



Differences in plasma and sputum biomarkers between COPD and COPD–asthma overlap

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ABSTRACT The pathophysiological features of chronic obstructive pulmonary disease (COPD)–asthma overlap are poorly understood and there has been no study of plasma or sputum biomarkers in overlap patients. In order to clarify the similarity and differences between overlap and COPD or asthma, we have investigated four potential biomarkers of COPD: surfactant protein A (SP-A), soluble receptor for advanced glycation end-products (sRAGE), myeloperoxidase (MPO) and neutrophil gelatinase-associated lipocalin (NGAL). SP-A and sRAGE are pneumocyte-derived markers. MPO and NGAL are neutrophil-derived molecules, but NGAL can also be expressed by respiratory epithelial cells.

Plasma levels of SP-A and sRAGE and induced sputum levels of MPO and NGAL were measured by enzyme immunoassay/ELISA in 134 subjects: nonsmokers (n=26), smokers (n=23), asthma (n=32), COPD (n=39) and COPD–asthma overlap patients (n=14).

In patients with COPD–asthma overlap, sputum MPO and plasma SP-A were significantly elevated whereas plasma sRAGE levels were reduced compared with asthma patients. Only sputum NGAL was significantly elevated in COPD–asthma overlap compared with COPD (p=0.00016) and could be used to differentiate patients with overlap from those with COPD.

Increased induced sputum levels of NGAL might be a characteristic feature of overlap, suggesting enhanced neutrophilic airway inflammation and/or airway epithelial injury in COPD–asthma overlap.



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Increased sputum levels of neutrophil gelatinase-associated lipocalin in COPD–asthma overlap versus COPD/asthma patients <http://ow.ly/qxC8X>

Received: Feb 07 2013 | Accepted after revision: May 28 2013 | First published online: June 21 2013

Support statement: This work was financially supported by the EVO funding of the Helsinki University Central Hospital, University of Helsinki, Finnish Antituberculosis Association Foundation, and partly by the SalWe Research programme for IMO (Tekes – the Finnish Funding Agency for Technology and Innovation grant number 648/10).

Conflict of interest: Disclosures can be found alongside the online version of this article at www.erj.ersjournals.com

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Introduction

Together, asthma and chronic obstructive pulmonary disease (COPD) are the most common chronic lung diseases worldwide. Differential diagnosis of asthma and COPD is important because of the different therapeutic approaches and the distinct clinical outcomes in morbidity and mortality [1, 2]. Although asthma and COPD differ from each other in their patterns of inflammation, their immunological mechanisms and the extent of the reversibility of airflow limitation, a significant number of patients exhibit symptoms and signs that are associated with both conditions [1–3]. The coexistence of asthma and COPD can frequently be seen in the general population, especially in elderly patients [4]. Importantly, these overlap patients might have distinct clinical characteristics, such as lower health-related quality of life or increased frequency of exacerbation when compared with patients with COPD alone with the same degree of lung function abnormality [5, 6].

Spirometry is essential in the diagnosis and assessment of severity of COPD, but it has limited efficacy in the evaluation of symptom burden and in helping to make a differential diagnosis from asthma. Furthermore, there is an urgent need to clarify the clinically relevant COPD phenotype as well as the underlying biological mechanisms [7]. In order to elucidate the pathophysiology of COPD–asthma overlap, we have devised a panel of four potential biomarkers. Two of the biomarkers studied here are previously well-characterised neutrophil-derived inflammatory molecules, myeloperoxidase (MPO) and neutrophil gelatinase-associated lipocalin (NGAL), both of which are associated with inflammation and early airway injury in smoking-related diseases [8]. Recently using two-dimensional electrophoresis and mass spectrometry we have shown that surfactant protein A (SP-A), which has multiple immunomodulatory roles in lung host defence, is associated with cigarette smoke-induced lung injury and in the development of COPD [9–11]. The receptor for advanced glycation end-products (RAGE) is a multi-ligand receptor abundantly expressed in type I pneumocytes. The expression of RAGE is significantly decreased in COPD lung, especially in severe disease [12]. The soluble isoform of RAGE (sRAGE) in plasma might represent a potential marker of COPD, as its levels decline in patients with COPD and decrease further during exacerbations [13–15].

We hypothesised that patients with asthma, COPD and COPD–asthma overlap would differ in their biomarker profiles. The aim of the present study was to determine the plasma and induced sputum levels of the four potential biomarkers of COPD in patients with asthma, COPD and COPD–asthma overlap. Another goal was to investigate whether the levels of these markers would be associated with variables such as pack-years, lung function and sputum cell profiles.

Methods

Subjects

Plasma and induced sputum samples were collected from 134 volunteer individuals. Based on their medical history and self-reported questionnaire data, the study subjects were categorised into five groups: healthy nonsmokers (NS, $n=26$) and asymptomatic healthy smokers with normal lung function (HS, $n=23$), patients with asthma (asthma, $n=32$), patients with COPD (COPD, $n=39$), and patients with asthma and irreversible obstruction (COPD–asthma overlap, $n=14$). The patients, who are part of the longitudinally followed cohort of Finnish asthma and COPD patients (FinnCADStudy) [5, 16], were recruited consecutively from Helsinki University Central Hospital (HUCH). The control subjects consisted of responders to an advertisement in HUCH and the local media. Spirometry and the diffusing capacity of the lung for carbon monoxide (DLCO) were performed on all participants. Spirometric values were assessed by standard spirometry (Medikro M 903; Medikro Oy, Kuopio, Finland) and were performed according to American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations [17]. Reference values were obtained from Finnish reference values for spirometry [18].

The diagnosis of asthma was based on the British Guidelines on Asthma Management [19], with one or more of the following findings together with the clinical history compatible of asthma: a postbronchodilator increase in forced expiratory volume in 1 s (FEV₁) of $\geq 12\%$, a bronchodilator response of $\geq 15\%$ or diurnal variation of $\geq 20\%$ in peak expiratory flow (PEF) recording, moderate-to-severe bronchial hyperreactivity, or a decrease in FEV₁ of $\geq 15\%$ in the exercise test. COPD was diagnosed according to the ATS/ERS Task Force recommendations [16, 20] and defined by irreversible chronic airway obstruction (FEV₁/forced vital capacity (FVC) < 0.70 or FEV₁/FVC $< 88\%$ predicted) in postbronchodilation spirometry related to long-term smoking [1, 18]. The diagnosis of COPD–asthma overlap was made when patients fulfilled both diagnostic criteria for asthma and COPD. All the cases with COPD and overlap had no history of α_1 -antitrypsin deficiency in their family. No patients were either receiving oral steroid treatment or experienced an exacerbation during the month before inclusion. Control subjects were never-smokers or current smokers with normal lung function and no history of lung disease.

This study was approved by the Ethics Committees Helsinki University Central Hospital and conducted in accordance with the ethical standards established in the Helsinki Declaration of 1975. All participants provided written informed consent.

Collection of blood and induction of sputum samples

Peripheral whole venous blood was collected into EDTA tubes, plasma was prepared by centrifugation for 10–15 min at 4500 rpm and stored at -80°C until analysed. Sputum was induced by inhalation of hypertonic saline and treated with dithioerythritol (Sigma, Munich, Germany) as recommended by the ERS Task Force and described in detail previously [21, 22]. The supernatant was frozen at -80°C for biochemical analyses. Cell viability was studied with trypan blue in a Burkler chamber [23]. Cytochrome preparations were made by Cytospin (Thermo Scientific, Wilmington, DE, USA) and centrifuged at $4053 \times g$ for 6 min. The slides were stained by May–Grunwald–Giemsa staining (Merck, Darmstadt, Germany) for cell differential counts with 400 cells being counted from each slide. Detailed cell profiles were assessed for all sputum samples. Only the samples with $<70\%$ of squamous epithelial cells were accepted for further assessments. The slides were frozen at -20°C [23].

Measurement of SP-A, sRAGE, MPO and NGAL in plasma and sputum supernatants

SP-A, sRAGE, MPO and NGAL levels were measured by commercially available enzyme immunoassay/ELISA kits (Kokusai-F kit (Sysmex, Kobe, Japan), R&D Systems (Minneapolis, MN, USA), Abnova Inc. (Walnut, CA, USA), and Uscn Life Science Inc. (Wuhan, China), respectively) according to the manufacturers' instructions. The detection limits of sRAGE, SP-A, MPO and NGAL were $78 \text{ pg}\cdot\text{mL}^{-1}$, $1 \text{ ng}\cdot\text{mL}^{-1}$, $0.78 \text{ ng}\cdot\text{mL}^{-1}$ and $0.039 \text{ ng}\cdot\text{mL}^{-1}$, respectively.

Statistical analysis

The results are expressed as the mean \pm SEM. Comparisons between groups were evaluated using repeated measures ANOVA followed by Fisher's protected least significant difference test (PLSD) *post hoc* comparison. Plasma and sputum biomarkers were further analysed by plotting receiver operating characteristic (ROC) curves for their predictive capability in distinguishing patients with overlap from those with COPD, and patients with overlap and COPD from those with asthma or NS. Correlations of the plasma and sputum markers with demographics, lung function and sputum cell profiles were calculated by Spearman's rank correlations. To obtain an independent predictor for each biomarker, multivariate stepwise regression analysis was performed. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed with the SPSS 16.0 software program (SPSS Inc., Chicago, IL, USA).

Results

Subject characteristics

The demographics and clinical characteristics of the subjects are shown in table 1. The patients were older than the control subjects. Patients with COPD and overlap had more pack-years and significantly lower FEV₁ and DLCO than those in the NS, HS and asthma groups (table 1) (ANOVA followed by *post hoc* Fisher's PLSD; data not shown). The overlap patients had a greater improvement in the reversibility test and diurnal variation of PEF compared with asthma or COPD patients. A confirmation of the presence of asthma was usually made by an improvement in FEV₁ after a bronchodilator, in 14 cases by measurements of diurnal variation of PEF and in six by an increase in bronchial responsiveness. None of the participants had experienced an exacerbation or a respiratory tract infection in the month preceding the study.

Sputum was induced from all the subjects, but, based on the evaluation of cell profiles, 99 samples were accepted for further analysis: NS (n=18), HS (n=17), asthma (n=25), COPD (n=28) and overlap (n=11). The patients with asthma and overlap had a higher percentage of sputum eosinophils when compared with NS, HS and COPD groups (table 1). The percentage of sputum neutrophils was higher in COPD and overlap compared with NS and HS.

Plasma sRAGE decreases and plasma SP-A increases in COPD and COPD–asthma overlap

The plasma levels of sRAGE were significantly decreased in COPD and overlap groups (715.8 ± 51.2 and $790.6 \pm 81.8 \text{ pg}\cdot\text{mL}^{-1}$, respectively) when compared with asthma groups (p=0.025 and p=0.013, respectively) (fig. 1a). The plasma levels of SP-A were elevated in the COPD and overlap groups (45.5 ± 4.1 and $54.5 \pm 7.5 \text{ ng}\cdot\text{mL}^{-1}$, respectively) compared with the NS, HS and asthma groups (fig. 1b). There was no significant difference in plasma SP-A levels between the COPD and overlap groups (p=0.124).

TABLE 1 Characteristics of the subjects

	NS	HS	Asthma	COPD	Overlap
Subjects n	26	23	32	39	14
Females/males n	10/16	11/12	16/16	13/26	5/9
Age years	46.9±2.6	47.6±2.5	58.3±1.5	62.4±1.1	61.2±1.5
BMI kg·m⁻²	28.2±1.0	26.9±1.0	27.5±1.0	25.5±0.7	28.3±1.9
Smoking status					
Former smoker	0	0	17 (53)	13 (33)	8 (57)
Current smoker	0	23 (100)	4 (13)	26 (67)	6 (43)
Pack-years	0	25.8±3.7	16.1±3.8	47.8±3.8	36.9±6.1
Allergy	4 (15)	9 (40)	18 (56)	11 (28)	5 (36)
ICS use	0	0	28 (88)	13 (33)	14 (100)
Postbronchodilator					
FVC L	4.7±0.2	4.4±0.3	3.5±0.2	3.2±0.2	3.2±0.3
FEV ₁ L	3.9±0.2	3.5±0.2	2.6±0.1	1.9±0.1	1.9±0.2
FEV ₁ % pred	103.8±2.1	96.0±3.6	77.0±3.0	57.9±3.2	55.3±3.1
FEV ₁ /FVC	83.9±1.1	80.1±1.3	80.9±1.7	62.6±2.4	69.9±4.1
DLC ₀ % pred	93.8±4.6	89.4±2.8	87.8±5.6	62.9±4.2	66.8±5.2
ΔFEV₁[#] mL	110±31	78±38	173±36	109±30	203±49
ΔFEV₁[#] %	3.1±0.9	2.4±1.1	7.8±1.6	7.5±1.3	13.0±4.0
PEF diurnal variability %			28.1±2.0	17.2±1.8	37.9±4.8
Differential sputum cell counts %					
Eosinophils	1.5±0.8	0.9±0.6	6.3±1.9	1.4±0.5	8.3±3.5
Neutrophils	34.7±2.7	41.9±5.4	53.4±4.6	63.1±3.6	68.2±4.1
Macrophages	59.11±3.3	54.3±5.6	41.3±4.4	32.9±3.7	21.8±3.5

Data are presented as mean±SEM or n (%), unless otherwise stated. NS: nonsmokers; HS: healthy smokers; COPD: chronic obstructive pulmonary disease; BMI: body mass index; ICS: inhaled corticosteroid; FVC: forced vital capacity; FEV₁: forced expiratory volume in 1 s; % pred: % predicted; DLCO: diffusing capacity of the lung for carbon monoxide; Δ: change in; PEF: peak expiratory flow. #: improvement in FEV₁ after 400 µg salbutamol.

Sputum NGAL increases in COPD–asthma overlap compared with COPD

The sputum levels of MPO were elevated in the overlap group ($22.3 \pm 5.1 \mu\text{g}\cdot\text{mL}^{-1}$) when compared with the NS, HS and asthma groups (fig. 2a). Sputum MPO levels were also significantly elevated in COPD compared with asthma. There was no significant difference in sputum MPO levels between COPD and

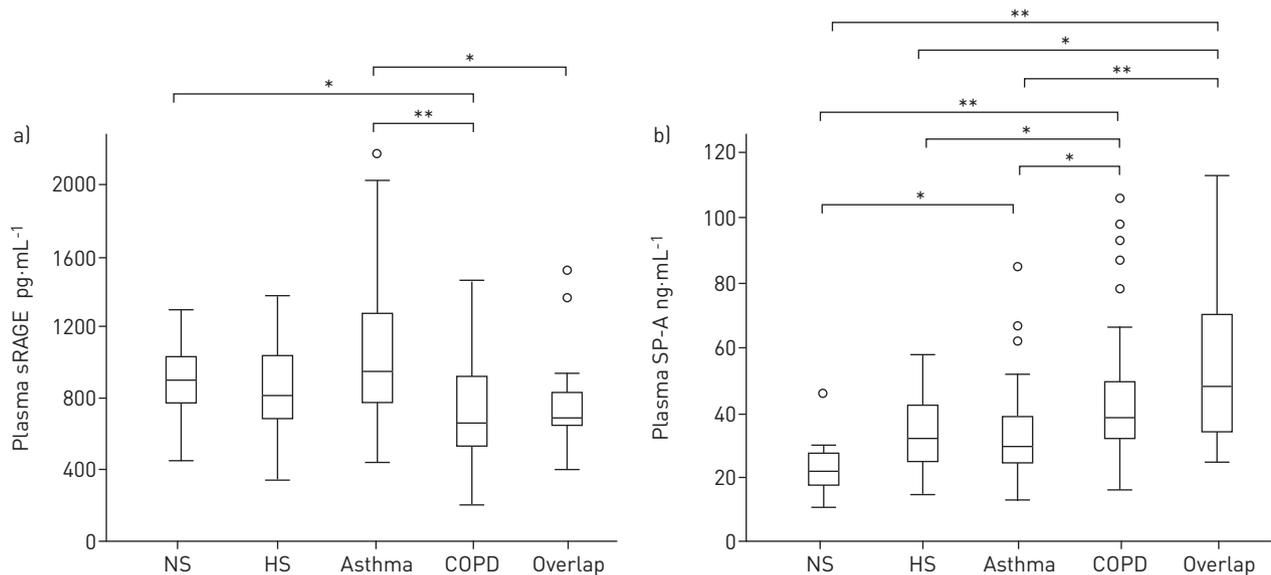


FIGURE 1 Plasma levels of a) soluble receptor for advanced glycation end-products (sRAGE) and b) surfactant protein A (SP-A). The boxes represent the 25th to 75th percentiles, the solid lines within the boxes show the median values, the whiskers are the 10th and 90th centiles, and the circles represent outliers. p-values were calculated by ANOVA followed by Fisher's protected least significant difference test. NS: nonsmoker; HS: healthy smoker; COPD: chronic obstructive pulmonary disease. *: p<0.05; **: p<0.01.

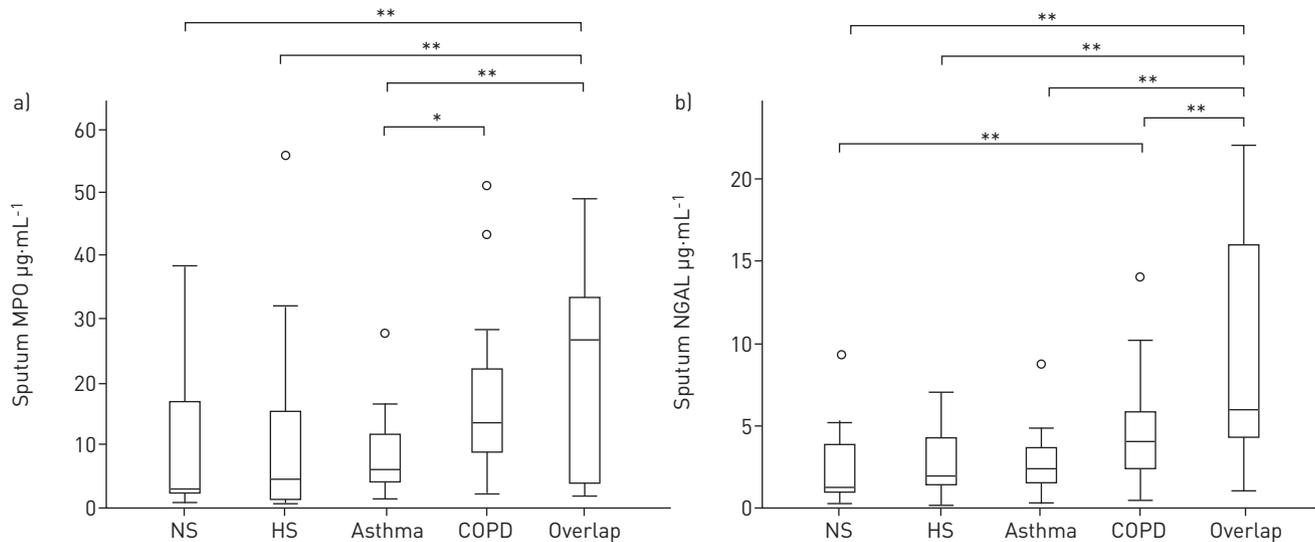


FIGURE 2 Sputum levels of a) myeloperoxidase (MPO) and b) neutrophil gelatinase-associated lipocalin (NGAL). The boxes represent the 25th to 75th percentiles, the solid lines within the boxes show the median values, the whiskers are the 10th and 90th percentiles, and the circles represent outliers. p-values were calculated by ANOVA followed by Fisher's protected least significant difference test. NS: nonsmoker; HS: healthy smoker; COPD: chronic obstructive pulmonary disease. *: $p < 0.05$; **: $p < 0.01$.

overlap ($p = 0.129$). In contrast, sputum levels of NGAL in overlap ($9.1 \pm 2.2 \mu\text{g}\cdot\text{mL}^{-1}$) were significantly higher than in COPD ($p = 0.00016$) (fig. 2b). There was also a significant difference in the sputum levels of NGAL between overlap and NS, HS and asthma, and NS and COPD. Sputum NGAL values did not differ significantly between COPD and asthma, although there was an increasing trend in COPD ($p = 0.058$).

Sputum NGAL can differentiate COPD–asthma overlap from COPD

An ROC curve analysis was carried out to evaluate the sensitivity, specificity and diagnostic accuracy of the biomarkers (table 2). The area under the curve (AUC) statistic showed that all of the four markers could differentiate COPD and overlap from nonsmokers (ROC AUC > 0.7). All these markers were also useful in distinguishing overlap from asthma patients, and plasma sRAGE and sputum MPO could distinguish patients with COPD from those with asthma. However, only sputum NGAL could distinguish patients with overlap from those with COPD.

Sputum NGAL is significantly associated with airflow obstruction independent of sputum neutrophil level

The study subjects were pooled in order to analyse the correlations between biomarker concentrations and different variables. Plasma sRAGE levels correlated with DLCO in both univariate and multivariate analyses (tables 3 and 4). Plasma SP-A levels correlated with pack-years with the highest correlation coefficients found in the univariate analysis. However, multivariate analysis revealed FEV₁/FVC to be the independent predictor for plasma SP-A. Interestingly, while sputum MPO levels were independently correlated with sputum neutrophil levels, sputum NGAL levels were associated with % pred FEV₁, independently of sputum neutrophil (table 4).

Discussion

The present study evaluated plasma and sputum levels of four inflammatory and lung injury-related biomarkers in patients with COPD–asthma overlap, COPD alone and asthma alone, and in control subjects. Patients with overlap and COPD displayed a similar pattern of abnormalities in plasma SP-A and sRAGE and sputum MPO. Plasma SP-A and sputum MPO levels were elevated, while plasma sRAGE levels were decreased in the patients with overlap and COPD compared with asthma patients. Overall, AUC statistics revealed that these three markers could differentiate COPD–asthma overlap and COPD from asthma and nonsmoking controls, but they did not distinguish between overlap and COPD. In contrast, only sputum levels of NGAL were significantly elevated in patients with overlap when compared with COPD, and sputum NGAL could differentiate patients with overlap from COPD patients, as well as from asthma and NS. Therefore, elevated levels of sputum NGAL might be a characteristic feature of COPD–asthma overlap.

TABLE 2 Receiver-operating characteristic (ROC) analysis for plasma and sputum biomarkers

	Overlap versus COPD	Overlap versus asthma	COPD versus asthma	Overlap versus NS	COPD versus NS
Plasma					
sRAGE					
AUC (95% CI)	0.570 (0.397–0.743)	0.719 (0.553–0.884)	0.737 (0.616–0.858)	0.703 (0.513–0.893)	0.703 (0.564–0.842)
Optimal threshold pg·mL ⁻¹	>673.9	<758.2	<903.5	<768.9	<819.7
Sensitivity/specificity	0.643/0.531	0.714/0.812	0.719/0.656	0.714/0.773	0.625/0.727
p-value	0.452	0.019	0.003	0.043	0.012
SP-A					
AUC (95% CI)	0.607 (0.424–0.790)	0.759 (0.608–0.910)	0.682 (0.550–0.813)	0.935 (0.857–1.013)	0.897 (0.810–0.985)
Optimal threshold ng·mL ⁻¹	>39.5	>39.0	>33.5	>29.0	>28.5
Sensitivity/specificity	0.643/0.562	0.643/0.750	0.656/0.625	0.857/0.913	0.844/0.913
p-value	0.252	0.006	0.013	0.00001	0.000001
Sputum					
MPO					
AUC (95% CI)	0.618 (0.362–0.873)	0.732 (0.492–0.972)	0.726 (0.589–0.862)	0.733 (0.517–0.950)	0.736 (0.568–0.904)
Optimal threshold µg·mL ⁻¹	>14.9	>13.0	>10.5	>12.5	>5.0
Sensitivity/specificity	0.600/0.607	0.700/0.880	0.679/0.640	0.700/0.722	0.893/0.667
p-value	0.274	0.034	0.005	0.044	0.007
NGAL					
AUC (95% CI)	0.708 (0.502–0.914)	0.818 (0.635–1.001)	0.697 (0.555–0.839)	0.869 (0.728–1.009)	0.781 (0.634–0.928)
Optimal threshold µg·mL ⁻¹	>4.4	>4.2	>3.5	>4.2	>2.0
Sensitivity/specificity	0.727/0.714	0.818/0.800	0.643/0.720	0.818/0.889	0.857/0.667
p-value	0.046	0.003	0.014	0.001	0.001

COPD: chronic obstructive pulmonary disease; NS: nonsmoker; sRAGE: soluble receptor for advanced glycation end-products; AUC: area under the curve; SP-A: surfactant protein A; MPO: myeloperoxidase; NGAL: neutrophil gelatinase-associated lipocalin.

Airway inflammation is important in the development and progression of COPD, with a vast body of evidence supporting the central role of neutrophils in the pathophysiology of this smoking-related syndrome [24]. Myeloperoxidase is a peroxidase enzyme abundantly expressed in the primary granules of neutrophils. Sputum MPO level has been shown to be elevated in patients with COPD and associated with exacerbation of COPD, evidence of a local activation of neutrophils [8, 25, 26]. Sputum levels of MPO were independently correlated with sputum neutrophils, which is in good agreement with this. Therefore, the present results of sputum MPO could indicate that both overlap and COPD are associated with enhanced airway neutrophilic inflammation.

TABLE 3 Correlations between the plasma and sputum markers and demographics, lung function and sputum cell profile

	Plasma sRAGE	Plasma SP-A	Sputum MPO	Sputum NGAL
Age	-0.046	0.420**	0.316**	0.379**
BMI	-0.064	-0.146	-0.001	0.049
Pack-years	-0.260**	0.541**	0.276*	0.319**
FEV ₁ % pred	0.262**	-0.428**	-0.291*	-0.440**
FEV ₁ /FVC %	0.117	-0.476**	-0.232*	-0.341**
DLco % pred	0.447**	-0.315*	-0.315*	-0.368**
Sputum neutrophils %	-0.302**	0.212*	0.394**	0.434**
Sputum eosinophils %	0.010	0.344**	0.126	0.166

sRAGE: soluble receptor for advanced glycation end-products; SP-A: surfactant protein A; MPO: myeloperoxidase; NGAL: neutrophil gelatinase-associated lipocalin; BMI: body mass index; FEV₁: forced expiratory volume in 1 s; % pred: % predicted; FVC: forced vital capacity; DLco: diffusing capacity of the lung for carbon monoxide. *: p < 0.05; **: p < 0.005.

TABLE 4 Multivariate stepwise analysis of all subjects with each plasma or sputum biomarker as the dependent variable[#]

	β	t-test	p-value
Plasma sRAGE			
DLCO % pred	0.353	2.615	0.012
Plasma SP-A			
FEV ₁ /FVC %	-0.370	-2.796	0.008
Sputum MPO			
Neutrophil in sputum %	0.371	2.908	0.005
Sputum NGAL			
FEV ₁ % pred	-0.427	-3.497	<0.001

sRAGE: soluble receptor for advanced glycation end-products; DLCO: diffusing capacity of the lung for carbon monoxide; % pred: % predicted; SP-A: surfactant protein A; FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity; MPO: myeloperoxidase; NGAL: neutrophil gelatinase-associated lipocalin. [#]: multivariate analysis was adjusted for age, body mass index, current smoking status, pack-years, inhaled corticosteroid use, and all the significant variables in the univariate analysis.

The most striking finding of the present study was related to sputum NGAL. Its levels were significantly increased in patients with COPD–asthma overlap compared with COPD. NGAL was originally recognised as an innate immunity antibacterial factor released from neutrophils, but it is also produced by tubular cells in the kidneys and epithelial cells in the intestinal and respiratory tracts [27, 28]. Urinary NGAL is now widely considered to be a sensitive marker for acute kidney injury, as it reflects tubular cell injury [28]. An early study by KEATINGS and BARNES [8] reported elevated sputum NGAL levels in patients with COPD. Bronchial lavage and plasma NGAL levels were also shown to be elevated in patients with COPD [29, 30], and bronchoalveolar lavage fluid NGAL has been associated with emphysema in smokers [31]. NGAL has been demonstrated to exert two functions of potential importance in the pathogenesis of COPD: inhibition of bacterial growth and enhancement of matrix degradation [32]. Thus NGAL could well be associated with COPD, as reflected by the independent correlation of sputum NGAL with airflow obstruction observed in our study. It should be noted that increased sputum NGAL might not be exclusively attributed to activated neutrophils, as the respiratory epithelial cells may also secrete NGAL in response to inflammatory stimuli [27, 33]. Therefore, the sputum NGAL level might be related to neutrophilic inflammation and/or ongoing damage to the respiratory epithelium.

Plasma levels of sRAGE were similarly decreased in the overlap and COPD groups, but not in asthma. In the multivariate analysis, plasma sRAGE levels were independently associated with DLCO. This association is in full agreement with a previous report that reduced plasma sRAGE levels were related to the severity of emphysema [14]. RAGE is primarily expressed on alveolar type 1 pneumocytes and could assist these cells in acquiring a spreading morphology [34, 35]. Therefore, RAGE might have a role in alveolar integrity, and decreased sRAGE levels could be associated with disrupted alveolar structures. Another possible explanation is that reduction in sRAGE might modify neutrophilic airway inflammation, as sRAGE has anti-inflammatory properties by capturing RAGE ligands [36]. This concept is in agreement with the fact that there were reduced plasma sRAGE levels in overlap and COPD with neutrophilic airway inflammation. Moreover, the recent study by SUKKAR *et al.* [15] has demonstrated a clear association between bronchoalveolar lavage fluid sRAGE and airway neutrophilic inflammation. Therefore, the present results indicate that peripheral lung destruction might be associated with decreased plasma sRAGE in patients with COPD–asthma overlap and COPD, but further investigations are needed to clarify its mechanisms and potential role of modulating the lung inflammation.

A glycoprotein SP-A, synthesised in alveolar type II cells and nonciliated bronchiolar cells of the distal pulmonary epithelium, is secreted into respiratory tract, so the increased circulatory SP-A can be caused by leakage of the lung epithelial barrier [37]. We have previously reported that plasma SP-A concentration is associated with cigarette smoking-related lung injury in smokers [10] and is elevated in patients with COPD [9], the results being in line with previous reports [37–39]. In the current study, both the COPD and overlap groups presented with increased levels of plasma SP-A when compared with patients with asthma and control groups. In agreement with our earlier findings [10], plasma SP-A levels were found to associate with smoking history and the degrees of airway obstruction and peripheral tissue damage. These results suggest that both COPD and COPD–asthma overlap might be associated with smoking-induced lung injury, with the mechanisms being different from those postulated for the pathophysiology of asthma [2].

Recently, we and others have reported that COPD–asthma overlap is associated with significantly lower health-related quality of life and increased exacerbation rates compared with COPD, which could not be explained by patients' demographic, lung function or computed tomography findings of emphysema [5, 6]. In the present study, plasma SP-A and sputum MPO levels were elevated, while plasma sRAGE was decreased in both COPD–asthma overlap and COPD when compared with asthma. Moreover, only sputum NGAL was significantly elevated in overlap patients compared with all of the other groups including COPD. Taken together, these results of the COPD-related biomarkers suggest that while airway neutrophilic inflammation and peripheral lung destruction are common pathological processes in overlap and COPD, COPD–asthma overlap is associated with enhanced airway inflammation, which can be detected by increased levels of sputum NGAL. These findings might have implications for the distinct clinical characteristics of COPD–asthma overlap, but further investigation will be needed to clarify the mechanisms of airway inflammation and the optimal monitoring and treatment strategy of overlap patients.

This was a cross-sectional study and, therefore, further prospective investigations would be required to evaluate the association of these plasma and sputum markers with the development of chronic airway diseases. Basic research studies are warranted to explore mechanisms behind this association. This study has significant strengths. First, none of the control subjects had any other exposures. Secondly, the diagnosis of asthma, COPD and COPD–asthma overlap was based on both clinical history and strict and objective criteria, which enables unbiased comparisons of the characteristics between those diseases. Although the sample size was limited, the present results do seem to reveal the difference in the biomarkers of COPD–asthma overlap compared with asthma and COPD alone.

In conclusion, we have demonstrated that patients with COPD–asthma overlap and COPD have a similar biomarker profile in plasma SP-A and sRAGE and sputum MPO levels, which differ from that in patients with asthma. Moreover, elevated levels of sputum NGAL might be a characteristic feature of overlap that could differentiate overlap from COPD, suggesting enhanced neutrophilic airway inflammation and/or airway epithelial injury in COPD–asthma overlap.

Acknowledgements

T. Marjomaa and S. Nummijoki (Dept of Medicine, Pulmonary Division, University of Helsinki and Helsinki University, Central Hospital, Helsinki, Finland) are acknowledged for their help and/or excellent technical assistance. We thank P. Piirilä (Clinical Physiology Laboratory, Dept of Clinical Physiology and Nuclear Medicine, Helsinki University Central Hospital, Helsinki, Finland) for her help in accessing lung function tests.

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