Exhaled breath condensate cytokine patterns in chronic obstructive pulmonary disease

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Summary Differences in cytokine patterns in stable chronic obstructive pulmonary disease (COPD), exacerbated COPD, smokers without apparent COPD, and healthy volunteers should be of interest for pathophysiological and therapeutic reasons. Methods including lavage, biopsy and sputum have been employed to investigate cytokines in the lung. For asystematic comparison, exhaled breath condensate (EBC) appears to be well suited.

We investigated healthy volunteers, smokers without apparent COPD, stable and exacerbated COPD patients (± inhalative steroids) and finally those whose exacerbation made mechanical ventilation inevitable, for a more complete picture of inflammatory cytokines in COPD. We chose EBC because it is non-invasive and can be used repeatedly in spontaneous breathing individuals and during mechanical ventilation. EBC cytokines (IL-1\textbeta, IL-6, IL-8, IL-10, IL-12p70, TNF-\alpha) were assayed from a single sample using a multiplex array test kit.

Keywords Cytokines; Chronic obstructive pulmonary disease; Exhaled breath condensate; Flow cytometry

Abbreviations: AECOPD, Acute exacerbation of a chronic obstructive pulmonary disease; AECOPD-GW, Acute exacerbation of a chronic obstructive pulmonary disease with general ward treatment; AECOPD-ICU, Acute exacerbation of a chronic obstructive pulmonary disease with intensive care unit treatment; BAL, Bronchoalveolar lavage; BALF, Bronchoalveolar lavage fluid; COPD, Chronic obstructive pulmonary disease; EBC, Exhaled breath condensate; FEV\textsubscript{1}, Forced expiratory volume in the first 1 s; FVC, Forced vital capacity; FEV\textsubscript{1}/FVC, Ratio of the forced expiratory volume in the first 1 s to the forced vital capacity of the lung; HS, Healthy smoking volunteers; ICS, Inhaled corticosteroids; ICU, Intensive care unit; IL-1\beta, Interleukin 1 beta; IL-6, Interleukin 6; IL-8, Interleukin 8; IL-10, Interleukin 10; IL-12p70, Interleukin 12p70; LABA, Long acting beta agonists; MMP-1, Matrix metalloproteinase 1; MMP-9, Matrix metalloproteinase 9; PaCO\textsubscript{2}, Carbon dioxide partial pressure in arterial blood; PaO\textsubscript{2}, Oxygen partial pressure in arterial blood; RV, Residual volume; SLPI, Antiproteases secretory leukoprotease inhibitor; TIMP-1, Tissue inhibitor of metalloproteinase 1; TLC, Total lung capacity; TNF-\alpha, Tumor necrosis factor alpha; VOL, Healthy non-smoking volunteers.

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We observed a significant increase of all cytokines in acute exacerbation compared to stable COPD, smokers, and volunteers. Stable COPD and volunteers exhibited only small differences in cytokine pattern with respect to IL-1β and IL-12 (P < 0.01). Smokers had increased levels of all investigated cytokines (P < 0.01) compared to non-smokers and, with the exception of IL-1β, to stable COPD. Inhaled steroids resulted in reduced levels of IL-1β, IL-6, IL-8, IL-10, and IL-12 (all: P < 0.01) in stable COPD (all: ex-smokers) with dose dependency for IL-8, IL-1β and IL-12.

EBC analysis successfully characterized important differences in stable COPD compared to exacerbation or smoking and non-smoking healthy individuals.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by airflow obstruction due to chronic bronchitis and/or emphysema which is progressive and associated with an abnormal inflammatory response of the lung. The final result of this process is destruction and loss of lung parenchyma. Various methods have been employed to investigate and possibly quantify inflammation in COPD. Among them invasive methods such as biopsy of the bronchial walls as well as bronchoscopy and bronchoalveolar lavage (BAL) with subsequent examination of cells and soluble markers. Induced sputum, a less invasive method, has been the source for many investigations aimed at describing inflammation. Exhaled breath condensate (EBC) constitutes another, entirely non-invasive method which has been used for the same purpose. Different stages and variations of the process called COPD were investigated, with a majority of the studies investigating stable disease although in some cases acute exacerbations of COPD (AECOPD) were also studied. The most severe and acute form of COPD, AECOPD that leads to ICU treatment and the need for invasive mechanical ventilation has also been studied by this technique with respect to pH, cytokines and nitrite. The different situations of exacerbation of COPD as well as stable disease were variably compared to either healthy volunteers and/or smokers without apparent signs of COPD.

In this study we wanted to characterize the cytokine pattern of inflammation in stable and exacerbated COPD (stable COPD, AECOPD with general ward treatment [AECOPD-GW], AECOPD with ICU treatment [AECOPD-ICU]) and compare it to healthy volunteers as well as smokers without apparent COPD. EBC was used to investigate the cytokine patterns, because this material is non-invasive and sampling does not lead to any alteration of the inflammatory situation of the airways. In this study EBC cytokine measurement proved to be useful in estimating the inflammatory status of the different patient groups. Volunteers, smokers, stable COPD patients and exacerbated COPD patients all exhibited significant differences. The understanding of these patterns may facilitate therapeutic decisions.

Methods

Study subjects and clinical scores

EBC was collected from:

(a) ICU patients with life-threatening exacerbation of COPD on mechanical ventilation (AECOPD-ICU), n = 11.
(b) Patients with severe and very severe exacerbated COPD with the need for hospital admission in a general ward (AECOPD-GW), n = 34.
(c) Patients with known and stable COPD (stable COPD), n = 40. (Stable COPD was defined by the lack of symptoms typical for an acute exacerbation and no need for a change in medication for at least 8 weeks prior to presentation.)
(d) Healthy smoking volunteers (HS), n = 21.
(e) Healthy non-smoking volunteers (VOL), n = 24.

Patient’s characteristics are depicted in Table 1. Patients on mechanical ventilation were included in this investigation at 8–48 h of ventilation. Mechanical ventilation was performed according to guidelines for protective ventilation with a tidal volume at 6 ml/kg ideal body weight. Ventilated patients fulfilled criteria described by Burge and co-workers. EBC of patients with AECOPD was collected before therapy at the time of diagnosis. All AECOPD-GW patients exhibited criteria for admission to a hospital as has been suggested: respiratory rate >25/min; pulse rate >110/min; PaO2 <8 kPa; abnormal chest radiograph; serious concomitant disease; altered mental state; living alone. All AECOPD patients (ICU and GW) were treated with i.v. antibiotics. EBC collection was
performed between 8 and 36 h following hospitalization. At the time of EBC collection corticosteroids had not yet been given. AECOPD diagnosis was based on criteria described by Anthonisen25: presence of at least one of the following three major symptoms: increase in dyspnoe, sputum volume increase, sputum change to purulence and at least one of the following minor symptoms: cough, wheeze, sore throat, nasal discharge, fever. All patients with stable COPD, AECOPD-GW, and AECOPD-ICU were nonsmokers for at least 1 year according to their own saying.

Lung function was performed on the day of EBC collection in all stable patients, within 1 week in AECOPD-GW patients and within 10 days following extubation in AECOPD-ICU patients. Capillary blood gas analysis was performed on all patients within the first few hours after admission. All COPD patients were staged according to current guidelines (GOLD), (1 update 2003) as stage III COPD or stage IV COPD. None of the patients in this series was treated with an oral steroid. All patients were on oral (theophylline) and inhaled therapy (LABA and/or long acting anticholinergic and/or inhaled corticosteroids (ICs)) according to the guidelines. All patients were regularly seen by pulmonary specialists. Approval for this investigation was obtained from the ethics committee of the University of Leipzig.

EBC collection and markers

EBC was collected by inserting a special conduit (FILT lung and Chest Diagnostics Ltd., Berlin, Germany) for the EcoScreen® breath condensate collecting device (ViaSys, Hoechberg, Germany) into the expiratory limb of the ventilator tubing directly after the Y-shaped connecting piece for a 20 min time period. Humidification of inspiratory gas was achieved using heat humidifiers. EBC from spontaneously breathings was similarly collected for 20 min with the EcoScreen® system as previously described.26

All EBC samples were examined for amylase activity (alpha-Amylase ESP1491300 kit; detection limit 0.05 μmol/l s; Boehringer Mannheim, Germany) in order to exclude contamination by saliva. Protein concentration in EBC was measured using the Micro BCA Protein Assay (Pierce, Rockford USA).

Bronchoalveolar lavage (BAL)

When BAL was scheduled for microbiological reasons in AECOPD patients, an aliquot (2 ml) was taken for the comparison of cytokines in BAL fluid (BALF) and EBC. A comparison of BALF and EBC was thereby achieved in 14 cases. In all cases, EBC collection was performed prior to bronchoscopy. BAL was done according to

### Table 1 Patient’s characteristics.

<table>
<thead>
<tr>
<th>Patients</th>
<th>VOL</th>
<th>HS</th>
<th>Stable COPD</th>
<th>AECB-GW</th>
<th>AECB-ICU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>24</td>
<td>21</td>
<td>40</td>
<td>34</td>
<td>11</td>
</tr>
<tr>
<td>Age (mean ± sd)*</td>
<td>54.7 ± 10.5</td>
<td>59.0 ± 12.2</td>
<td>60.3 ± 15.8</td>
<td>62.1 ± 12.3</td>
<td>62.7 ± 9.6</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>13</td>
<td>12</td>
<td>27</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>9</td>
<td>13</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Smoking status †</td>
<td>Non-smoker</td>
<td>Smoker</td>
<td>Ex-smoker</td>
<td>Ex-smoker</td>
<td>Ex-smoker</td>
</tr>
<tr>
<td>Pack years</td>
<td>0</td>
<td>26 ± 5</td>
<td>30 ± 9</td>
<td>28 ± 7</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>FEV1 (l)‡</td>
<td>3.79 ± 0.46</td>
<td>3.38 ± 0.58</td>
<td>1.33 ± 0.60</td>
<td>1.33 ± 0.59</td>
<td>1.21 ± 0.38</td>
</tr>
<tr>
<td>FVC (l)‡</td>
<td>4.14 ± 0.49</td>
<td>3.89 ± 0.58</td>
<td>2.53 ± 0.96</td>
<td>2.28 ± 0.82</td>
<td>2.36 ± 0.76</td>
</tr>
<tr>
<td>FEV1/FVC (%)‡</td>
<td>91.4 ± 2.50</td>
<td>86.7 ± 4.46</td>
<td>51.9 ± 7.61</td>
<td>57.2 ± 9.36</td>
<td>52.6 ± 8.11</td>
</tr>
<tr>
<td>PaO₂ (kPa)§</td>
<td>10.09 ± 0.40</td>
<td>9.44 ± 0.40</td>
<td>7.37 ± 0.46</td>
<td>6.33 ± 0.28</td>
<td>5.36 ± 0.61</td>
</tr>
<tr>
<td>PaCO₂ (kPa)§</td>
<td>5.15 ± 0.29</td>
<td>5.40 ± 0.31</td>
<td>5.84 ± 0.31</td>
<td>6.58 ± 0.24</td>
<td>8.58 ± 0.63</td>
</tr>
<tr>
<td>Protein in EBC (μg/ml) (mean ± sd)*</td>
<td>13.9 ± 8.9</td>
<td>13.4 ± 5.5</td>
<td>11.5 ± 4.4</td>
<td>10.7 ± 5.3</td>
<td>14.4 ± 9.1</td>
</tr>
<tr>
<td>ICS therapy</td>
<td>0</td>
<td>0</td>
<td>21</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Yes</td>
<td>24</td>
<td>21</td>
<td>19</td>
<td>14</td>
<td>11</td>
</tr>
</tbody>
</table>

All data are shown as mean ± sd.

*P < 0.05 (no significant difference between investigated groups) in ANOVA test.

†Smoking was defined as current smokers or ex-smokers that discontinued smoking no longer than 12 month, non-smoking as no smoking longer than 1 year.

‡Results were measured after AECB.

§Results were measured at time of hospitalization.

ICS: inhalative corticosteroid.
published guidelines\textsuperscript{27} (5 x 20 ml aliquots). The cell free supernatant was used for further analysis.

Cytometric bead array

A multiplex fluorescent bead immunoassay (cytometric bead array [CBA] Becton Dickinson, San Jose, CA, USA) has been adapted to analysis in breath condensate to detect cytokine concentrations. A mixture of six bead populations with distinct fluorescence intensities and coated with capture antibodies specific for IL-8, IL-1\textbeta, IL-6, IL-10, TNF-\textalpha, and IL-12p70 proteins were incubated with 2 ml of lyophilized breath condensate reconstituted with 50 \mu l of ddH\textsubscript{2}O (duplicate samples were prepared). Cytokines in EBC samples and recombinant standards bound to capture beads were detected by PE-conjugated detection antibodies in a flow cytometer (FACS Calibur\textsuperscript{TM}, Becton Dickinson). Effects of cryoconservation, lyophilization, and reduction of reagent amounts as well as requirements to buffer composition were investigated in a group of 9 samples in order to ensure correct detection of cytokines by this commercially available system: defined cytokine concentrations were dissolved in water and measured before and after cryoconservation and lyophilization. A loss of 10.6\% of activity was observed. By adding 1\% of BSA this loss was reduced to 1.8\%.

Mean intra-assay reproducibility and inter-assay reproducibility were 92.8\% (IL-8: 89.5\%; IL-1\textbeta: 94.5\%; IL-6: 90.7\%; IL-10: 96.5\%; TNF-\textalpha: 91.6\%; IL-12: 93.9\%) and 87\% (IL-8: 80.6\%; IL-1\textbeta: 85.7\%; IL-6: 90.4\%; IL-10: 92.7\%; TNF-\textalpha: 88.9\%; IL-12: 83.8\%), respectively.

Statistical analysis

Statistical analysis was performed with the SPSS software package (SPSS Inc., Chicago, USA). Linear regression analysis was applied to investigate the correlation of cytokine levels in EBC and BALF. Comparison of patient groups (three or more) was performed by Kruskal–Wallis test and Mann–Whitney test. Data in box plots are median ± 25/75\% (box) and 5/95\% confidence intervals, with outlying data points indicated as closed circles. Statistical significance was accepted at the 5\% level.

Results

Patients’ characteristics

Patient characteristics are listed in Table 1.

General exhaled breath condensate characteristics

None of the condensate samples exhibited amylase concentrations measurable with the assay used. Therefore a relevant saliva contamination can be excluded because amylase concentration in saliva is proximally 10,000 times higher than those in EBC with respect to detection limit of assays.\textsuperscript{28,29} Total EBC protein concentration was regularly measured. Results are shown in Table 1. There was no significant difference in any of the subgroups analyzed (P = 0.27).

Inflammatory cytokines in EBC

The rates of detection of the various cytokines in all of the patient subgroups are listed in Table 2. In AECOPD-ICU and AECOPD-GW patients all cytokine levels were considerably higher and cytokines were more often detectable than in patients with stable COPD, healthy smokers or healthy volunteers (Fig. 1, Table 2). Cytokines in relation to protein in EBC showed comparable results shown in Fig. 2.

<table>
<thead>
<tr>
<th>Marker</th>
<th>VOL d/nd (%)</th>
<th>HS d/nd (%)</th>
<th>Stable COPD d/nd (%)</th>
<th>AECOPD-GW d/nd (%)</th>
<th>AECOPD-ICU d/nd (%)</th>
<th>All patients d/nd (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8</td>
<td>14 (58)/10 (42)</td>
<td>19 (90)/2 (10)</td>
<td>31 (77)/9 (23)</td>
<td>34 (100)/0 (0)</td>
<td>11 (100)/0 (0)</td>
<td>109 (84)/21 (16)</td>
</tr>
<tr>
<td>IL-1\textbeta</td>
<td>19 (79)/5 (21)</td>
<td>21 (100)/0 (0)</td>
<td>39 (97)/1 (3)</td>
<td>34 (100)/0 (0)</td>
<td>11 (100)/0 (0)</td>
<td>124 (95)/6 (5)</td>
</tr>
<tr>
<td>IL-6</td>
<td>11 (46)/13 (54)</td>
<td>20 (95)/1 (5)</td>
<td>15 (37)/25 (63)</td>
<td>33 (97)/1 (3)</td>
<td>10 (91)/1 (9)</td>
<td>89 (68)/41 (32)</td>
</tr>
<tr>
<td>IL-10</td>
<td>13 (54)/11 (46)</td>
<td>19 (90)/2 (10)</td>
<td>31 (77)/9 (23)</td>
<td>34 (100)/0 (0)</td>
<td>11 (100)/0 (0)</td>
<td>108 (83)/22 (17)</td>
</tr>
<tr>
<td>TNF-\textalpha</td>
<td>16 (67)/8 (33)</td>
<td>20 (95)/1 (5)</td>
<td>33 (82)/7 (18)</td>
<td>34 (100)/0 (0)</td>
<td>11 (100)/0 (0)</td>
<td>114 (88)/16 (12)</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>19 (79)/5 (21)</td>
<td>21 (100)/0 (0)</td>
<td>37 (92)/3 (8)</td>
<td>34 (100)/0 (0)</td>
<td>11 (100)/0 (0)</td>
<td>122 (94)/8 (6)</td>
</tr>
</tbody>
</table>

d, cytokine detectable; nd, cytokine not detectable.
Differences between stable COPD and healthy smokers were observed in all cytokines investigated with the exception of IL-1β: Healthy smokers showed significantly increased levels for all cytokines compared with healthy volunteers. For stable COPD and healthy volunteers differences were only observed for IL-1β and IL-12p70 (Figs. 1 and 2).

Subgroup analysis of COPD stage III versus COPD stage IV in both the AECOPD as well as in the stable COPD patient groups did not reveal significant differences with respect to cytokines analyzed (data not shown).

Correlation of EBC cytokines with lung function or ventilatory parameters

Results of lung function as well as blood gas analysis are shown in Table 1 for the following parameters: FEV₁, FVC, FEV₁/FVC, PaO₂, and PaCO₂. There was

Figure 1 IL-8, IL-1β, IL-6, IL-10, TNF-α, and IL-12p70 levels in EBC of patients at ICU with life-threatening exacerbation of COPD on mechanical ventilation (AECOPD-ICU: n = 11), patients with severe and very severe exacerbated COPD with the need for hospital admission in a general ward (AECOPD-GW: n = 34), patients with known and stable COPD (stable COPD: n = 40), healthy smoking volunteers (HS: n = 21), and healthy non-smoking volunteers (VOL: n = 24). Results are median 75/75% (box) and 5/95% confidence intervals, with outliers indicated as closed circles.
no strong, significant correlation observed between lung function parameters VC, $R_{tot}$, FEV₁, FVC, FEV₁/FVC, TLC, RV, $P_{aO_2}$, and $P_{aCO_2}$ and the level of cytokines in EBC of either all patients or any of the subgroups. This same result was also true for all the parameters of ventilation examined in this study: positive end expiratory pressure (6.7 mbar ± 3.4), peak inspiratory pressure (20.6 mbar ± 3.3), tidal volume adapted to ideal body weight (6.3 ml/kg body weight ± 1.0), expiratory minute volume...
(9.8 L/min ± 2.5) and breathing frequency (21.5 ± 4.5) when correlated to cytokine levels in ventilated patients.

**Influence of inhaled corticosteroid in COPD patients at cytokines in EBC**

Stable COPD patients (n = 40) and patients with AECOPD-GW (n = 34) were evaluated for the influence of ICS on the level of cytokines in EBC. We found lower levels for IL-1β, IL-6, IL-8, IL-12, and IL-10 (all: P < 0.01) but not for TNF-α in patients with stable COPD on ICS (n = 21) compared with stable COPD patients without ICS (n = 19; Fig. 3). ICS-dose dependence (lower dose ≤1000 μg, higher dose > 1000 μg/day) was recognized for IL-8, IL-1β and IL-12 (Fig. 4). In contrast to these results, patients with AECOPD exhibited no difference in cytokine levels depending on the use of ICS (Fig. 3). Although a lower level of EBC cytokines was prominent in stable COPD patients on ICS, no

**Figure 3** EBC cytokines in patients with severe and very severe exacerbated COPD with the need for hospital admission in a general ward (AECOPD) with (n = 18) and without (n = 11) ICS therapy and patients with known and stable COPD (stable COPD) with (n = 21) and without (n = 19) ICS therapy (median ± 25/75% [box] and 5/95% confidence intervals, with outliers indicated as closed circles).
change in lung function parameters was apparent in comparison with stable COPD patients without ICS.

Correlation of cytokine levels in BALF and EBC

The correlation of BALF ingredients with the same molecules in EBC is largely unknown. We found no significant correlation between cytokine levels in BALF and those in EBC (Table 3). However the results were not equal for all of the cytokines investigated and clearly the correlation was closer for IL-12p70 ($r = 0.52; P = 0.06$; Fig. 5) than for any of the remaining cytokines investigated. A larger number of samples might well have demonstrated a significant correlation in this case and possibly also for IL-6 and IL-10.

Discussion

This study was aimed at characterizing inflammation by analyzing cytokine profiles in healthy individuals, smokers, stable COPD, acute...
exacerbation without and with the need for mechanical ventilation. We chose to measure cytokine profiles in EBC because this material is acquired non-invasively. Investigating a potential influence of ICS on the cytokine profiles was a secondary aim of our study.

Chronic inflammation, although different to that in asthma is the predominant feature of COPD. The nature of this inflammatory process has been thoroughly investigated by, e.g. invasive methods such as biopsies and bronchoscopy.\(^6,^8\) Several investigators have relied upon induced sputum as a source for monitoring of inflammation.\(^4,^19,^30\) Detection of cytokines in EBC has only been used in one study of COPD patients.\(^16\) This is the first study comparing a systematic profile of cytokines in a spectrum of healthy volunteers, smokers without COPD, stable COPD patients and patients with AECOPD using EBC.

A prominent finding of this study is the order of magnitude in differences of cytokine levels of COPD patients with acute exacerbations in comparison with either stable COPD patients, healthy smokers or non-smoking volunteers. This difference in magnitude relates to all of the cytokines examined. A similarly strong increase of cytokine levels, approx. 15 fold in this study, was only paralleled in an analysis of Nys et al. in which a 5–25 fold increase in IL-1\(\beta\) and IL-8 was observed in BALF of patients with acute lung injury with pneumonia versus patients with acute lung injury without pneumonia.\(^31\) A much smaller difference was observed in investigations using induced sputum where AECOPD-GW versus stable COPD resulted in an IL-6 increase of approx. 1.5 fold\(^32\) or an IL-8 increase of 2 fold and a TNF-\(\alpha\) increase of 4 fold.\(^22\) EBC cytokine analysis using the bead array used here appears to be very sensitive in picking up large differences in cytokine levels in acute inflammation compared to more chronic inflammation. This of course would be a prerequisite of any standard tool for monitoring inflammation.

Cytokines expressed in relation to protein concentration in EBC as a means of standardization showed absolutely comparable results with regard to the differences between subgroups. Protein concentration in EBC has been suggested to be one of the potential ways to standardize concentrations in EBC. However, no reliable way of standardization has yet been agreed upon. We therefore decided to present the raw cytokine concentrations as measured in EBC in this paper.

Much smaller magnitudes of cytokine levels were observed in clinically stable and clinically healthy situations. However, volunteers were different from smokers with respect to all cytokines demonstrating that smoking without any sign of COPD does induce an inflammatory lesion. In BALF a similar subset of cytokines was analyzed before and did not result in significant differences between stable COPD patients, smokers and non-smokers at comparable group sizes.\(^33\) Although not significant, the relative levels observed in this study fit well to our data. An IL-6 increase (2 fold) due to smoking comparable to the one described in our study was previously reported in EBC.\(^34\) We also observed an increase in IL-8 in smokers compared with non-smokers. In contrast IL-8 was not different between these two groups in a study using BALF to analyze neutrophil chemokines in smokers and non-smokers.\(^7\)

Cytokine levels in stable COPD patients who were all seen by pulmonary specialists and who were treated according to current guidelines were rather low in comparison with those of smokers and

<p>| Table 3 Correlation of EBC cytokines and BALF cytokines in a subgroup of 14 patients. |
|----------------------------------------|------------------|------------------|
| Marker      | Correlation of EBC and BALF |     |</p>
<table>
<thead>
<tr>
<th></th>
<th>(R)</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8</td>
<td>0.03</td>
<td>0.93</td>
</tr>
<tr>
<td>IL-1(\beta)</td>
<td>0.06</td>
<td>0.83</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.40</td>
<td>0.15</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.41</td>
<td>0.15</td>
</tr>
<tr>
<td>TNF-(\alpha)</td>
<td>0.21</td>
<td>0.46</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>0.52</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Figure 5 Correlation of IL-12p70 in EBC with concentration in BAL (\(r = 0.52; P > 0.06\)) in a subgroup of 14 patients.

Exhaled breath condensate cytokine in COPD 1237
volunteers. A significant difference between stable COPD and healthy volunteers was only seen for IL-1β and IL-12. In fact, in stable COPD the level of inflammatory cytokines appeared to be lower than in smokers possibly due to the fact that all patients with stable COPD had quit smoking at least 1 year ago. A study that has found elevated levels of, e.g. IL-8 in BALF of smokers is not really comparable to any of our groups, because all COPD patients were smokers whereas in this study all COPD patients were ex-smokers and smokers were clinically apparent. Another explanation for the small difference in cytokine levels between stable COPD patients and healthy volunteers might have been therapeutic intervention. Because steroids have an influence on cytokine levels in many systems we evaluated the effect of ICS on cytokine levels in stable COPD and AECOPD-GW patient subgroups. In stable COPD ICS therapy in this study was correlated with decreased levels of IL-1β, IL-8, IL-6, IL-10 and IL-12 but not TNF-α. Other authors have not observed an ICS induced effect on IL-8, MMP-1, MMP-9, SLIP, and TIMP-1 in induced sputum indicating again, that EBC analysis of cytokines may be a method with increased sensitivity. In fact the effect of ICS in COPD is a matter of ongoing debate. From this study it can be concluded, that there is definitely an effect of ICS on cytokines in EBC and this effect can even be underscored by demonstrating an ICS-dose dependency for IL-1β, IL-8 and IL-12. Patel and coworkers similarly found lower IL-6 and IL-8 concentrations in supernatant of human bronchial epithelial cells harvested from biopsies of stable COPD patients and a negative correlation of at least IL-6 with the ICS dose. The effect of dexamethasone on basal cytokine release by alveolar macrophages varied in another study between smokers and COPD patients. Dexamethasone 10⁻⁵ M resulted in a significant reduction in both smoking COPD patients (approx. 45% reduction of basal GM-CSF release in macrophages) and smokers without COPD (approx. 57% reduction). In contrast, dexamethasone 10⁻⁷ M did not result in a significant reduction of the strongly increased IL-8 release in smoking COPD patients, but did reduce the much lower basal release of IL-8 in smokers without COPD by approx. 30%. Further work of Ito and coworkers demonstrated a significant difference in the effect of dexamethasone 10⁻⁶ M on IL-1β induced release of IL-8 and TNF-α between smokers (without COPD) and non-smokers: dexamethasone failed to reduce the stimulated release of both cytokines in smokers. This and experiments aiming at the activity and expression of the enzyme histone deacetylase (HDAC) has generated the hypothesis, that smoking inhibits HDAC expression and promotes cytokine expression and the lack of steroid responsiveness. It was suggested that even in COPD patients that have stopped smoking, this mechanism might continue. Our findings with COPD patients that all were ex-smokers appear to argue against that assumption. Our findings in fact seem to fit well with the clinical impression that inhaled steroids do reduce the frequency of COPD exacerbations and that lower levels of cytokines are related to less frequent COPD exacerbations. However, there was no relation of IL-8 level in induced sputum and exacerbation frequency in another investigation. The significance of this finding will have to be further investigated. In AECOPD-GW patients, no impact of ICS on cytokine levels was observed, but cytokine levels were much higher as mentioned before and the small doses of inhaled steroids might not be enough to influence this level of inflammation. Oral steroids have proven use in this situation.

A methodological question we wanted to answer in this study concerned the correlation of cytokine levels of EBC with BAL fluid of the same patient. We did not find any significant correlation of cytokine levels in EBC compared with BAL although the numbers varied for each cytokine and significance seemed within reach for IL-12p70. This is in some contrast to another marker, nitrite, for which we previously described a significant but not very strong correlation between EBC and BAL. We cannot offer a good explanation for the dissimilar behavior of different cytokines. Instead we postulate that a correlation of ingredients of EBC and BAL will have to be demonstrated for each ingredient separately.

An increasing number of papers indicate the usefulness of EBC in respiratory medicine. An ERS statement on this subject is currently in preparation. For the purpose of estimating the extent of inflammation of the airways/lung in COPD, EBC seemed to perform well and might enable physicians to monitor the disease and rationally follow therapy.

We conclude that AECOPD-GW and AECOPD-ICU exhibit similar strong increases in cytokine levels in COPD patients. Stable COPD (ex-smokers) and volunteers were similar with respect to cytokine patterns with the exception of elevated IL-1/β and IL-12. Smokers have increased levels of all cytokines when compared to non-smokers. Inhaled steroids resulted in reduced levels of IL-1/β, IL-6, IL-8, IL-10 and IL-12 in stable COPD (ex-smokers) with dose dependency demonstrated for IL-1/β, IL-8 and IL-12. EBC therefore appears to be able to characterize important differences in various COPD patient groups.
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References


