

# A comparative study of sTREM-1, IL-6 and IL-13 concentration in bronchoalveolar lavage fluid in asthma and COPD: A preliminary study

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## Abstract

**Background.** Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) is a cell-surface receptor presented on neutrophils, macrophages and monocytes. Elevated sTREM-1 levels are a marker of acute microbial infections, and have also been reported in chronic lung diseases. IL-6 and IL-13 are effective markers in distinguishing these diseases. IL-6 has been shown to play an important role in chronic obstructive pulmonary disease (COPD), while IL-13 is described as one of the key mediators of allergic asthma.

**Objectives.** The aim of the study was to evaluate the level of sTREM-1 in bronchoalveolar lavage fluid (BALF) from stable mild-to-moderate asthma and COPD patients and to compare the utility of BALF sTREM-1 measurements in asthma/COPD differentiation with those of IL-6 and IL-13.

**Material and methods.** The concentration of sTREM-1, IL-6 and IL-13 was evaluated by ELISA in the BALF of stable mild-to-moderate asthma and COPD patients.

**Results.** There were no significant differences in BALF sTREM-1 levels between asthma and COPD patients (52.5 pg/mL for asthma vs 73.4 pg/mL for COPD,  $p = 0.492$ ), in contrast to the differences in the IL6/IL13 ratio (0.68 in COPD, 0.22 in asthma,  $p = 0.043$ ).

**Conclusions.** The study showed that BALF sTREM-1 levels do not discriminate between mild-to-moderate asthma and COPD. In contrast, the IL-6/IL-13 ratio measured in BALF effectively differentiated these two diseases in their stable state. These results suggest that the most important marker of inflammation in mild-to-moderate obstructive lung disease is not microbiology-dependent.

**Key words:** IL-6, asthma, COPD, IL-13, sTREM-1

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Triggering receptor expressed on myeloid cells (TREM-1) is a cell-surface receptor presented on neutrophils, macrophages and monocytes. There is evidence that TREM-1 is involved in host immune response to microbial infection.<sup>1</sup> However, little is known about the natural ligands for TREM-1. Recently, peptidoglycan recognition protein 1 (PGLYRP1), a neutrophil granule protein with antibacterial properties, was found to be a functional ligand for TREM-1.<sup>2</sup> Activation of TREM-1 causes rapid neutrophil degranulation, increasing pro-inflammatory cytokine production (CXCL8, tumor necrosis factor  $\alpha$  [TNF- $\alpha$ ], IL-1 $\beta$ ) and inhibiting IL-10 expression in monocytes and macrophages.<sup>3,4</sup> Upregulation of TREM-1 is associated with the release of its soluble form (sTREM-1), which can be detected in various biological fluids, including serum, sputum and bronchoalveolar lavage fluid (BALF).<sup>5,6</sup> Soluble TREM-1 is generated through proteolytic cleavage of membrane-anchored TREM-1 by matrix metalloproteinases with the subsequent release of the TREM-1 ectodomain into the microenvironment.<sup>7</sup> Alternatively, sTREM-1 may be secreted by neutrophils in response to lipopolysaccharide (LPS) stimulation during de novo protein synthesis.<sup>8</sup> Soluble TREM-1 acts as a decoy for the natural TREM-1 ligand and reduces its activation.<sup>9</sup> Soluble TREM-1 is detectable in infectious diseases caused by extracellular microorganisms. Earlier studies have shown that an elevated level of serum sTREM-1 might be an important marker of sepsis, as well as bacterial and fungal pneumonia.<sup>5,10</sup> TREM-1 is also an amplifier of the inflammatory reaction. This probably explains the increase in TREM-1 or sTREM-1 expression found in non-infectious inflammatory disorders such as COPD, asthma, inflammatory bowel disease and acute pancreatitis.<sup>11–14</sup>

The role of sTREM-1 in airway inflammatory diseases, asthma and COPD, seems interesting not only in terms of the mechanisms of the inflammatory reaction. If TREM-1 is involved in airway inflammation, it might be hypothesized that it could be a marker of asthma or COPD. To date, there are no established and reliable biochemical factors that are used in the differential diagnosis or monitoring of asthma and COPD.<sup>15</sup> Two cytokines, IL-6 and IL-13, are probably the most effective in distinguishing these diseases. Sputum IL-6 level is increased in asthma and COPD compared to healthy subjects.<sup>16</sup> IL-6 has been shown to play a particular role in COPD: Elevated concentrations of it correlate with impaired lung function and are related to high frequencies of COPD exacerbations.<sup>17</sup> On the other hand, IL-13 is described as one of the key mediators of allergic asthma, promoting eosinophil predominance, goblet cell hyperplasia and mucus hypersecretion in the respiratory tract.<sup>18</sup> Elevated levels of IL-13 have been found in samples from the respiratory tract of asthma patients compared to healthy people. It has previously been shown that the ratio of IL-6 and IL-13 concentration in induced sputum is significantly different in asthmatics than in COPD patients.<sup>19</sup>

To date, the concentration of sTREM-1 in asthma and COPD patients has been evaluated in serum and plasma only. Systemic sTREM-1 levels correlated positively with asthma and COPD severity and negatively with lung function, and are a good marker of neutrophilic inflammation.<sup>11,12,20</sup> To the authors' knowledge, there have been no studies on sTREM-1 concentrations in respiratory samples from asthma and COPD patients. Also, the level of sTREM-1 has not been compared with other asthma or COPD biomarkers. The aims of this study were therefore (1) to evaluate sTREM-1 concentrations in BALF from asthma and COPD patients; and (2) to compare the utility of BALF sTREM-1 measurements in asthma/COPD differentiation with that of IL-6 and IL-13.

## Material and methods

### Characteristics of the patients

Eight patients with mild to moderate asthma (classified according to the Global Initiative for Asthma recommendations) and 11 patients with stage I-II COPD (classified according to the Global Initiative for Chronic Obstructive Lung Disease recommendations) were enrolled in the study.<sup>21,22</sup> All the patients were stable and had been free from exacerbations for at least 8 weeks before the start of the study. None of the patients had had respiratory infections for at least 1 month preceding the enrollment. The only concomitant respiratory inflammations in the participants were related to the upper airways: 6 patients (2 with asthma, 4 with COPD) had chronic sinusitis, and 4 patients (1 with asthma, 3 with COPD) had chronic rhinitis.

An important inclusion criterion was treatment with bronchodilators only, with no systemic or local anti-inflammatory treatment with corticosteroids. The only treatment used by the asthma patients before inclusion in the study were short-acting  $\beta$ 2-agonists (SABAs) as needed (7 patients), and long-acting  $\beta$ 2-agonists (LABAs) and SABAs as needed (one patient). In the COPD group, 6 patients were treated with SABAs only, 2 with long-acting muscarinic antagonists (LAMAs), 2 with LABAs and 1 with LAMAs and LABAs.

The study was approved by the Ethical Committee of the Medical University of Warsaw, and written informed consent was obtained from all the patients enrolled.

### Bronchoalveolar lavage

Flexible bronchoscopies (using an Olympus BF-1T160 bronchoscope, Olympus Corporation, Tokyo, Japan) were performed under local anesthesia (2% lidocaine) after premedication with inhaled salbutamol (400  $\mu$ g), intramuscular atropine (0.5 mg) and oral midazolam (7.5 mg). Supplemental oxygen was applied during the

entire procedure and blood oxygen saturation was monitored with a pulse oximeter (Pulsox DP-8, Konica Minolta Inc., Tokyo, Japan). Following macroscopic inspection of the bronchial tree, BAL was performed by wedging the bronchoscope in the bronchi of the middle lobe or the lingula and administering 4 × 50 mL sterile NaCl warmed to 37°C. Gentle suction was applied to recover the BALF into sterile containers. The subsequent steps of BALF processing were completed according to American Thoracic Society (ATS) recommendations.<sup>23</sup> The cells were evaluated and counted in May-Grünwald-Giemsa stained smears. The supernatants were stored at –70°C.

## sTREM-1, IL-6 and IL-13 analysis

Soluble TREM-1, IL-6 and IL-13 concentrations in BALF were measured using appropriate ELISA kits (IQ Products, Groningen, Netherlands for sTREM-1; R&D Systems, Minneapolis, USA for IL-6 and IL-13) according to the manufacturers' instructions. The lower limits of detection were 7 pg/mL for sTREM-1, 0.76 pg/mL for IL-6 and 13.2 pg/mL for IL-13.

## Statistical analysis

The results are presented as median and range of values. The statistical analysis was performed using STATISTICA 12.0 software (StatSoft Inc., Tulsa, USA). Differences between continuous variables were tested using Fisher's exact test or the nonparametric Mann-Whitney U test. Correlations between variables were analyzed with Spearman's rank test. Differences were considered statistically significant at  $p < 0.05$ .

## Results

### Clinical characteristics of the asthma and COPD patients

The basic comparative characteristics of the asthma and COPD patients are shown in Table 1. The patients with COPD were significantly older than the asthmatics. Spirometry results showed a significantly stronger impairment of respiratory function in COPD than in asthma.

### Cellular composition of BALF in asthma and COPD

A significantly lower proportion of lymphocytes was found in the BALF of the COPD patients when compared to the asthmatics (Table 2).

**Table 1.** Demographic and clinical data of patients with asthma and COPD. The values are expressed as median and range of values; p-values were obtained using Fisher's exact test or the Mann-Whitney U test

Characteristics	Asthma	COPD	p-value
Gender (male/female)	6/2	8/3	0.664
Atopy (n)	7	5	0.079
Age (yrs)	43.5 (21–65)	65 (52–78)	0.0005
Smoking exposure (pack/years)	0 (0–21)	45 (30–100)	0.00002
Disease duration (months)	44.5 (12–264)	48.5 (12–216)	0.6574
FEV <sub>1</sub> (% predicted)	92 (52–121)	73 (65–95)	0.0008
FVC (% predicted)	113 (87–143)	104 (83–123)	0.0327
FEV <sub>1</sub> /FVC (%)	67 (49–90)	56 (43–64)	0.00005

COPD – chronic obstructive pulmonary disease, FEV<sub>1</sub> – forced expiratory volume at first second, FVC – forced vital capacity.

**Table 2.** The cellular composition of the bronchoalveolar lavage fluid (BALF) of asthma and COPD patients. The sum of all non-epithelial cells was counted as 100%. The values are expressed as median and range of values; p-values were obtained using the Mann-Whitney U test

BALF cells	Asthma	COPD	p-value
Macrophages (%)	84.5 (73–92)	92 (51–98)	0.146
Lymphocytes (%)	12 (7–16)	4 (1–25)	0.027
Neutrophils (%)	1.5 (1–7)	3.5 (1–23)	0.101
Eosinophils (%)	1.5 (0–11)	0 (0–4)	0.274

### Comparison of BALF sTREM-1, IL-6 and IL-13 concentration in asthma and COPD

There were no significant differences in the BALF sTREM-1 concentration between asthma and COPD (Fig. 1). The median sTREM-1 concentration was 52.5 pg/mL (38.3–102.5 pg/mL) for asthma and 73.4 pg/mL (20.4–226.9 pg/mL) for COPD. No correlations between sTREM-1 concentration and the proportions of inflammatory cells in asthma or COPD were noted.

The median concentrations of IL-6 in BALF were: 14.3 pg/mL (0–62.6 pg/mL) and 24.6 pg/mL (7.8–75.5 pg/mL) for asthma and COPD respectively; and the median concentrations of IL-13 were 40.4 pg/mL (35.5–71.5 pg/mL) and 42.8 pg/mL (32.2–53.5 pg/mL) for asthma and COPD, respectively. The IL-6/IL-13 ratio was calculated for both groups. In 2 cases (from the COPD group) an undetectable level of IL-6 concentration was arbitrarily set as 0.76 pg/mL (the lower detection limit of the ELISA kit). IL-6/IL-13 ratio was significantly higher in the COPD (0.68 [0.15–1.55]) than in the asthma group (0.22 [0.02–0.99]) ( $p = 0.043$ ) (Fig. 2).

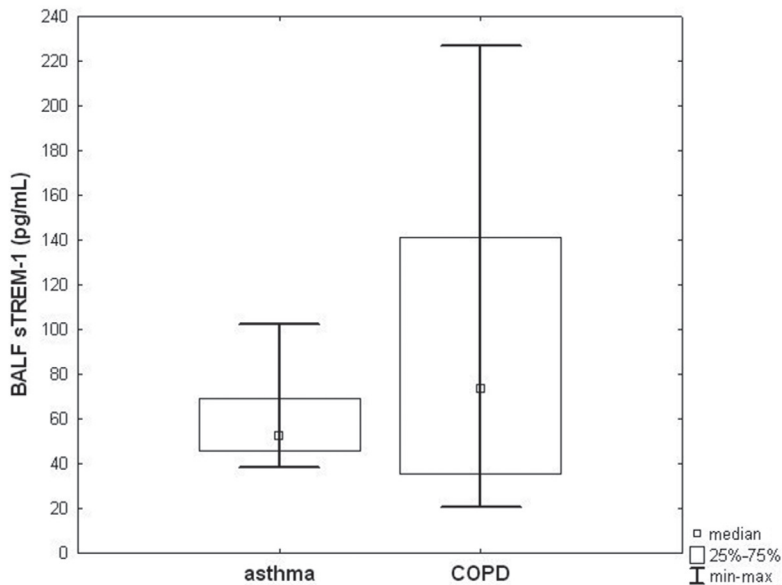


Fig. 1. sTREM-1 levels in the BALF of asthma and COPD patients. The values are expressed as median and range of values. The statistical analysis was performed using the Mann-Whitney U test

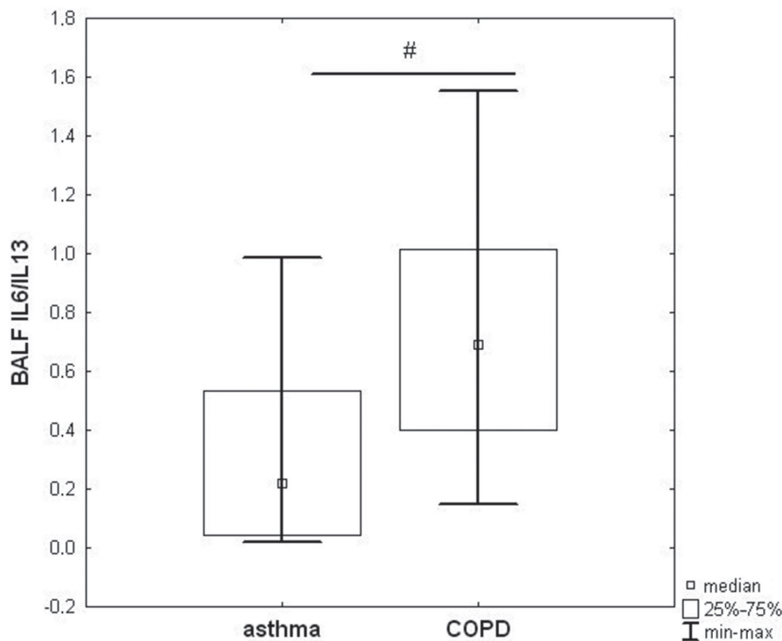


Fig. 2. The IL-6/IL-13 ratio in the BALF of asthma and COPD patients. The values are expressed as median and range of values. The statistical analysis was performed using the Mann-Whitney U test ( $p < 0.05$ )

## Discussion

The study showed a measurable concentration of sTREM-1 in lavage fluid from peripheral airways. The results demonstrated that the level of sTREM-1 in BALF was not significantly different in asthma and COPD patients. This finding suggests that sTREM-1 is not suitable for the differentiation of obstructive lung diseases in their stable phase. Simultaneously, the study confirmed that measurement of other BALF biomarkers, IL-6 and IL-13, can more efficiently distinguish between asthma and COPD.

To the authors' knowledge, this is the first study comparing the BALF concentration of sTREM-1 in asthma and COPD patients. To date, only serum/plasma levels

of sTREM-1 in asthma and COPD have been evaluated. Previous studies have demonstrated increased systemic sTREM-1 concentrations in asthma and COPD patients, and a relationship between serum sTREM-1 levels and the severity of the disease.<sup>12,20</sup> Bucova et al. found that in asthma patients, plasma sTREM-1 correlated with the clinical stage and disease severity, and was highly elevated during disease exacerbations.<sup>12</sup> On the other hand, a study by Rohde et al. that included COPD patients showed no differences in serum sTREM-1 levels between patients with advanced stable disease and those with COPD exacerbations.<sup>11</sup> As sTREM-1 is regarded as a marker of infection and a significant proportion of COPD exacerbations are related to airway infections, this finding seems surprising. The authors of the study concluded that the lack of any

increase in sTREM-1 during acute exacerbations may be explained by the high baseline levels of sTREM-1 caused by bacterial colonization present in patients with moderate to severe COPD, making any further increase during acute exacerbation COPD undetectable.

Serum levels of sTREM-1 in patients with asthma and COPD exacerbations were compared by Phua et al. Although the authors found no differences between the entire COPD and asthma groups, they noted a higher sTREM-1 level in Anthonisen type 1 COPD exacerbations compared to asthma and Anthonisen type 2 and 3 COPD exacerbations.<sup>24</sup> This seems to be consistent with the role of sTREM-1 in bacterial infections.

The results of the studies cited above prompted the present authors to investigate whether sTREM-1 concentrations in local respiratory samples (BALF) differ in patients with stable asthma and stable COPD. Interestingly, the BALF levels of sTREM-1 in the present study are comparable with serum/plasma sTREM-1 levels reported by other authors in mild stable asthma and COPD.<sup>11,12</sup> This may suggest that the systemic sTREM-1 level reflects the local expression of sTREM-1 in the respiratory tract. Although higher sTREM-1 concentrations were observed in the BALF of the COPD patients than in the asthma patients, the difference was not statistically significant. The lack of statistical significance may be at least partially related to the small number of patients in this study. Nevertheless, based on the preliminary data, it might be concluded that sTREM-1 level in lower airway respiratory samples does not discriminate stable asthma and COPD. Since, to the authors' knowledge, the BALF sTREM-1 levels in stable asthma and COPD have not been reported before, no comparison of the current results with other studies is possible.

In light of the existing data, the major clinical application of sTREM-1 measurement seems to be the differentiation between bacterial and viral infections.<sup>11,25</sup> This might be of particular interest in airway and pulmonary infections. Shu et al. reported that sTREM-1 may differentiate between mycobacterial colonization and active mycobacterial pulmonary disease.<sup>26</sup> Because of potential relationships between airway infections and chronic obstructive airway diseases, the measurement of sTREM-1 concentrations in respiratory samples seems to have both clinical and research applications. It has been shown that the microbial composition of the airways may be an important determinant of the risk of developing asthma or COPD.<sup>27</sup> If sTREM-1 is a sensitive marker of microbial colonization in the respiratory tract, this may add some new data on the pathogenesis of obstructive airway diseases. The need for a sensitive biomarker of bacterial infection might be exemplified by the fact that over 70% of the bacterial species on body surfaces cannot be cultured by currently available techniques.<sup>28</sup> The results of the present study suggest that the microbial burden in stable mild-to-moderate asthma and COPD is similar. The rela-

tionship between the sTREM-1 concentration in the lungs and microbiological dependency in asthma and COPD certainly needs further investigation.

To compare BALF sTREM-1 levels with other recognized asthma and COPD biomarkers this study also investigated the BALF concentrations of 2 cytokines – IL-6 and IL-13. It has previously been reported that concentrations of IL-6 are increased in COPD, whereas IL-13 is elevated in asthma.<sup>29,30</sup> However, IL-6 and IL-13 are not specific for a single disease. Therefore, it was assumed that the IL-6/IL-13 ratio characterizes asthma and COPD more accurately than the levels of either single cytokine, since the level of an individual cytokine may change dynamically in the course of the disease.<sup>19</sup> The results obtained supported this hypothesis. The IL-6/IL-13 ratio in BALF was significantly different in asthma than in COPD, even though there was no difference between BALF sTREM-1 levels in these 2 diseases.

The study has some limitations. The major flaw is the small number of patients included in the study. This was due to the preliminary nature of the research and to the relatively invasive bronchoscopic procedure required to collect BALF. The small number of patients did not permit a further sub-analysis of sTREM-1 levels in patients with different asthma and COPD phenotypes. The fact that only one type of biological sample was used in the study might be considered a second limitation. It could have been interesting to compare BALF sTREM-1 concentrations with sTREM-1 levels in induced sputum, exhaled breath condensate and serum. Third, since the study did not include a control group, no comparison could be made between BALF sTREM-1 concentrations in patients with obstructive pulmonary diseases and in healthy subjects.

## Conclusions

The study showed that BALF sTREM-1 levels are similar in mild-to-moderate asthma and COPD. In contrast, the IL-6/IL-13 ratio measured in BALF effectively differentiated asthma and COPD. Further studies that involve patients with different asthma and COPD phenotypes and different degrees of disease severity are warranted to elucidate whether BALF sTREM-1 concentrations might be considered one of the local inflammatory markers in obstructive lung diseases.

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