Longitudinal follow-up of systemic inflammation after acute exacerbations of COPD

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Abstract

Background: Acute exacerbations are important in the clinical course of COPD, yet the underlying mechanisms are poorly understood. Systemic inflammation is now considered as an important component in the disease process. In this study we evaluated longitudinally the systemic inflammation during hospital treatment for acute exacerbation and after clinical recovery.

Methods: Blood was collected on day 0, 1, 4 and 8 in 21 patients admitted for an acute exacerbation of COPD and at 1 month, 3 months and 6 months after discharge. Systemic inflammation was determined by measurement of the pro-inflammatory markers interleukin (IL)-6, soluble tumor necrosis factor (TNF) receptors sTNFR55 and sTNFR75, the anti-inflammatory mediator sIL-1RII, and bactericidal permeability increasing protein (BPI) as a marker of neutrophil activation. In addition, plasma level of Trolox antioxidant capacity (TEAC) was determined. Healthy age-matched controls were measured for the same markers at one time-point.

Results: All inflammatory markers analyzed were elevated on first day of admission for exacerbation of COPD, as compared to healthy controls. During treatment, levels of IL-6, and sTNFR75 rapidly decreased, whereas sTNFR55 and BPI remained elevated. Moreover, sIL-1RII and TEAC increased during first 8 days of treatment. In the stable condition all inflammatory markers returned to values comparable to healthy controls, with the exception of BPI, which remained persistently elevated compared to healthy controls.

Abbreviations: ATS, American Thoracic Society; BAL, broncho alveolar lavage; BMI, body mass index; BPI, bactericidal permeability increasing protein; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; ECP, eosinophilic cationic protein; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; IL-6, interleukin 6; IL-8, interleukin 8; IVC, inspiratory vital capacity; MPO, myeloperoxidase; sIL-1 RII, soluble InterLeukin-1 receptor II; sTNF R55, soluble tumor necrosis factor receptor 55; sTNF R75, soluble tumor necrosis factor receptor 75; TEAC, trolox equivalent antioxidant capacity; TNF-α, tumor necrosis factor-α.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality throughout the world. Hospital admission for acute exacerbations forms the major component of the economical burden of COPD in western countries.1 Generally, acute exacerbations are characterized by varying combinations of symptoms as increase in cough, sputum production, worsening of dyspnea or changes in sputum purulence. Despite the important role of these acute exacerbations in the clinical course of COPD, underlying pathogenic mechanisms are poorly understood.

Inflammation is a prominent feature of COPD as shown by the presence in the airways of activated neutrophils and macrophages and increased numbers of inflammatory mediators.2,3 Exacerbations of COPD are generally considered to reflect a flare-up of these underlying inflammatory processes, although information about the inflammatory response in the lungs, particularly during severe exacerbations, is still limited. Recent studies showed increased airway inflammation during exacerbations, as measured by increased sputum neutrophil, eosinophil and lymphocyte counts, increased sputum myeloperoxidase (MPO) and IL-8, and activation of NF-kappa B in sputum macrophages, compared to the stable phase.4–7

Besides the abnormal local inflammatory response, systemic inflammation is now considered an important component in the disease process. This systemic inflammation is related to the systemic consequences of COPD, including involuntary weight loss, muscle dysfunction and wasting, and increased cardiovascular morbidity.8 Increased systemic inflammation during acute exacerbations of COPD is indicated by increased levels of the acute phase proteins C-reactive protein (CRP) and fibrinogen, elevated levels of cytokines as IL-6, and the neutrophil marker MPO.9–11 In addition, the anti-inflammatory mediator sIL-1RII was shown to increase progressively during treatment of exacerbation.12 Recently, Hurst et al. published an extensive evaluation of a panel of potential biomarkers at exacerbation, from which CRP was the most selective biomarker.13 Moreover, a decreased plasma anti-oxidant capacity was observed during exacerbations.14

Most of the presently available data are obtained in cross-sectional studies not taking into account the time to recovery to the baseline stable state. The aim of our study was to evaluate longitudinally the course of a panel of systemic inflammatory mediators, and the anti-oxidant capacity, during hospital treatment for acute exacerbation and after clinical recovery, in patients with severe COPD. As a control group healthy age-matched control subjects were analyzed. Our hypothesis was a demonstration of a differential time response pattern of a panel of inflammatory mediators as well as of anti-oxidative capacity during and in the recovery phase of acute exacerbations and assessment of COPD related disease markers in the stable disease condition.

Materials and methods

Study population

Patients with acute exacerbation: The study population consisted of 21 patients consecutively admitted to the University Hospital Maastricht for an acute exacerbation of COPD. COPD was defined as forced expiratory volume in 1 second (FEV1) < 80% predicted for age and height, ß2 agonist reversibility of <11% of predicted and airflow obstruction evidenced by a ratio of FEV1 to forced vital capacity (FVC) of <0.70 of predicted according to ATS criteria.15 Patients with important co-morbid conditions as malignancies, diabetes mellitus, thyroid and cardiovascular diseases, were excluded from the study in order to avoid non-COPD related interfering factors. Patients, already treated with antibiotics and/or corticosteroid therapy on admission, were also excluded from the study. Furthermore, if patients had a recurrent exacerbation within the follow-up period of 6 months, they were excluded from the study.

The presence of an acute exacerbation was defined as the presence of one or more the following symptoms: (1) increased cough and sputum volume, (2) increased sputum purulence, and (3) increased dyspnea. All 21 patients were classified as a type 1 exacerbation, based on the Anthonisen criteria.16 Patients were treated according to a standard protocol with nebulized salbutamol and ipratropiumbromide, intravenous prednisolone in a dosage of 0.5 mg/kg. Duration of intravenous therapy was 4 days. On day 4 patients were switched to an oral tapering schedule of prednisolone. Specific antibiotic treatment was administered to patients in case of positive sputum cultures. A sputum culture was considered as positive (proving bacterial infection) if significant bacterial growth was present as defined by the number of bacteria (higher than 105 colony forming units – cfu) in a representative sample. Antibiotic treatment was based on resistance assessment of identified bacteria. Patients were evaluated for inflammatory markers on day 0 (admission) before the start of treatment, day 1, day 4 and day 8 of hospitalization for acute exacerbation. Patients were discharged based on clinical judgment of an experienced, independent chest physician.

After discharge, patients were evaluated after 1 month, 3 months and 6 months. The study was approved by the medical ethical committee of the University Hospital Maastricht and all participants have given their written informed consent.

Healthy controls: The healthy control group consisted of 20 subjects who were age and gender matched and without any evidence of COPD based on questionnaires and lung
function tests. They were not suffering from acute or chronic diseases, based on analysis of the clinical history and medical examination. Control subjects were living in the same area as the patients. Control subjects were evaluated for inflammatory markers at 1 time-point. Control subjects without manifested morbid conditions were chosen to find out COPD-related changes in systemic mediators during the recovery period and after the supervised 6-month period when the disease was considered stable.

Lung function measurements: On admission for an acute exacerbation, arterial blood gases at rest were assessed by puncture of the radial artery during room air breathing. On days 1, 4 and 8 of admission and 1, 3 and 6 months after discharge, forced expiratory volume in one second (FEV₁) and inspiratory vital capacity (IVC) were calculated from the flow-volume curve, using a portable pneumotachograph (Jaeger instruments, Würzburg, Germany). Flow-volume curves were performed at a standardized time point, one hour after medication. Lung function values were expressed as a percentage of the reference values.

Measurement of inflammatory parameters and TEAC in plasma: Blood was collected in EDTA containing tubes. (Sherwood Medical, St. Louis, MO, US) The blood samples were put on ice immediately and kept on ice during the entire preparation. Plasma was separated by centrifugation at 167 g for 10 min at 4 °C and two aliquots were centrifuged at 3000 rpm. Supernatants were collected and stored at −70 °C until analysis for cytokines and anti-oxidants.

Inflammatory mediators were measured in plasma by sandwich enzyme-linked immunosorbent assay (ELISA), as described previously. Briefly, for detection of sTNF-R55 and sTNF-R75, monoclonal antibodies MR1-1 and MR2-2 were used for coating and specific biotin labeled polyclonal rabbit anti-human (h)-sTNF-R IgG as detector reagents. For sIL-1RII measurements, plates were coated with monoclonal antibody 8.5 against shIL-1RII and detection was carried out with a biotinylated polyclonal rabbit anti-shIL-1RII IgG. For IL-6 and BPI measurements, plates were coated with murine monoclonal antibody SE1 and human BPI specific monoclonal antibody 4E3, respectively. Biotinylated polyclonal rabbit anti-human IL-6 antisera and biotinylated polyclonal rabbit anti-human BPI IgG were used as detection antibodies. All plasma samples were measured in the same run.

For analysis of TEAC, plasma was deproteinized. An aliquot of 150 μl of plasma was deproteinized by mixing with an equal volume of 10% (w/v) TCA, and after centrifugation for 5 min at 14,000 rpm the supernatant was used for spectrophotometrical analysis. TEAC was determined enzymatically (Sigma) as described by Van den Berg et al. Statistics: Differences in parameters within an individual patient between two time points were compared using Wilcoxon matched pairs signed rank test. Because we have performed planned comparisons in a relatively small sample size, no multiple comparisons tests were performed. Differences between patients and controls were measured using the Mann–Whitney U-test. Significance was determined at the 5% level. Data were analyzed using the SPSS for Windows statistical package (version 12.0, SPSS Inc., Chicago, IL, US). Data were expressed as mean (±SD).

Results

Clinical parameters: The characteristics of the COPD patients on admission for an acute exacerbation and healthy controls are summarized in Table 1. On average, the duration of admission was 9.4 days (range 5–14). There was a small improvement in FEV₁ during the clinical course of acute exacerbation, which was not statistically significant (from 53.9 ± 0.03 l on admission to 0.97 ± 0.04 l on day 8; p = 0.19). There was a significant improvement in PaO₂ during admission (from 53.9 ± 10.2 mmHg on admission to 64.7 ± 8.7 mmHg on day 8; p = 0.001). PaCO₂ values did not change significantly (data not shown).

Twelve of 21 patients had a positive sputum culture, 9 patients with 1 microorganism and 3 patients with 2 microorganisms. Microorganisms cultured were Streptococcus pneumoniae (6 patients), Haemophilus influenzae (8 patients), and Moraxella catharralis (1 patient). No differences existed in any of the measured parameters between patients with and without positive sputum cultures.

Inflammatory markers: Fig. 1 shows the time course of the inflammatory mediators on day 0, 1, 4 and 8 during admission for an acute exacerbation of COPD and at 1

| Table 1 | Characteristics of COPD patients with acute exacerbation and healthy controls |
|---------|-----------------------------|-----------------------------|-----------------------------|
| Patients (n = 21) | Controls (n = 20) | p-Value |
| Age (yr) | 66.7 ± 9.0 | 60.6 ± 3.4 | NS |
| Sex F/M | 6/15 | 6/14 | NS |
| BMI (kg/m²) | 23.5 ± 4.7 | 25.9 ± 2.7 | NS |
| FEV₁% pred | 35.0 ± 14.4 | 108.2 ± 14.2 | 0.0001 |
| IVC % pred | 62.9 ± 22.9 | 114.8 ± 12.4 | 0.0001 |
| Smokers current/ex/none | 7/13/1 | 1/11/8 | NS |
| Pack-years | 40 ± 20 | 20 ± 15 | 0.0001 |
| Inhaled steroids Y/N | 19/2 | 0/20 | 0.0001 |
| Bacterial infection Y/N | 12/9 | NA |  |
| PaO₂ (mmHg) | 53.2 ± 10.5 | NA |  |
| PaCO₂ (mmHg) | 47.2 ± 16.5 | NA |  |

Values of patients are values on admission. Data are presented as mean ± SD.
month, 3 months and 6 months after discharge. As comparison, levels were also measured in healthy controls. The cytokine IL-6 was not detectable in the healthy control subjects with the assay used, therefore, detection limit of the assay (20 pg/ml) was indicated in the figure. On the day of admission, IL-6 was significantly enhanced in patients as compared to healthy controls \((p = 0.033)\). After one day of treatment levels dropped strongly (day 0 to day 1: \(p = 0.043\)) and were no longer different from control subjects. Surprisingly, enhanced levels of IL-6 were seen 1 month after discharge, however, this enhancement was not significant. Likewise, the sTNFR75 was enhanced at admission of exacerbation, and declined thereafter (day 0–day 1: \(p = 0.01\); day 1–day 4: \(p = 0.019\)). After 1 month of discharge, increased levels of sTNFR75 were observed (day 8 to month 1: \(p = 0.028\)), and declined thereafter (month 1–month 6: \(p = 0.013\)). Soluble TNFR55 was elevated during all time points of acute exacerbation of COPD, with exception of the day 4 time point (day 1–day 4: \(p = 0.004\)). After 1, 3 and 6 months, levels were no longer different as compared to healthy controls. The anti-inflammatory marker sIL-1RII was significantly increased on the day of admission and increased further during treatment of exacerbation (day 0–day 1: \(p = 0.031\); day 1–day 4: \(p = 0.038\)). In the recovery period sIL-1RII decreased to levels comparable to healthy controls (day 8 to 1 month: \(p = 0.004\)). Also the neutrophil marker BPI was elevated on admission (\(p = 0.0001\)), and remained elevated during and

**Fig. 1** Course of inflammatory mediators (A. IL-6, B. sTNFR75, C. sTNFR55, D. sIL-1RII, E. BPI) during and after acute exacerbation of COPD. Data shown are means ± SD. Dots represent patient data; open squares represents values from healthy controls. \(*: p<0.05\) versus healthy controls. \(\#: p<0.05\) versus value on day 0.
after recovery of exacerbation, as compared to control subjects. Highest BPI levels were seen on day 8 and lowest at 3 months after exacerbation (day 8 to month 3: p = 0.01). Anti-oxidant capacity: The change in levels of systemic anti-oxidant TEAC during treatment of acute exacerbation and after recovery is shown in Fig. 2. There was a significant increase in TEAC levels during treatment of acute exacerbation, compared to admission values (day 0–day 8: p = 0.003). After discharge, levels decreased significantly (month 3–month 6: p = 0.017).

Discussion

This study showed elevated levels of systemic inflammatory markers IL-6, sTNFR55, sTNFR75, and sIL-1RII as well as neutrophil protein BPI, on admission for acute exacerbation of COPD. During treatment, there was a rapid decrease in IL-6, and sTNFR75, whereas in contrast sTNFR55 and BPI remained elevated. Moreover, sIL-1RII and TEAC increased during first 8 days of treatment. In the stable condition, inflammatory markers returned to values comparable to healthy controls, with the exception of BPI, which remained persistently elevated compared to healthy controls.

Growing evidence exists about the upregulation of systemic inflammation, as manifested by an elevated acute phase response, as part of the underlying pathogenetic processes ongoing during acute exacerbations. In this study, elevated levels of IL-6 were found on admission for an acute exacerbation, compared to healthy controls. During recovery and follow-up, IL-6 is in the same range as in healthy controls, suggesting a short flare-up of systemic inflammation at the beginning of the exacerbation. IL-6 is the most important cytokine in the hepatic synthesis of acute phase proteins. Moreover, IL-6 is important in regulating levels of fibrinogen, the precursor to fibrin in the coagulation cascade. Elevated fibrinogen levels and IL-6 are associated with increased cardiovascular mortality and morbidity. It has already been demonstrated that during acute exacerbation levels of fibrinogen and IL-6 rise further. In this way, increased systemic inflammation can predispose to cardiovascular events.

TNFα is a pro-inflammatory cytokine, which has been found in increased levels in airways and circulation of COPD patients. TNFα mediates intracellular signaling via two receptors: a 55 kDa receptor (TNFR55) and a 75 kDa receptor (TNFR75). A variety of inflammatory stimuli, including endogenous TNFα formation, is known to induce proteolytic shedding of the extracellular cytokine-binding domains of the TNF receptors. Therefore soluble TNF receptors are considered as pro-inflammatory markers. Earlier studies of our group have shown an increase in soluble TNF receptors during treatment of acute exacerbation. In this study we found that on admission for acute exacerbation, before the start of treatment, levels of both sTNF receptors were elevated. During treatment sTNFR75 levels rapidly decreased whereas sTNFR55 remained elevated. One, 3 and 6 months after acute exacerbation, levels of both TNF receptors were not different from healthy controls.

Besides monitoring of the changes in inflammatory mediators to predict diagnosis, outcome and prognosis of acute exacerbations, further studies are needed to integrate these findings in order to better understand ongoing processes during exacerbations of COPD. Acute phase responses are involved in activation of the classical complement pathway. Biochemical markers as CRP, IL-6 and TNF-α are generally considered as biochemical markers of endothelial dysfunction; particularly CRP represents one of the strongest independent predictors of vascular morbidity and mortality. The role of systemic inflammation to shift the hemostatic balance in favor of the activation of coagulation during acute exacerbations of COPD remains largely unexplored.

IL-1 is a pro-inflammatory cytokine, and has two receptors: type I receptor which mediates cellular activation and type II receptor which acts as a decoy receptor. Both IL-1 receptors are present in soluble forms and the soluble receptors inactivate sIL-1. In patients with stable COPD normal levels of sIL-1RII have been found, which increase during treatment of acute exacerbations, indicating that an upregulation of anti-inflammatory cytokines is present during treatment of COPD exacerbations. In this study we have confirmed and expanded those findings. We found elevated levels of sIL-1 RII on admission for acute exacerbation.
exacerbation, before start of treatment. These levels increased further during treatment. This supports the hypothesis that recovery of acute exacerbations is associated with an increase in anti-inflammatory capacity. After a follow-up period of 1, 3 and 6 months, sIL-1RII levels are comparable to healthy controls. Future studies should include the course of other anti-inflammatory mediators such as IL-10 in the course of acute exacerbation. Modulation of anti-inflammatory effects can contribute to fasten the recovery after acute exacerbations.

BPI is stored within the azurophilic granules of neutrophils and can be released upon direct stimulation of the neutrophil by bacterial endotoxins, but also by inflammatory stimuli like TNF-α. Therefore it can be used as a marker for neutrophilic activation. So far no data exist on BPI levels in COPD patients. We demonstrated increased systemic levels of BPI in COPD patients with an acute exacerbation in comparison to healthy control subjects. These data suggest ongoing neutrophilic activation in COPD patients, in clinically stable patients and during acute exacerbations. This is in line with other reports indicating that recruitment and activation of neutrophils is an important event in the pathogenesis of COPD. Elevated levels of serum MPO and serum ECP have been found during exacerbations indicating neutrophilic and eosinophilic activation. Noguera et al. reported that circulating neutrophils from COPD patients produced more reactive oxygen species and the level of expression of several surface adhesion molecules in circulating neutrophils is higher in stable COPD patients than in healthy controls. Interestingly, this difference disappeared during acute exacerbations, suggesting neutrophil sequestration in the pulmonary circulation. A recent study found activated neutrophils in the circulation of stable COPD patients, which correlated with disease severity.

The question is whether and to what extent the course of inflammatory parameters was influenced by the anti-inflammatory treatment our patients received. Corticosteroids are potent immunosuppressive agents that are known to affect T-cell-mediated inflammation by the inhibition of proliferation and cytokine production, as well as the immuno-stimulatory function of monocytes and macrophages. Moreover, in vitro experiments have shown that exposure of cells to the steroid analogue dexamethasone resulted in enhanced membrane expression of IL-1R11 followed by augmented release of the receptor over a period of 18–24 h. Treatment with systemic steroids thus may be partially responsible for the course of systemic inflammatory mediators that we have observed after admission. In line herewith, studies in cardiac surgery patients have shown a decrease in circulating levels of TNF and IL-6 after treatment with corticosteroids. Since our patients did not receive corticosteroids before admission, the enhanced levels of inflammatory parameters observed on day 0 cannot be attributed to treatment effects. Steroid treatment of patients was, according to a standard schedule, tapered after 4 days during a period of 15 days. Interpretation of the course of systemic inflammatory parameters in the recovery period is therefore also most likely not influenced by steroid therapy.

A number of studies have demonstrated an increased oxidative stress in COPD patients, especially during exacerbations. Our study demonstrates an increase in total anti-oxidant capacity during treatment of acute exacerbation, suggesting that improvement in antioxidant capacity may be a contributing factor in recovery from acute exacerbations.

These results were found in a group of patients with severe COPD, admitted for an acute exacerbation, which remained stable during 6 months of follow-up. We realize this is probably a selected population, since patients with exacerbations in the follow-up period and patients with cardiovascular co-morbidity were excluded. Therefore these results are probably not generally applicable in all populations of COPD patients. Otherwise, our results after the 6-month follow-up period clearly illustrate the importance of careful assessment and monitoring of the clinical condition in the evaluation of inflammatory systemic mediators. This is particularly important when systemic inflammation will be evaluated as part of the phenotyping of COPD.

In conclusion, this study clearly demonstrated upregulation of systemic inflammation in acute exacerbations of COPD. During recovery pro-inflammatory markers declined whereas levels of sIL-1RII and TEAC increased. In the stable, state inflammatory markers were no longer enhanced with the exception of neutrophil marker BPI. Attenuation of systemic inflammation may offer new perspectives in the management of COPD patients to reduce the burden of exacerbations.

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