaCl). As a safety precaution, patients inhaled 200 µg of salbutamol prior to sputum induction. Subsequently, patients with an FEV1 <60% inhaled nebulized physiological saline, and patients with an FEV1 ≥60% inhaled nebulized hypertonic saline. Sputum samples were collected 15 min 30 sec after starting the inhalation. The number of inflammatory cells in sputum samples was measured using a Fuchs-Rosenthal chamber and recorded as total cell count (TCC). Eosinophils, neutrophils, and macrophages were also counted based on morphological criteria. Sputum samples in which ≥80% of the cells were squamous cells were excluded from the analyses. Sputum IL-4, IL-5, IL-9, IL-1β, IL-8, and IL-6, and serum IL-6, and serum and sputum sCD163 levels were evaluated using commercially available ELISA kits (R&D Systems; Minneapolis, MS, USA) according to the manufacturer's instructions. Peripheral blood mononuclear cells (PBMCs) (10<sup>6</sup>/tube) were stained with FITC-anti-CD14 and PE-anti-CD163 at room temperature for 30

minutes, washed with PBS, and subjected to flow cytometry analysis.

**Statistical Analysis.** Statistical analyses were conducted with SPSS v19.0 using Bonferroni correction, Student's t-test, Kruskal-Wallis test, Mann-Whitney U test, or the chi-squared test. The relationships between inflammatory mediators were identified using bivariate correlation analysis.  $P < 0.05$  was considered statistically significant.

## Results and Discussion

### Study Population.

Sputum collection was successful in 85 patients with asthma (38 males, 47 females) and 21 healthy controls (10 males, 11 females). Among these, 40 patients (18 males, 22 females) were classified as severe asthma and 45 patients (20 males, 25 females) were categorized as mild/moderate asthma. Comparisons with healthy controls, patients with asthma had a significantly higher TCC ( $P = 0.016$ ) and eosinophil count ( $P < 0.001$ ) in sputum samples. Forty-seven patients had a positive allergy test, yet no significant difference was observed in the lung function and level of inflammatory mediators between atopic and nonatopic patients.

**Table 1. Correlations (r value) between inflammatory cells and inflammatory mediators in sputum of patients with asthma.**

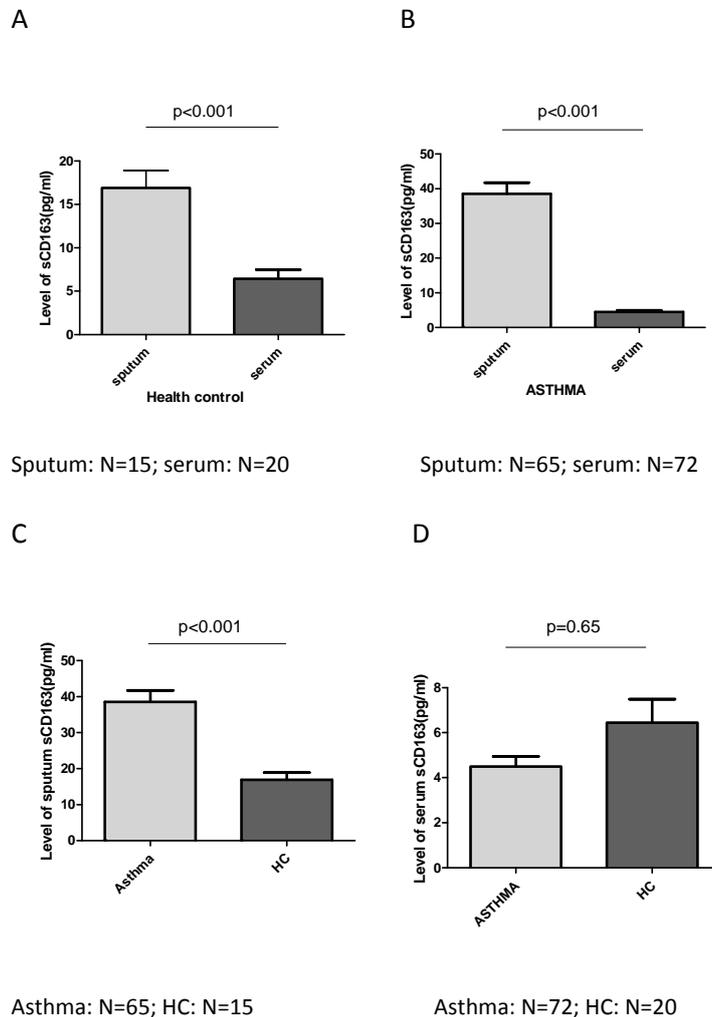
	Blood eosinophils	TCC	Sputum macrophages	Sputum neutrophils	Sputum eosinophils
<b>Log IL-4</b>	NS	0.311**	0.455**	NS	NS
<b>Log IL-5</b>	0.310*	0.455**	0.399**	0.295*	0.520**
<b>Log IL-9</b>	0.548**	0.425**	0.352*	NS	NS
<b>Log sCD163</b>	-0.288*	NS	NS	NS	-0.459*

The data were analyzed by bivariate correlation analysis, \*:  $P < 0.01$ ; \*\*:  $P < 0.05$ ; NS:  $P > 0.05$ .

### Inflammatory Mediators in Sputum and Clinical Characteristics in Patients with Asthma.

In all asthma patients, sputum IL-6 (median, 13.15 pg/ml; IQR, 4.0-51.77) level was significantly increased compared to serum IL-6 (median, 1.65 pg/ml IQR, 0.76-14.81) level ( $P < 0.001$ ) (Figure 1). In all asthma patients, sputum sCD163 level (22.4 pg/ml; IQR, 11.52-42.91) was significantly augmented ( $P < 0.001$ ) in comparison with the controls (10.54 pg/ml, IQR, 9.85-23.5) (Figure 1). These data are in accordance with a previous report (10). Sputum sCD163 level may be elevated in response to inflammation in the airways in asthma, because sCD163 is a marker of inflammatory response (11). Serum sCD163 levels in asthma (3.75 pg/ml; IQR, 2.11-8.46) and in healthy controls (4.28 pg/ml; IQR, 1.81-14.77) were similar ( $P = 0.65$ ), suggesting that sputum sCD163 is a more accurate marker of inflammatory response than serum sCD163 in asthma. In all asthma patients, sputum sCD163 level was negatively associated with post-bronchodilator FEV1 ( $r = -0.482$ ,  $P = 0.022$ ) and post-bronchodilator FCV ( $r = -0.358$ ,  $P = 0.013$ ). Sputum IL-6 level was negatively associated with post-bronchodilator FEV1 ( $r = -0.562$ ,  $P = 0.01$ ). Sputum sCD163 level was negatively

associated with the 6MWT ( $r=-0.481$ ,  $P=0.015$ ). These data are in accordance with the findings of the previous studies. In cystic fibrosis, an impairment of lung function was independently associated with elevated plasma IL-6 levels (12).

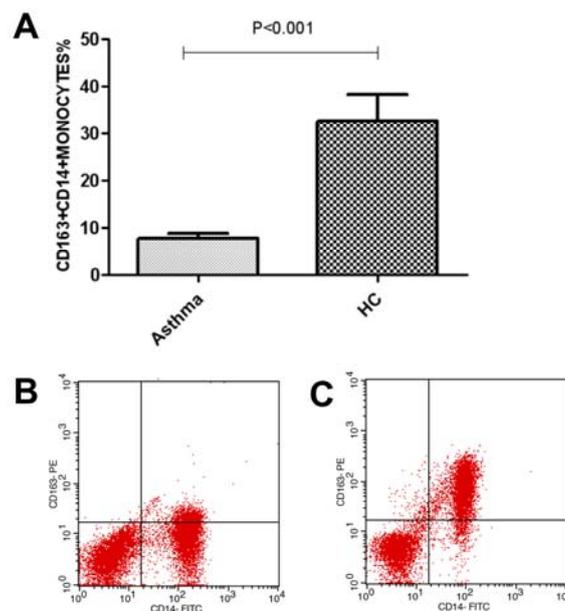


**Figure 1. Sputum and serum sCD163 levels in patients with asthma and healthy controls.** Sputum sCD163 levels were significantly increased compared to serum sCD163 levels in patients with asthma (A) and healthy controls (B). In patients with asthma, sputum sCD163 level was significantly increased compared to healthy controls (C), there were no significant differences in serum sCD163 levels between groups (D).

In obese female children with asthma, plasma sCD163 levels were inversely associated with FEV1%/predicted (11). In the present study, it was found that both serum and sputum CD163 levels, but only serum IL-6 levels, were significantly higher in patients with severe asthma compared to patients with mild/moderate asthma. In patients with severe COPD (GOLD III-IV), serum sCD163 levels were elevated compared to COPD (GOLD I-II) (13). These findings suggest that sCD163 is associated with a decline in lung function in patients with respiratory diseases, providing evidence supporting a

novel relationship between macrophage activation and lung function impairment (14). Furthermore, in an Inuit indigenous population, a 10% increase in plasma sCD163 level was associated with a 5.7–8.0 ml decrement in lung function (14). We propose that sCD163 level increases prior to the decline in lung function and that sCD163 is a biomarker of early stage lung function impairment.

In all asthma patients, sputum IL-4 and IL-9 levels were positively associated with TCC and the number of sputum macrophages; sputum IL-5 level was positively associated with the number of sputum TCC, macrophages, neutrophils, eosinophils, and blood eosinophils, and sputum IL-9 level was positively associated with the number of blood eosinophils. However, sputum sCD163 level was negatively associated with the number of blood and sputum eosinophils (Table 1), while serum sCD163 level was not. In contrast, house dust mite-challenged CD163(-/-) mice displayed increased airway eosinophils (15). Data from the latter study suggest that eosinophils may activate or sustain macrophages in the airways of patients, resulting in the release of CD163. Taken together, these findings indicate that increased sCD163 in sputum in asthma patients may protect the airways from inflammation caused by eosinophils.



**Figure 2. Expression of CD163 on the surface of macrophages from patients with asthma and healthy controls.** (A) Percentage of CD163+CD14+ monocytes in asthma and healthy controls. (B) Representative patient with asthma. (C) Representative healthy control.

In all patients with asthma, sputum sCD163 level had a significantly positive association with sputum IL-9 level ( $r=0.301$ ,  $P=0.028$ ), an inverse association with sputum IL-6 ( $r=-0.493$ ,  $P=0.032$ ) level, and a significantly positive relationship with sputum IL-5 ( $r=0.588$ ,  $P=0.005$ ) level (Table 2). Serum IL-6 ( $P=0.011$ ), sputum sCD163 ( $P=0.022$ ), and serum sCD163 ( $P=0.025$ ) levels were significantly higher in patients with severe asthma compared to patients with mild/moderate asthma.

**Table 2. Correlations (r value) between inflammatory mediators in sputum of patients with asthma.**

	IL-8	IL-9	IL-6	IL-5
IL-1 $\beta$	0.510**	NS	NS	NS
IL-8	-	0.278*	0.352**	NS
IL-6	0.402**	0.623**	-	NS
CD163	NS	0.301*	-0.493*	0.588**

The data were analyzed by bivariate correlation analysis, \*: P<0.01; \*\*: P<0.05; NS: P>0.05.

### Expression of CD163 on Monocytes.

In all asthma patients, the percentage of CD163+CD14+ monocytes was significantly lower compared with the healthy controls (P<0.001), yet the two groups were similar in terms of the percentage of CD14+ monocytes (Figure 2). Previous studies showed that the expression of CD163 on macrophages in sputum and BALF was decreased in asthma (15,16) and COPD (17), and ex-smokers with COPD had a higher percentage of CD163+ macrophages in BALF than current smokers with COPD (18). In the present study, sputum sCD163 level in asthma patients was noticeably higher than healthy controls, while CD163+CD14+monocytes were decreased in asthma patients. A previous study reported a strong inverse correlation between membrane CD163 expression and plasma sCD163 levels, indicating that plasma sCD163 may be derived from circulating monocytes (19).

To our knowledge, this is the first study to simultaneously investigate the level of serum sCD163 and expression of CD163 on macrophages in asthma. We speculate that the reduction in CD163+CD14+M2 monocytes in the blood of asthmatic patients is because sCD163 released by plasma CD163+CD14+ monocytes infiltrated into the lung alveoli, and CD163+CD14+monocytes were consumed. Several mediators, such as IL-4 or IL-13 (20), induce the release of sCD163 from macrophages in the airways or lung tissues of asthma patients into the pulmonary alveoli, resulting in an elevated level of sCD163 in the sputum. These data suggest that sCD163 has an anti-inflammatory role in the airways.

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