Clinical characteristics of eosinophilic asthma exacerbations

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ABSTRACT

Background and objective: Airway eosinophilia is associated with an increased risk of asthma exacerbations; however, the impact on the severity of exacerbations is largely unknown. We describe the sputum inflammatory phenotype during asthma exacerbation and correlate it with severity and treatment response.

Methods: Patients presenting to hospital with an asthma exacerbation were recruited during a 12-month period and followed up after 4 weeks. Induced sputum was collected at both visits. Patients underwent spirometry, arterial blood gas analysis, fractional exhaled nitric oxide analysis, white blood cell counts and a screening for common respiratory viruses and bacteria.

Results: A total of 47 patients were enrolled; 37 (79%) had successful sputum induction at baseline, of whom 43% had sputum eosinophils ≥3% (EE). Patients with EE had a significantly lower forced expiratory volume in 1 s (FEV1) % predicted (70.8%, P = 0.03) than patients with NEE (83.6%). Furthermore, EE patients were more likely to require supplemental oxygen during admission (63% vs 14%, P = 0.002). The prevalence of respiratory viruses was the same in EE and NEE patients (44% vs 52%, P = 0.60), as was bacterial infection (6% vs 14%, P = 0.44). Fractional expiratory nitric oxide (FeNO) correlated with sputum %-eosinophils (ρ = 0.57, P < 0.001), and predicted airway eosinophilia with a sensitivity of 86% and a specificity of 70%.

Conclusion: Our findings suggest that eosinophilic asthma exacerbations may be clinically more severe than NEEs, supporting the identification of these higher risk patients for specific interventions.

SUMMARY AT A GLANCE

Patients with airway eosinophilia during an exacerbation of asthma had lower forced expiratory volume in 1 s (FEV1) and were more likely to require supplemental oxygen during admission than those without eosinophilia. This suggests that eosinophilic asthma exacerbations are more severe than non-eosinophilic exacerbations.

Abbreviations: ACQ, Asthma Control Questionnaire; ATS, American Thoracic Society; AUC, area under the curve; BMI, body mass index; COPD, chronic obstructive pulmonary disease; ED, emergency department; EE, eosinophilic exacerbation; ERS, European Respiratory Society; FeNO, fractional expiratory nitric oxide; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; GM, geometric mean; ICS, inhaled corticosteroid; Ig, immunoglobulin; IL, interleukin; NEE, non-eosinophilic exacerbation; pCO2, partial pressure of carbon dioxide; PCR, polymerase chain reaction; PEF, peak flow; pO2, partial pressure of oxygen; ROC, receiver operating characteristic; RT, reverse transcriptase; VAS, visual analog scale.

INTRODUCTION

Asthma is a chronic inflammatory disease of the airways, with variable airflow obstruction and episodic exacerbations.1 While the inflammatory phenotype of stable asthmatic patients has been well described,2 the inflammatory phenotype during an exacerbation has been much less examined. Infections naturally alter the cellular composition of sputum but the relationship between respiratory pathogens and sputum phenotype during naturally occurring exacerbations has not been reported.

Asthma patients with sputum eosinophilia during stable disease have severe airflow obstruction despite optimal treatment1,3,4 and more frequent exacerbations.5 However, it remains largely unknown whether the airway inflammation phenotype during asthma exacerbations is associated with symptoms and lung function. If eosinophilic asthma is indeed associated with both more frequent and also more severe exacerbations, it would further highlight the need for preventive...
measures in this group, including new therapeutic options, such as anti-IL-5 treatment.

Therefore, the primary aim of the study was to evaluate whether patients presenting with airway eosinophilia during an asthma exacerbation have more pronounced bronchoconstriction than patients without eosinophilia.

METHODS

Study design

The study was conducted at the Bispebjerg University Hospital in Copenhagen, Denmark. Patients between 16 and 45 years of age, presenting with acute asthma at the emergency department (ED), were recruited during a 12-month period beginning July 2013. Patients with pulmonary disease other than asthma (i.e. COPD) were excluded.

The main outcome was forced expiratory volume in 1 s (FEV1) at exacerbation and follow-up. Secondary outcomes included other markers of severity (peakflow, arterial blood gas analyses, etc.) as well as the Asthma Control Questionnaire (ACQ). Furthermore, we wanted to evaluate the use of fractional expiratory nitric oxide (FeNO) and blood eosinophils as markers of the eosinophilic phenotype during acute asthma. Finally, we wished to determine the role of respiratory pathogens on the inflammatory phenotype during an asthma exacerbation.

Participants were examined within 24 h of admission and again after 4 weeks in the research unit at the hospital, regardless of their clinical status. On admission, all patients were treated with salbutamol/ipratropium and given 80 mg methylprednisolone. The study was approved by the local scientific ethics committee (protocol nr H-2-2013-046). All subjects gave informed consent.

Markers of airway inflammation

Sputum was induced according to the European Respiratory Society (ERS) guidelines and processed as described by Pavord et al.6 Cell counts were obtained from 400 non-squamous cells. A cut-off value of 3% was used to classify patients as having either an eosinophilic exacerbation (EE) or a non-eosinophilic exacerbation (NEE).7

FeNO was analysed on a NioxMinor (Aerocrine, Solna, Sweden) following the recommendations of ERS/American Thoracic Society (ATS)8 using the mean of two measurements, and applying the ATS-defined lower cut-off value of 25 ppb. Blood samples were taken in the ED shortly after admission including a full white blood cell differential count and measurement of total IgE by the hospital laboratory.

Assessment of exacerbation severity

Peakflow and peripheral saturation were measured before any treatments were given. An arterial blood gas was collected in the course of the initial stabilization of subjects in the ED, during which time oxygen was administered if required. Spirometry was performed according to the ERS recommendations, within 24 h of admission, using a daily-calibrated Jaeger spirometer (CareFusion, San Diego, CA, USA). Asthma control was assessed with the ACQ.

Detection of respiratory pathogens

Nasal secretion was collected using a flocked swab (Copan, Brescia, Italy) and tested using a tandem multiplex real-time PCR assay to detect a comprehensive range of respiratory viruses (human adenovirus species B-D; human bocavirus; coronaviruses OC43, 229E, HKU1 and NL63; influenza viruses A, B and C; parainfluenzaviruses 1–4; KI and WU polyomaviruses; respiratory syncytial virus types A and B and human metapneumovirus).9,10 Rhinoviruses were detected with a separate reverse transcriptase (RT)-PCR.11 Bacterial cultures on spontaneous expectorate, gathered at exacerbation, were assessed by the hospital laboratory.

Statistical analysis

Data were analysed with SPSS version 22.0 (IBM, Armonk, New York, USA). Normally distributed data are reported as mean ± SD and analysed using student’s t-test for continuous variables and chi-square test for categorical variables. Measurements of FeNO were normally distributed after log transformation, and are reported as geometric mean (GM) with 95% CI. Cell counts, pack-years of smoking and usage of inhaled corticosteroids (ICSs) are reported as median (range) and comparisons are made using Mann-Whitney test for unpaired analyses, Wilcoxon signed-rank test for paired analyses and Spearman’s $\rho$ for correlations.

To assess the ability of FeNO and blood eosinophils to predict sputum eosinophils ≥3% at exacerbation, multivariable logistic regression analyses were performed correcting for age, pack-years and daily dose of ICS. A receiver operating characteristic (ROC) curve was constructed for both variables and the overall accuracy of the test was measured as the area under the ROC curve (AUC).12 For all analyses, a $P$-value of <0.05 was considered significant.

RESULTS

Patient characteristics and sputum phenotyping

During the study period, a total of 271 patients were admitted with acute asthma to ED, of whom 141 were of 16–45 years. A total of 47 patients agreed to participate. The main reason given for declining to participate was the logistics of having to come back for the follow-up visit. Of these, 37 (79%) had a successful sputum induction and were included in the exacerbation analyses. At follow-up, 7 patients had dropped out, and of the remaining 40 patients, 34 (85%) had a successful sputum induction. A total of 26 patients (55%) had a sputum cell differential count at both visits, and were used for the follow-up analyses.

The characteristics of the study subjects are shown in Table 1. At the time of exacerbation, 16 (43%) had
Blood eosinophils (×10^9 cells/L) 6.58 (0.47) 0.24 (0.42) 0.10
Blood neutrophils (×10^9 cells/L) 7.36 (3.19) 0.50

Exacerbation severity
As spirometry with reversibility was done during admission, beta-2 agonists could not be withheld if patients required them. Time from admission to spirometry was not different between the EE and NEE groups (Tables 1 and 2).

Respiratory pathogens
A respiratory virus was detected in 22 (47%) patients at the time of exacerbation, of whom 20 had rhinovirus (91%) and 2 (9%) had human coronavirus. The prevalence of detectable respiratory viruses was the same in EE (44%, P = 0.60) and NEE patients (52%). Furthermore, the percentage of eosinophils and neutrophils was similar in patients with and without viral infection (P = 0.46 and 0.20, respectively).

Thirty-six patients (77%) were able to produce spontaneous sputum for bacterial culture. Six patients had a positive culture with either Haemophilus influenzae (n = 2), Serratia marcescens (n = 1), Staphylococcus aureus (n = 1) or Streptococcus pneumoniae (n = 2). The prevalence of bacterial infection was not different between the groups (EE: 1/16 vs NEE: 3/21, P = 0.44).

Excluding patients with bacterial infection did not change any of the conclusions of this study.
Table 3  Characteristics of EE and NEE patients at exacerbation and follow-up after 4 weeks

|                      | Exacerbation |                      | 4-Week follow-up |                      |                      |                      |                      |                      |
|----------------------|--------------|----------------------|------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
|                      | EE (n = 16)  | NEE (n = 21)        | **P-value**      | EE (n = 12)          | NEE (n = 14)         | **P-value**          | EE (n = 12)          | NEE (n = 14)         | **P-value**          |
| ACQ                  | 3.0 ± 1.2    | 3.7 ± 1.2           | 0.11             | 0.7 ± 0.8            | 1.7 ± 1.2            | 0.03                 | <0.001              | <0.001              | 0.002               |
| FeNO, GM (95% CI)    | 60 (40–90)   | 22 (17–30)          | <0.001           | 35 (23–53)           | 14 (9–23)            | 0.007                | <0.001              | 0.03                | 0.03                |
| ICS budesonide       | 0 (0–1600)   | 0 (0–2400)          | 0.94             | 1200 (0–1600)        | 800 (800–4000)       | 0.87                 | 0.01                | 0.001               |                      |
| % on ICS             | 37.5         | 33.3                | 0.71             | 83.3                | 100.0                | 0.12                 | 0.01                | 0.002               |                      |
| FEV1 (L)             | 2.78 (0.86)  | 2.90 (0.61)         | 0.62             | 3.25 (1.09)          | 3.17 (0.62)          | 0.80                 | 0.03                | 0.01                | 0.01                |
| FEV1 % predicted     | 70.8 ± 18.9  | 83.6 ± 15.2         | 0.03             | 84.2 ± 17.3          | 89.9 ± 12.8          | 0.35                 | 0.04                | 0.007               |                      |
| FVC (L)              | 3.83 (1.10)  | 3.61 (0.77)         | 0.50             | 4.11 (1.23)          | 3.84 (0.78)          | 0.49                 | 0.01                | 0.04                |                      |
| FVC % predicted      | 82.0 ± 17.5  | 87.8 ± 15.8         | 0.31             | 90.0 ± 17.3          | 92.0 ± 13.5          | 0.74                 | 0.02                | 0.05                |                      |
| FEV1/FVC ratio       | 0.75 ± 0.11  | 0.81 ± 0.07         | 0.07             | 0.91 ± 0.20          | 0.89 ± 0.10          | 0.77                 | 0.03                | 0.01                |                      |
| % Reversibility to beta-2 agonist | 13.9 ± 9.8 | 6.6 ± 5.9 | 0.02 | | | | | | |
| Sputum total cell count (∗10⁶/mL) | 0.36 (0.05–3.65) | 0.87 (0.02–7.87) | 0.13 | 0.52 (0.17–8.44) | 0.84 (0.41–3.76) | 0.07 | 0.37 | 0.65 | |
| Sputum eosinophils (%) | 20.1 (3.5–59.8) | 0 (0–2.8) | <0.001 | 5.5 (0.3–69.8) | 0 (0–58.0) | 0.007 | 0.16 | 0.11 | |
| Sputum neutrophils (%) | 48.2 (7.8–78.5) | 68.8 (15.9–93.3) | 0.21 | 25.2 (0.72–0.0) | 46.8 (3.0–19.1) | 0.12 | 0.39 | 0.31 | |

1Two patients had stopped taking their prescribed ICS (without consulting with the study doctor) because of relief of symptoms.

ACQ, Asthma Control Questionnaire; CI, confidence interval; EE, eosinophilic exacerbation; FeNO, fractional expiratory nitric oxide; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; GM, geometric mean; ICS, inhaled corticosteroid; NEE, non-eosinophilic exacerbation.

Treatment response at 4-week follow-up

At exacerbation, 14 of the 16 patients in the EE group and 18 of the 21 patients in the NEE group were prescribed 37.5 mg oral prednisolone, to be taken once daily for 10 days (ns). At follow-up, seven EE patients (58%) and four NEE patients (29%) had sputum eosinophils ≥3%. No patients had to be readmitted, or re-visit their GP, during the observation period.

At follow-up, the NEE patients had poorer asthma control than EE patients (ACQ score: 1.7 vs 0.7, P = 0.03) despite being on a similar ICS dose (Table 2).

Between exacerbation and follow-up, FEV1 improved in both the EE (70.8 ± 18.9 to 84.2 ± 17.3, P = 0.04) and NEE group (83.6 ± 15.2 to 89.9 ± 12.8, P = 0.007). At follow-up, FEV1 was similar between the two groups (P = 0.35).

In both the EE and NEE groups, total blood leukocytes decreased from exacerbation to follow-up (EE: 10.0 to 6.5 × 10⁹ cells/L, P = 0.01; NEE: 9.1 to 6.5 × 10⁹ cells/L, P = 0.002), driven by a decrease in neutrophils (EE: 6.2 to 2.8 × 10⁹ cells/L, P = 0.005; NEE: 6.6 to 3.7 × 10⁹ cells/L, P = 0.005), while blood eosinophils did not change (EE: 0.4 to 0.5 × 10⁹ cells/L, P = 0.39; NEE: 0.1 to 0.1 × 10⁹ cells/L, P = 0.84).

Biomarkers of airway eosinophilia at the time of exacerbation

FeNO was highest in the EE patients (60 ppb, 95% CI: 40–90 vs 22 ppb, 95% CI: 17–30, P < 0.001) (Table 2), and FeNO correlated with sputum %eosinophils (ρ = 0.57, P < 0.001). A multivariable logistic regression analysis revealed that FeNO predicted the presence of sputum eosinophilia ≥3% in a model correcting for age, pack-years of smoking and dose of ICS (R² = 0.49, P = 0.002).

In an ROC curve for FeNO to predict EE, the AUC was 0.84 (95% CI: 0.70–0.98, P = 0.001) for the whole population. Using a cut-off value of 25 ppb, the sensitivity was 86% and the specificity was 70% to detect EE. At exacerbation, patients with FeNO >25 ppb had lower FEV1 (72.6 ± 17.7, P = 0.03) than patients with FeNO <25 ppb (85.2 ± 15.8).

Blood eosinophils also correlated with sputum %eosinophils (ρ = 0.41, P = 0.03), but were less predictive than FeNO of sputum eosinophilia (R² = 0.22, P = 0.09) when adjusting for confounders as described above. The ROC curve for blood eosinophils to predict EE had an AUC of 0.69 (95% CI: 0.48–0.91, P = 0.09).

Both FeNO and blood eosinophils showed similar predictive abilities at the follow-up visit (data not shown).

DISCUSSION

In this study, we have demonstrated that eosinophilic airway inflammation during an acute asthma exacerbation is associated with lower FEV1 measured within 24 h of admission to hospital and a higher requirement for oxygen supplementation. Furthermore, a significant proportion of patients with airway eosinophilia at the time of exacerbation continued to have elevated airway inflammation during the follow-up period.
Eosinophilic asthma exacerbations

Eosinophils at the 4-week follow-up, despite being treated with systemic steroids. These findings suggest that eosinophilic asthma exacerbations may be clinically more severe than NEEs, and that eosinophilic inflammation may persist despite standard anti-inflammatory treatment.

More pronounced bronchoconstriction in patients with sputum eosinophilia during exacerbation, compared with those without, has been described by Turner et al. (FEV1: 70% vs 88%, P = 0.01). A similar study by Di Franco et al. showed that the increase in FEV1 from exacerbation to recovery correlated with a decrease in sputum eosinophils, and that unstable patients at follow-up still had high sputum eosinophils, despite treatment.

Peripheral oxygen saturation and arterial pH were within the normal range for most patients in both groups, but was slightly lower in the EE group, and these patients were more likely to require supplemental oxygen during their admission to hospital. At admission, however, respiration frequency was not different between the groups, and neither were the partial pressures of oxygen and carbon dioxide. Eosinophilic airway inflammation is associated with more pronounced airway smooth muscle hypertrophy, and severe asthma in general is known to be associated with pronounced structural changes in the peripheral airways. Studies of fatal or near-fatal asthma have indicated that increased inflammation during exacerbations aggravates this chronic state, causing pronounced mucus oedema, and lumen occlusion by mucus plugs, in a setting of predominantly eosinophilic infiltration. In light of this, even subtle changes in oxygenation could represent differences in the pathology involved and prove to be of prognostic value, but further studies are required to investigate this.

One of the main limitations of this study is the sample size prohibiting elaborate subgroup analyses. With a relatively low participation rate, there is a risk of introducing a selection bias, however, the reasons given for not wanting to participate in the study were not related to asthma severity, and are unlikely to be specific to patients with a certain sputum inflammatory phenotype. Several studies have shown that while FEV1 improves upon initial treatment in hospital, it does not fully recover to the pre-exacerbation levels until 7–14 days, even when patients are receiving appropriate treatment, and therefore measurements of FEV1 within 24–48 h of admission are commonly used in studies of acute asthma. While used in previous studies of acute asthma, we acknowledge that measuring FEV1 during admission reflects an interaction between severity and response to initial treatment; however, in the subset of patients with documented PEF on arrival this was highly correlated to subsequent FEV1. The strength of this study is that sputum was collected together with clinical information, inflammatory markers and samples for detection of respiratory pathogens, at both exacerbation and recovery in a heterogeneous, real-life population.

Eosinophilic airway inflammation, during stable disease, is a predictor of steroid response, and adjusting treatment based on sputum cell counts reduces the frequency and severity of exacerbations, without increasing the overall ICS dose. In this study, ACQ at follow-up was highest in the non-eosinophilic patients, despite similar FEV1 between the groups. This could indicate that treatment response, at least regarding symptoms following an exacerbation, is also lower in the non-eosinophilic group. It could also be that symptoms other than wheeze (e.g. coughing), captured by the ACQ but not spirometry, are more prominent in the non-eosinophilic patients.

In this study, FeNO and blood eosinophils were able to predict sputum eosinophils ≥3% during exacerbation, with correlations similar to previous studies in stable asthma. A large proportion of patients had persistent eosinophilia at follow-up. While it could be due to insufficient treatment, all but five patients were on medium or high doses of ICS. The patients with eosinophilia at follow-up could represent an underlying phenotype with an increased risk of future exacerbations, however, to clarify if this is the case, or eosinophilia is the result of a prolonged response to exacerbation, requires a study with assessment of the inflammatory profile in stable phase and then subsequently during an exacerbation.

Activated eosinophils are thought to play a direct role in tissue remodelling and anti-IL-5 reduces the rate of exacerbations by upwards of 50% in selected asthmatic patients with substantial eosinophilia and frequent exacerbations. Such treatment might also attenuate exacerbation severity in patients with a predominantly eosinophilic inflammatory response – in our study 41% of patients. This however remains to be explored.

In conclusion, FEV1, during admission was significantly lower in patients with sputum eosinophilia during an acute exacerbation compared with patients with non-eosinophilic inflammation. Our findings add to the limited data available on eosinophilia during acute asthma, and suggest that the new biological treatment options that are becoming available may have an attenuating effect on exacerbations. Whether evaluating the presence of airway eosinophilia during exacerbations, either directly or using biomarkers such as FeNO, will prove useful in the clinical setting will have to be evaluated in larger multicentre trials.

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