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RESEARCH ARTICLE

Identification of exacerbations in obstructive lung disease through biomarkers

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Abstract

Inflammation has been identified as an important factor for disease exacerbation in obstructive lung disease. In this study, we used neutrophil and eosinophil counts as biomarkers for exacerbation in obstructive lung disease. We conducted a case–control study within a cohort of patients frequenting an outpatient clinic of Respiratory Medicine using data from the Utrecht Patient Oriented Database (UPOD). Cases were patients with a hospital admission for obstructive lung disease in 2005. For each case, one control patient was sampled from the same study base. We identified 143 cases (118 patients with chronic obstructive pulmonary disease and 25 asthma patients) and 143 controls. Admission was associated with both neutrophilia (adjusted odds ratio (OR) 4.3; 95% confidence interval (CI) 2.2–8.5), and eosinophilia (adjusted OR 2.6; 95% CI 1.1–6.2). The association with eosinophilia was only seen in asthma patients. In conclusion, neutrophil and eosinophil counts seem to be useful biomarkers for identifying exacerbations in pharmacoepidemiological studies on obstructive lung disease.

Keywords: *Eosinophil; exacerbation; hospital admission; molecular epidemiology; neutrophil; obstructive lung disease*

Introduction

Worldwide, obstructive lung diseases are leading causes of morbidity and mortality (GINA 2008, GOLD 2008). Bronchodilators and inhaled glucocorticoids are central in the treatment of obstructive lung disease. However, response to pharmacotherapy varies and a minority of these patients continue to have symptoms and exacerbations in spite of being treated according to guidelines (GINA 2008, GOLD 2008, Sutherland 2004). While various factors (e.g. environment, genetic factors, lifestyle) can contribute to exacerbation in obstructive lung disease (Andenaes & Kalfoss 2004, Baibergenova et al. 2005), so far no valid biomarkers have been made available to identify such exacerbations in pharmacoepidemiological studies.

Exacerbations in obstructive lung disease can be subdivided in difficult-to-treat asthma and non-respondent chronic obstructive pulmonary disease (COPD) and have been studied in pharmacoepidemiology so far mainly through identifying short courses of glucocorticoids and hospital admissions (Blais et al. 1998, Roede et al. 2008, Van Ganse et al. 1995). Although hospital admissions are widely acknowledged as a useful parameter for this purpose, they also carry a number of limitations, e.g. indication for hospital admission may vary significantly in different populations and countries, a trend towards outpatient care, underestimation of

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disease exacerbations not leading to a hospital admission (Boyter & Steinke 2005, Roede et al. 2008, Van Ganse et al. 1995).

Molecular epidemiology and biomarkers are gaining importance in this research field (Cho & Kim 2009, Franciosi et al. 2006, Mahajan et al. 2008, Velthove et al. 2009, Wild et al. 2008). Neutrophils and eosinophils play an important role in many of the inflammatory processes in obstructive lung diseases and are important factors for disease exacerbations in obstructive lung disease, also seen in many other studies (Chung et al. 1999, Jatakanon et al. 1999, Pauwels 2004, Wenzel et al. 2000). However, these studies mostly deal with invasive methods such as lung biopsies or induced sputum. Measuring inflammation parameters in peripheral blood would provide a simpler and more easily accessible biomarker.

In this study we investigated the possible association between neutrophils and eosinophils and hospital admission for disease exacerbation in a cohort of patients visiting an outpatient clinic of the Respiratory Medicine Department on a regular basis.

Materials and methods

Setting

Data were obtained from the Utrecht Patient Oriented Database (UPOD). UPOD is an infrastructure of relational databases comprising administrative data on patient characteristics, laboratory test results, medication orders, discharge diagnoses and medical procedures for all patients treated at the University Medical Centre Utrecht, a 1042-bed tertiary teaching hospital in the centre of the Netherlands. All UPOD research is in accordance with current Dutch privacy and ethical regulation. A more complete description of UPOD has been published elsewhere (Ten Berg et al. 2007).

Study population

From UPOD, we identified all adult (aged ≥18 years) patients, who were treated in the outpatient clinic of the Respiratory Medicine Department ($n = 3088$). Of those patients, 903 had at least one haematological blood test for any clinical reason in 2005 and had no history of chemotherapy.

Hospital admission was used as an outcome measure for exacerbation in this study. Within the ICD-9-CM code group of diseases of the respiratory system (ICD-9-CM codes 460-519), admissions for otorhinolaryngological diseases (ICD-9-CM codes 460-478), (non-chronic) infectious disorders (ICD-9-CM codes 480-488, 490, 494 and 510-519) and respiratory diseases due to external agents (ICD-9-CM codes 495, 500-508) were excluded. Therefore, cases were defined as patients with an admission for obstructive lung disease (ICD-9-CM code 491, 492, 493 and 496). The date of the first admission in 2005 was defined as the index date. During this admission, the first blood measurement during admission was included in the analyses.

For each case, one control patient was sampled from the same study base. Controls had a blood test requested by the outpatient department of Respiratory Medicine within a period of 1 month around the index date of a case patient, and had no history of hospital admissions for obstructive lung disease. This control patient could have asthma, COPD or respiratory-related diagnoses other than asthma or COPD. For all patients, the diagnosis was retrieved from the clinical records.

Exposure and covariate assessment

Cases and controls were compared with respect to absolute eosinophil and neutrophil counts. The absolute eosinophil and neutrophil count, on the one hand, and hospital admission, on the other, are associated through a non-linear relationship. Therefore, the absolute count was dichotomised according to the upper limit of our laboratory references, using our lab references of $1.6–8.3 \times 10^9$ l^{-1} for neutrophils and $<0.4 \times 10^9$ l^{-1} for eosinophils.

Data on outpatient medication use at the time of the blood test was retrieved from the clinical records of both cases and controls. Glucocorticoid use at time of the blood test could be chronic use or a short course and daily dose exposure was expressed as nasal and inhaled beclomethasone equivalents or systemic prednisone equivalents, using defined daily dosages (Anonymous 2007).

For each patient the lung function at the time of the blood test was retrieved from the Department of Respiratory Medicine. We used forced expiratory volume in 1 s (FEV_1), peak expiratory flow (PEF), and the FEV_1/forced vital capacity (FVC) ratio as indication parameters for the lung function (GINA 2008, GOLD 2008).

Statistical analysis

Mann–Whitney tests and χ^2 tests were used as appropriate. Inhaled beclomethasone equivalents, systemic prednisone equivalents and the lung function measurements were categorised into tertiles, and missing equivalents were grouped in a separate category. Nasal beclomethasone equivalents were categorised into two groups for known equivalents and a third for missing doses. Unconditional multivariate logistic regression analysis was used to estimate the strength of the association between eosinophil and neutrophil counts and hospital admission in separate models, expressed as odds ratios (ORs) with 95% confidence intervals (CIs).

Results

A total of 143 (4.6%) patients from the study cohort could be qualified as cases, including 118 patients with COPD and 25 asthma patients; in addition 143 controls were sampled from the study base. As shown in Table 1, the gender distribution was equal, but cases were older compared with controls. The lung function parameters FEV_1, PEF and the FEV_1/FVC ratio for cases were lower compared with controls ($p < 0.001$; Table 1). Also, cases used β_2-agonists and glucocorticoids more frequently at the time of blood sampling compared with controls.

The distributions of the absolute neutrophil and eosinophil counts are depicted in Figure 1A and B. No cases and one control had neutropenia ($<1.6 \times 10^9$ l^{-1}). As shown in Table 2, 19 (13.3%) cases had eosinophilia and 73 (51.0%) had neutrophilia. Of all controls, 15 (10.5%) patients had eosinophilia and 21 (14.7%) had neutrophilia, respectively. Overall, Table 2 shows that eosinophilia was associated with a more than twofold increased risk and neutrophilia was associated with a fourfold increased risk of hospital admission. Stratifying on diagnosis, neutrophilic COPD cases had a fourfold increased risk of admission, and admission was not associated with eosinophilia for COPD patients. With regard to asthma cases, admission was associated with eosinophilia (adjusted OR 15.3, 95% CI 3.9–60.0) and with neutrophilia (adjusted OR 5.9, 95% CI 2.0–17.5).

In clinical practice, positive predictive values (PPV) and negative predictive values (NPV) are valuable as these indicate the risk of hospital admission for patients with neutrophilia or eosinophilia. Overall, neutrophilia had a PPV of 77.7%. After stratification on diagnosis, similar results were found for COPD cases (PPV = 74.1%). For asthma patients the neutrophilia NPV (93.1%) and eosinophilia NPV (90.8%) were most informative, implying that asthma patients without neutrophilia and without eosinophilia are not likely to be hospitalised.

Of all patients, 36 (25.2%) cases and 11 (7.7%) controls had immature granulocytes. Subgroup analyses, including only patients without immature granulocytes, showed an adjusted OR of 3.5 (95% CI 1.6–7.6) including only patients without immature granulocytes compared with an adjusted OR of 4.3 (95% CI 2.2–8.5, Table 2) including all cases and controls. Nine of the 143 (6.3%) cases used glucocorticoids during admission before the

Table 1. Basic characteristics of the study population.

Characteristic	Cases(n = 143)	Controls(n = 143)	p-Value(two-sided)
Age (years), mean (SD)	65.6 (14.2)	49.9 (18.2)	<0.001[a]
Gender, *n* (%)			0.722[b]
Male	78 (54.5)	75 (52.4)	
Female	65 (45.5)	68 (47.6)	
Lung function, median (IQR)			
FEV_1 (l)	1.3 (0.9–1.9)	2.4 (1.6–3.3)	<0.001[a]
FEV_1 (% predicted)	52.6 (36.1–67.5)	79.5 (57.4–98.5)	<0.001[a]
PEF ($l\ s^{-1}$)	3.8 (2.7–6.0)	6.5 (4.7–8.5)	<0.001[a]
FEV_1/FVC (%)	57.6 (44.7–69.2)	75.2 (62.2–81.0)	<0.001[a]
Unknown	37 (25.9)	17 (11.9)	
Drug use, n (%)			
Short-acting β_2-agonist	73 (51.0)	33 (23.1)	<0.001[b]
Long-acting β_2-agonist	91 (63.6)	33 (23.1)	<0.001[b]
Nasal glucocorticoid[d]	6 (4.2)	19 (13.3)	0.006[b]
≤200	3 (50.0)	3 (15.8)	1.000[c]
>200	1 (16.7)	1 (5.3)	
Dose unknown	2 (33.3)	15 (78.9)	
Inhaled glucocorticoid[d]	108 (75.5)	52 (36.4)	<0.001[b]
<800	25 (23.1)	10 (19.2)	
800–1336	13 (12.0)	9 (17.3)	0.530[c]
≥1336	47 (43.5)	15 (28.8)	
Dose unknown	23 (21.3)	18 (34.6)	
Systemic glucocorticoid[e]	76 (53.1)	32 (22.4)	<0.001[b]
<10	17 (22.4)	8 (25.0)	
10–25	18 (23.7)	17 (53.1)	0.091[c]
≥25	27 (35.5)	4 (12.5)	
Dose unknown	14 (18.4)	3 (9.4)	

[a]Mann–Whitney test; [b]χ^2 test; [c]χ^2 test for trend analysis; [d]in μg beclomethasone equivalents; [e]in mg prednisone equivalents.
IQR, interquartile range; FEV_1, forced expiratory volume in 1 s; PEF, peak expiratory flow; FVC, forced expiratory volume in 1/s forced vital capacity ratio.

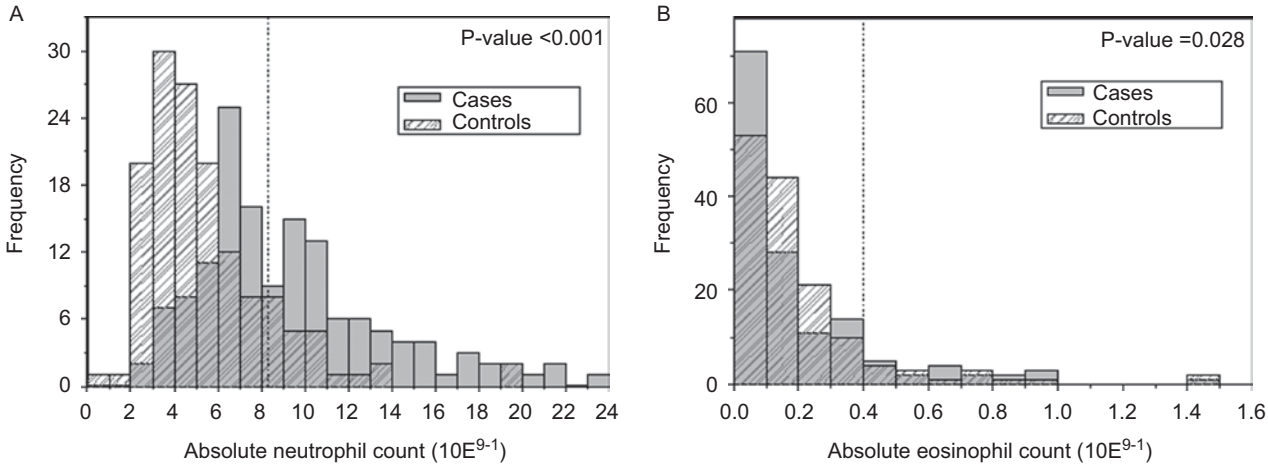

Figure 1. Distribution of neutrophil and eosinophil counts for cases and controls. Cases have an increased absolute neutrophil count compared with controls (A), with a median (interquartile range) of 8.4×10^9 l^{-1} (6.2–11.4 $\times 10^9$ l^{-1}) for cases and 4.6×10^9 l^{-1} (3.6–6.6 $\times 10^9$ l^{-1}) for controls. This was statistically significant with a *p*-value <0.001. The histogram for the eosinophil count is more similar for cases and controls (B) with median (interquartile range) of 0.10×10^9 l^{-1} (0.03–0.28 $\times 10^9$ l^{-1}) for cases and 0.13×10^9 l^{-1} (0.07–0.26 $\times 10^9$ l^{-1}) for controls. This was statistically significant with a *p*-value of 0.028. The vertical reference lines represent the upper limits of our lab references of 8.3×10^9 l^{-1} for neutrophils (A), and 0.4×10^9 l^{-1} for eosinophils (B).

Table 2. Associations between hospital admission and eosinophilia and neutrophilia.

	Cases *n* (%)	Controls *n* (%)	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
All cases	143	143		
Eosinophilia[b]	19 (13.3)	15 (10.5)	1.3 (0.6–2.7)	2.6 (1.1–6.2)
Neutrophilia[c]	73 (51.0)	21 (14.7)	6.0 (3.4–10.6)	4.3 (2.2–8.5)
COPD	118	143		
Eosinophilia[b]	10 (8.5)	15 (10.5)	0.8 (0.3–1.8)	0.9 (0.3–2.4)
Neutrophilia[c]	60 (50.8)	21 (14.7)	6.0 (3.3–10.7)	3.8 (1.8–8.0)
Asthma	25	143		
Eosinophilia[b]	9 (36.0)	15 (10.5)	4.8 (1.8–12.7)	15.3 (3.9–60.0)
Neutrophilia[c]	13 (52.0)	21 (14.7)	6.2 (2.5–15.5)	5.9 (2.0–17.5)

[a]Adjusted for age, gender, nasal glucocorticoid use, inhalation glucocorticoid use, systemic glucocorticoid use and lung function (forced expiratory volume in 1 s, peak expiratory flow, forced vital capacity); [b]eosinophilia is defined as eosinophils >0.4×10^9 l^{-1} vs ≤0.4×10^9 l^{-1} as reference range; [c]neutrophilia is defined as neutrophils >8.3×10^9 l^{-1} vs 1.6–8.3×10^9 l^{-1} as reference range. COPD, chronic obstructive pulmonary disease; OR, odds ratio; CI, confidence interval.

first blood test was conducted. However, this glucocorticoid use did not have major effects on the associations found with an adjusted OR of 4.6 (95% CI 2.3–9.3) for neutrophilia when including only pretreatment blood tests in the case group compared with an adjusted OR of 4.3 (95% CI 2.2–8.5, Table 2) including all cases and controls. For eosinophilia, the adjusted ORs were 2.7 (95% CI 1.1–6.6) and 2.6 (95% CI 1.1–6.2, Table 2), respectively. Moreover, excluding respiratory-related diagnoses other than asthma or COPD among the controls did not have major influence on the results (data not shown).

Discussion

In this study, we found an association between hospital admission and neutrophil and eosinophil counts. Stratified on diagnosis, we found a sixfold increased risk of admission for neutrophilic asthma patients with a NPV of 93.1%. The results showing that admission was associated with neutrophilia for COPD patients and that hospital admission was associated with eosinophilia for asthma patients, but not for COPD patients are reassuring as these latter results are in line with current knowledge about asthma and COPD (GINA 2008, GOLD 2008) and were the positive and negative controls in this study.

As hospital admission was associated in asthma but not in COPD patients, there seem to be pathophysiologically different phenotypes. Phenotyping is needed for understanding the molecular mechanisms of the diseases, and therefore better prediction of outcomes in patients with these phenotypes, new therapeutic innovations and for phenotype-based treatment.

Our results support the hypothesis that biomarkers, such as neutrophil and eosinophil counts, may be useful as markers for disease exacerbation. However, using hospital admission as an outcome measure for exacerbation could lead to an underestimation of exacerbation

frequency, because not all exacerbations result in admission and not all admissions are coded correctly (Blais et al. 2006, Movig et al. 2003). Timely detection and identification of exacerbations without hospital admission is warranted in pharmacoepidemiological studies of lung diseases to avoid misclassification, but also in order to study severity pathways in patients with an exacerbation without hospital admission. Also from a clinical point of view these results might be interesting. Timely identification of disease deterioration might prevent admissions for obstructive lung disease in the future. Molecular epidemiology and biomarkers are promising in this research field (Biomarkers Definitions Working Group 2001, Califf & Ginsburg 2008, Cho & Kim 2009, Valet 2006). Because of the transverse time perspective of this case–control study we cannot draw conclusions about the time relationship of the association between hospital admission and neutrophil and eosinophil counts. A replicate study should be conducted in a prospective, blinded fashion and the accuracy of the neutrophil and eosinophil counts as early exacerbation markers for early identification of exacerbations should be confirmed.

It appears from previous studies that many authors struggle with the issue of whether or not the observed neutrophilia is primarily a characteristic of asthma severity or secondary to the treatment with glucocorticoids (Douwes et al. 2002, Green et al. 2002, Jatakanon et al. 1999, Louis et al. 2000). Glucocorticoids have a complex mechanism of action (Barnes & Adcock 2003, Czock et al. 2005). These drugs reduce the absolute count of many inflammatory cells, such as T lymphocytes, mast cells and eosinophils, but neutrophils were found to be less responsive to glucocorticoids (Barnes & Adcock 2003, Jatakanon et al. 1999). Some studies have shown that glucocorticoids increase the absolute neutrophil count by inhibiting apoptosis (Czock et al. 2005, Zhang et al. 2001). However, these findings were done in *in vitro* experiments, and *in vivo* studies mainly focus on healthy volunteers and short-term effects of glucocorticoids on the neutrophil count (Chakraborty et al. 1999, Steele et al. 1987). Green et al. (2002) concluded that, in some asthma subjects at least, neutrophilia is not due to the glucocorticoid treatment. Also Louis et al. (2000) found in their study that severe asthmatics, treated with systemic glucocorticoids, had a lower absolute neutrophil count in sputum compared with severe asthmatics who did not use systemic glucocorticoids. In this study we found a strong association between hospital admission and neutrophilia for asthma patients. These results persisted after adjustment for glucocorticoid use and lung function and add to the evidence that neutrophilia among difficult-to-treat asthma patients is not solely caused by glucocorticoid treatment, but is an inflammatory characteristic of this asthma phenotype.

There are some methodological issues. First, controls sampled for this study visited the outpatient clinic of the Department of Respiratory Medicine, but their diagnosis could be different from asthma or COPD. However, excluding respiratory-related diagnoses other than asthma or COPD among controls did not have major influence on the results.

Furthermore, as UPOD is a database containing routinely collected data, there is always a clinical indication for a blood test. A possible consequence of this diagnostic suspicion bias is that it causes an over-representation of more severely ill patients. Yet, this issue applies more for controls, as admitted patients always have blood tests because of their health status. As a result, potential controls with a mild underlying disease might be underrepresented among our controls. This will lead to bias towards the null.

In conclusion, we were able to use neutrophil and eosinophil counts as a biomarker to identify exacerbation in obstructive lung disease. This shows promising possibilities in exploring the use of biomarkers in pharmacoepidemiology using routinely collected data.

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