BACKGROUND: Blood eosinophil count is a marker of eosinophilic airway inflammation and disease severity in asthma. However, blood neutrophil count might also be associated with disease severity. We tested the hypothesis that high blood eosinophil and neutrophil counts are both associated with the risk of asthma exacerbations among individuals with asthma from the general population.

METHODS: From the Copenhagen General Population Study with 81,351 participants, we included 4838 with self-reported asthma. We recorded baseline blood eosinophil and neutrophil counts, and asthma exacerbations during follow-up in 2003–2011, defined as moderate (short-course treatment of prednisolone) or severe (hospitalization).

RESULTS: The multivariable-adjusted incidence rate ratios (IRRs) were 1.28 (95% CI, 1.06–1.55) for moderate exacerbations and 1.55 (1.20–2.00) for severe exacerbations (IRRs) were 1.28 (95% CI, 1.06–1.55) for moderate eosinophil counts vs individuals with blood neutrophil counts (0.89–1.55) for severe exacerbations for individuals with blood neutrophils, the multivariable-adjusted IRRs were 2.14 (1.74–2.63) for moderate exacerbations and 1.18 (0.89–1.55) for severe exacerbations for individuals with blood neutrophil counts >3.77 × 10^9/L (lowest tertile). Blood neutrophils and neutrophil counts interacted on moderate exacerbations (P = 3 × 10^-4), but not on severe exacerbations.

CONCLUSIONS: High blood eosinophil counts are associated with an increased risk of both moderate and severe asthma exacerbations, while high blood neutrophil counts are associated with an increased risk of moderate, but not severe exacerbations.

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Sputum cell counts can distinguish between eosinophilic, neutrophilic, mixed, and paucigranulocytic inflammation in asthma (1), which are inflammatory subtypes with possibly different exacerbation risks (2). Sputum eosinophilia is found in approximately half of patients with asthma (3–6) and is associated with uncontrolled or severe disease (7) as well as frequent exacerbations (4). A high blood eosinophil count is associated with sputum eosinophilia (8) and therefore the use of blood eosinophil counts has gained ground as a marker of severe disease (9). Among asthma patients, high blood eosinophil counts have been associated with increased mortality (10), a better response to corticosteroids (11), and are used to adjust therapy with oral corticosteroids in severe asthma, resulting in improved asthma control and reduced exacerbation frequency (12). The advent of anti–interleukin-5 (anti–IL-5)7 therapy targeting eosinophilic inflammation will mean that it will be easier to identify potentially responsive patients and monitor therapy using blood eosinophil counts (13). Additionally, blood eosinophil count predicts response to omalizumab, anti-IgE therapy (14). In a recent study of 130,248 asthma patients identified from medical records in the UK, a high blood eosinophil count was associated with more severe exacerbations and poorer asthma control (15), which is in keeping with results from other observational studies from the general population that have
found high blood eosinophil counts to be associated with an increased risk of future asthma exacerbations (16, 17), and with historical self-reported asthma attacks (18, 19).

As with eosinophils, bronchial infiltration of neutrophils is a feature of asthma, which also may be driving the pathogenesis of the disease (20, 21). Few epidemiological studies have investigated associations between both blood eosinophil and neutrophil counts with lung function measurements and respiratory symptoms in the general population (22–24), but in a recent cross-sectional study of 381 individuals with asthma, neutrophil inflammation assessed in blood together with an eosinophilic inflammatory pattern was associated with specific characteristics of asthma, including a more active disease (25). Although the correlation between sputum and blood neutrophil counts is weak (26) there is evidence to suggest that both blood eosinophil and blood neutrophil counts are associated with respiratory symptoms in asthma patients and therefore they might both influence the risk of exacerbations.

We hypothesized that high blood eosinophil and blood neutrophil counts are associated with an increased risk of moderate and severe asthma exacerbations. We included 4838 individuals with self-reported asthma selected from 81351 individuals from the general population, and prospectively assessed the risk of exacerbations according to blood eosinophil and neutrophil counts as well as interactions of these 2 inflammatory patterns.

**Methods**

**STUDY POPULATION**

We included participants from the Copenhagen General Population Study (CGPS), an ongoing, prospective population study initiated in 2003. Individuals aged 20–100 years were randomly selected from the general population through the national Danish Civil Registration System. For this study, we included participants who attended the CGPS between 2003 and 2011. Follow-up ended on December 31, 2011. The response rate was 42%.

All participants completed a questionnaire, which was validated by an interviewer at Herlev Hospital, Denmark, were the study took place. All participants underwent a physical examination including spirometry, and provided blood samples. The study was conducted according to the declaration of Helsinki and approved by the Regional ethics committee (H-KF 01–144/01). Current asthma was defined as an affirmative answer to the specific question: “Do you have asthma?”

**BLOOD EOSINOPHILS AND NEUTROPHILS**

Blood samples for white blood cell (WBC) counts were collected in EDTA tubes at baseline, the day of study attendance, and measured on fresh samples using the ADVIA™ 120 Hematology system, which was monitored with daily precision testing by using both internal QC material and monthly accuracy testing with an external QC program. Eosinophil and neutrophil counts were reported in total numbers (×10⁹/L) together with other leukocyte subpopulations. The CV was 10% for blood eosinophil count and 2% for blood neutrophil count.

For 260 individuals with asthma, we had repeated measurements of blood eosinophil and blood neutrophil counts collected at a second visit. Mean time from baseline to the second measurement was 10 years (5–12 years). Repeated measurements of WBC counts were also measured on fresh samples using the ADVIA™ 120 Hematology system.

**SPIROMETRY AND CHARACTERISTICS**

Forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC) were determined without the use of a bronchodilator. All characteristics were recorded at baseline.

See the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol63/issue4 for more information.

**EXACERBATIONS**

We prospectively analyzed risk of asthma exacerbations from 2003–2011. A moderate exacerbation was defined as a short-course treatment with prednisolone and a severe asthma exacerbation was defined as a hospitalization due to asthma. Information on hospitalization and medication was obtained by linking the CGPS to the National Danish Patient Registry, which records all hospital contacts in Denmark and to the Danish Registry of Medicinal Product Statistics. Asthma hospitalizations were identified using the WHO International Classification of Diseases ICD-10 code DJ45-DJ46. We identified treatment with prednisolone (H02AB06) by using the Anatomic Therapeutics Chemical code. Moderate exacerbations had to be minimum 4 weeks apart to be considered as separate exacerbations. Individuals with any use of systemic corticosteroids (H02AB) 1 month before baseline were excluded from the analyses as this treatment might result in a low blood eosinophil count at baseline. We recorded all exacerbations, that is, one individual could have more than one exacerbation during follow-up.

**STATISTICAL ANALYSES**

Analyses were done in Stata/SE version 14 for Windows. FEV₁ in percentage of the predicted value was calculated separately for each spirometer as well as separately for men and women using internally derived references based on a subsample of healthy, asymptomatic, never-smokers without self-reported asthma in a linear regression with age and height as covariates.
Blood Eosinophil and Neutrophil Counts in Asthma

Differences in baseline characteristics and symptoms were assessed using Kruskal–Wallis test.

Follow-up for every participant began at study entry and ended at death (n = 309), emigration (n = 16), or end of follow-up on December 31, 2011, whichever came first.

We calculated the incidence rate ratios (IRRs) with 95% CI separately for moderate and severe exacerbations in a negative binomial regression model assessing the risk of exacerbations according to blood eosinophil counts and blood neutrophil counts separately. The IRR is defined as a relative difference used to compare incidence rates of events occurring at any given point in time between groups. Owing to overdispersion, we used negative binomial regression [nbreg y x covariates, exposure (follow-up time) irr]. We used tertiles of blood counts to assess associations across different concentrations. Individuals in the lowest tertile of blood eosinophil count and neutrophil count, respectively, were the reference group. The models were multivariable adjusted for age, sex, FEV1 in percentage of predicted, smoking status, cumulative smoking, and body mass index (BMI). In sub analyses, we further adjusted for inhaled corticosteroids, which could potentially influence blood eosinophil and neutrophil counts although it might also induce reverse causation. In a negative binomial regression model adjusted for the same confounders as mentioned above, we assessed the risk of moderate and severe exacerbations in 9 groups formed by combining tertiles of blood eosinophil and neutrophil counts, by using individuals in the lowest tertile of both blood eosinophils (<0.18 × 10^9/L) and blood neutrophils (<3.77 × 10^9/L) as the reference group.

Regression dilution ratio was calculated according to Clarke et al. (27).

Interactions were tested using the Likelihood-ratio test and continuous measurements of blood counts. Imputation of missing covariates for adjustments was done by multivariable normal regression. Information on age, sex, FVC, FEV1, and FEV1 in percentage of predicted gave similar results to those reported.

BASELINE CHARACTERISTICS AND SYMPTOMS
Baseline characteristics of individuals with and without asthma in the CGPS, see online Supplemental Table 2.

Men had higher blood eosinophil counts than women in the asthma population. Individuals with blood eosinophil counts in the highest tertile had a slightly lower ratio of FEV1:FVC and a lower FEV1 in percentage of the predicted value than individuals in the lower tertiles. In contrast, a higher proportion of individuals with blood eosinophil counts in the lower tertile experienced dyspnea compared with individuals in the higher tertiles. Fewer individuals with blood eosinophil counts in the lowest tertile reported asthma, allergy, and/or eczema during childhood as well as wheezing during a cold and exercise. There was an increasing percentage of individuals with familial disposition to asthma with high blood eosinophil count and the proportion of individuals using inhaled medications was also higher. The median blood neutrophil count did not vary among tertiles of blood eosinophil count.

EXACERBATIONS
Individuals with asthma were on average followed for a median of 4.0 years (IQR 2.1–6.0). In total, 2728 moderate and 447 severe exacerbations were recorded. Of the 918 individuals with exacerbations during follow-up, 19% had more than 1 severe exacerbation and 47% had more than 1 moderate exacerbation. Mean annual exacerbation rate by tertiles of blood eosinophil and neutrophil counts are shown in Table 1. Owing to the skewed distribution of blood eosinophil counts, each tertile does not include exactly one-third of the individuals.

The multivariable-adjusted IRR of moderate exacerbations for individuals in the middle tertile of blood eosinophil counts (0.18–0.29 × 10^9/L) was 0.80 (95% CI 0.65–0.98) using individuals with the lowest blood eosinophil counts as the reference (Fig. 2). In comparison, the multivariable-adjusted IRR for individuals with the highest blood eosinophil count (>0.29 × 10^9/L) was 1.28 (1.06–1.55). Corresponding values for severe exacerbations were 0.99 (0.75–1.31) and 1.55 (1.20–2.00). Further adjusting the estimates for blood neutrophil counts gave similar results. Adjusting the estimates for inhaled corticosteroids likewise gave similar results (see
online Supplemental Fig. 3). When restricting our analyses to users of inhaled corticosteroids or never-smokers, results also remained similar (see online Supplemental Fig. 4).

By using individuals with the lowest blood neutrophil counts as the reference, the multivariable-adjusted IRR of moderate exacerbations increased from 1.40 (1.13–1.72) for individuals in the middle tertile of blood neutrophil counts (3.77–4.85 × 10⁹/L) to 2.14 (1.74–2.63) for individuals with the highest blood neutrophil counts (>4.85 × 10⁹/L). For severe exacerbations, however, the risk was not higher with higher blood neutrophil counts. Further adjusting the models for blood eosinophil counts gave similar estimates.

Owing to the surprisingly increased risk of moderate exacerbations among individuals with low blood eosinophil counts, we investigated the interaction between blood eosinophil and blood neutrophil counts in stratified analyses. By using individuals with the lowest blood eosinophil and blood neutrophil counts as the reference group, the multivariable-adjusted IRR of moderate exacerbations increased from 2.02 (1.39–2.94) to 3.15 (2.20–4.51) with higher blood neutrophil counts within the group of individuals with low blood eosinophil counts (<0.18 × 10⁹/L) (Fig. 3). Corresponding values with increasing tertiles of blood eosinophil counts within individuals with low blood neutrophil counts (<3.77 × 10⁹/L) were 1.32 (0.88–1.97) and 1.93 (1.31–2.84), respectively. There was an interaction of blood neutrophil counts and blood eosinophil counts on moderate exacerbations (P = 3 × 10⁻⁴), but not on severe exacerbations (P = 0.27). For estimates in the model with the interaction term, see online Supplemental Table 3.

When comparing the 4 corners of the table in Fig. 3, that is, individuals with blood eosinophil counts in the highest and lowest tertiles in combination with blood neutrophil counts in the highest and lowest tertiles, individuals with high blood neutrophil counts smoked more compared to individuals with low blood neutrophil counts regardless of blood eosinophil count (see online Supplemental Table 4). Individuals with high blood neutrophil counts also had a higher degree of airflow limita-

Fig. 1. Distribution of blood eosinophil and neutrophil counts.
Table 1. Baseline characteristics of individuals with asthma.a

<table>
<thead>
<tr>
<th>Blood eosinophil counts (×10⁹/L) in tertiles</th>
<th>Blood neutrophil counts (×10⁹/L) in tertiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.18, n = 1714</td>
<td>&lt;3.77, n = 1625</td>
</tr>
<tr>
<td>0.12 (0.09–0.15)</td>
<td>0.22 (0.14–0.33)</td>
</tr>
<tr>
<td>0.23 (0.20–0.26)</td>
<td>0.23 (0.15–0.33)</td>
</tr>
<tr>
<td>0.40 (0.34–0.52)</td>
<td>0.22 (0.14–0.35)</td>
</tr>
<tr>
<td>0.18–0.29, n = 1573</td>
<td>3.77–4.85, n = 1609</td>
</tr>
<tr>
<td>4.22 (3.42–5.26)</td>
<td>4.23 (2.84–3.51)</td>
</tr>
<tr>
<td>4.24 (3.55–5.15)</td>
<td>4.27 (4.02–4.55)</td>
</tr>
<tr>
<td>4.32 (3.55–5.29)</td>
<td>5.77 (5.26–6.66)</td>
</tr>
<tr>
<td>&gt;0.29 , n = 1551</td>
<td>−</td>
</tr>
<tr>
<td>0.12 (0.09–0.15)</td>
<td>−</td>
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<tr>
<td>0.22 (0.14–0.15)</td>
<td>−</td>
</tr>
<tr>
<td>0.40 (0.34–0.52)</td>
<td>−</td>
</tr>
</tbody>
</table>

P value across tertiles

Eosinophil count, ×10⁹/L: 0.12 (0.09–0.15), 0.23 (0.20–0.26), 0.40 (0.34–0.52). Neutrophil count, ×10⁹/L: 0.22 (0.14–0.33), 0.23 (0.15–0.33), 0.22 (0.14–0.35).

Sex, men: 523 (31%), 632 (40%), 754 (49%). Age, years: 56 (47–66), 56 (47–65), 57 (47–68). FEV₁, FVC, %: 75 (68–81), 75 (67–80), 73 (66–79). FEV₁, % of predicted: 88 (74–99), 88 (75–100), 85 (71–96). Degree of air flow limitation: FEV₁ >80, 50 ≤ FEV₁ <80, FEV₁ <50. BMI, kg/m²: 26 (23–29), 26 (24–30), 26 (24–29). Current smokers: 418 (24%), 421 (27%), 487 (31%). Pack-years: 119 (7%), 103 (6%), 108 (7%). Dyspnea: 447 (26%), 351 (22%), 339 (22%). Low education: 760 (44%), 687 (44%), 717 (46%). Allergy: 1311 (76%), 1257 (80%), 1228 (79%). Familial disposition to asthma: 672 (39%), 706 (45%), 665 (43%). Wheezing: During a cold, 682 (40%), 703 (44%), 689 (44%). During exercise, 634 (37%), 646 (41%), 680 (44%). In unknown situations, 368 (21%), 332 (21%), 352 (23%). Any inhaled medication, 1101 (64%), 1073 (68%), 1109 (72%). Inhaled corticosteroids, 920 (54%), 902 (57%), 935 (60%).

P values for comparison across tertiles were calculated using Kruskal-Wallis test.

a Data are n (%) for categorical values and median (IQR) for continuous values. Baseline characteristics were at the date of examination except for inhaled medication which was up to 1 year prior. 

b Pack-years were calculated for current and former smokers only. Dyspnea was defined as ≥2 on the modified Medical Research Council Dyspnea Scale. Familial disposition to asthma was the reporting of a mother and/or father with asthma. Low education was less than 3 years of education following the mandatory primary school. P values for comparison across blood eosinophil and neutrophil counts in tertiles were calculated using Kruskal-Wallis test.
tion, a higher proportion reported dyspnea, and they were less educated.

For information on the overlap of individuals with moderate and severe exacerbations, see online Supplemental Fig. 5. For baseline characteristics of individuals according to exacerbations, see online Supplemental Table 5.

REGRESSION DILUTION
To estimate stability of blood eosinophil and neutrophil counts in the same person with self-reported asthma over 10 years, we calculated regression toward the mean for both counts based on tertiles determined at the first examination for 260 individuals that each were remeasured 10 years later (Fig. 4) (see online Supplemental Tables 6 and 7). The regression dilution ratio was 0.61 for blood eosinophil counts and 0.54 for blood neutrophil counts. Main results corrected for the regression dilution ratio are shown in online Supplemental Fig. 6. Of the individuals with asthma in the highest tertile at baseline, 51% remained in the highest tertile of blood eosinophil counts and 64% remained in the highest tertile of blood neutrophil counts 10 years later.

Discussion
In 4838 individuals with asthma from the general population, we found that high blood eosinophil counts were associated with an increased risk of both moderate and severe exacerbations, while high blood neutrophil counts were associated with an increased risk of moderate, but not of severe exacerbations. A surprisingly higher risk of moderate exacerbations among individuals with low blood eosinophil counts could be explained by high blood neutrophil counts. This interaction between blood eosinophil and blood neutrophil counts in relation to asthma exacerbations in the general population is novel.

The association between high blood eosinophil counts and increased risk of exacerbations in the general population has been assessed before. Two studies using data from the National Health and Nutrition Examination Survey found that high blood eosinophil counts were associated with self-reported historical asthma attacks (18, 19). Furthermore, a prospective cohort study with 1 year of follow-up including 2392 adults with persistent asthma found that high blood eosinophil counts...
were associated with the risk of both exacerbations and excessive use of short-acting β₂-agonist (16). In a recent large scale cohort study by Price et al. using medical record data from 130,248 adult primary care asthma patients, blood eosinophil counts above $0.4 \times 10^{9}/L$ were associated with more severe exacerbations and poorer asthma control, illustrating that blood eosinophilia can be used as a marker of future exacerbation risk (15). Like
Price et al. found that individuals with high blood eosinophil counts were more likely to be men and current nonsmokers, but we found no difference in pack-years. In accordance with others, we also found high reporting of wheezing with high blood eosinophil counts (15) as well as a slightly lower FEV₁:FVC and FEV₁ in percentage of the predicted value in individuals with high blood eosinophil counts (28).

We were not able to find a stepwise higher risk of moderate exacerbations with higher blood eosinophil counts. Instead, we found a high number of moderate exacerbations in individuals with low blood eosinophil counts and a significant interaction with the level of blood neutrophil counts on the association with moderate exacerbations. Corresponding to airway inflammation in asthma, which is heterogeneous and with inflammatory subgroups that can be categorized on the basis of eosinophil and neutrophil counts in sputum (1), blood counts of eosinophils and neutrophils may also reflect these inflammatory patterns. In our study, we found that in individuals with asthma, exacerbation risk varies according to the inflammatory phenotype found in blood. There is evidence that exacerbations are less severe in noneosinophilic asthma (29). This is supported by our study, as we found high blood eosinophil counts but not blood neutrophil counts, to be associated with severe exacerbations. Individuals with blood eosinophil counts in the highest tertile might represent individuals with sputum eosinophilia as a cut-point of $0.27 \times 10^9/L$ have been reported in asthma to differentiate eosinophilic and noneosinophilic inflammation with a sensitivity of 78% and a specificity of 91% (8). In our study, high blood neutrophil counts were however associated with an increased risk of moderate exacerbations and may identify a phenotype with a higher symptomatic score leading to prednisolone treated exacerbations not requiring hospital admission.

Approximately half of asthma patients with mild to moderate disease do not have airway eosinophilia and the inflammation process of these individuals remains to be clarified as the risk of exacerbations likely differs according to inflammatory phenotype (2). In the epidemiological study on genetics and environment in asthma (EGEA) including 381 adults with asthma, 4 inflammatory phenotypes were described in peripheral blood corresponding to the same inflammatory phenotypes found in sputum (26). In that study, an eosinophilic inflammatory pattern was associated with a higher percentage of self-reported asthma attacks in the last 12 months as well as lower FEV₁ and more breathlessness compared to individuals with a noneosinophilic pattern. A neutrophil pattern together with an eosinophilic pattern, however, was associated with more active disease, more dyspnea, and higher symptomatic score similar to our findings. In our study, we grouped exacerbations as moderate if the individuals received a short-course treatment with prednisolone and found that high blood neutrophil counts were associated with the risk of moderate, but not of severe exacerbations. It is plausible, that a high blood neutrophil count associated with more symptoms might...
result in seeing a doctor more regularly, thereby increasing the risk of having prednisolone prescribed.

The neutrophilic phenotype in asthma identified by sputum and not blood counts has previously been associated with resistance to inhaled corticosteroids (30), which could also lead to increased symptoms and exacerbation rate. Among the individuals in our study with high blood neutrophil counts, a higher percentage was using inhaled corticosteroids. We can, however, not exclude that a high neutrophil blood count could be a consequence of the inhaled corticosteroid treatment, which delays neutrophil apoptosis (31). In a study by Green et al., participants with the neutrophilic asthma phenotype defined through sputum counts were found to be older, predominantly female, and more likely to be non-atopic (30). Although we do not have asthma subtypes identified by sputum counts, these characteristics are similar to what we found in the group with high blood neutrophil counts and low blood eosinophil counts. Of notice, remodeling of the airways increases with age and is also associated with neutrophilia (25, 32). The neutrophilic phenotype is associated with smoker’s asthma (33) as smoking worsens asthma symptoms, increases neutrophilic inflammation, and oxidative stress leading to more air trapping (34). In our study, the percentage of current smokers was higher with higher blood neutrophil counts. We cannot fully exclude that some of these individuals could have chronic obstructive pulmonary disease reported as asthma, however, a high blood neutrophil count was also associated with an increased risk of moderate exacerbations in never smokers alone.

Strengths of our study include the large sample size from a general population as well as the prospective design. We were able to include more than 4800 individuals with asthma selected from 81351 individuals from the general population, and to follow them through the Danish registries with no one lost to follow-up. We are limited by only having prebronchodilator spirometry measurements. As we rely on self-reported asthma with no measurements of reversible airflow limitation, we cannot exclude that some individuals in fact did not have asthma. However, self-reported asthma has been reported to have both high specificity and sensitivity in epidemiology (35–37) and a 6% prevalence of asthma is in accordance with other studies in the adult Danish population (38). Furthermore, a change in asthma admission rate during follow-up time could potentially influence our results, however, we have no knowledge indicating that this change should be other than nondifferential. Lastly, our classification of individuals is based on a single blood sample. We had repeated blood counts from 260 individuals with self-reported asthma with a median of 10 years apart. In an attempt to correct our analyses for regression dilution bias (27), we calculated regression toward the mean based on these data and found larger effects sizes than for uncorrected estimates. After a median of 10 years, more than half of the individuals with baseline blood counts of eosinophils and neutrophils in the highest tertile remained in the highest tertile suggesting some stability of the measurements.

In conclusion, our data support the view that high blood eosinophil and neutrophil counts may reflect airway inflammation and provide a noninvasive way of assessing this inflammation. Furthermore, our data show that exacerbation risk depends on both inflammatory patterns, which interact with each other on moderate exacerbations and thus both eosinophil and neutrophil counts should be considered as markers of future asthma exacerbation risk. As sputum eosinophilia can be difficult to assess in the daily clinical practice, blood eosinophils may gain ground as a marker of disease severity and the advent of anti-IL-5 therapy will make monitoring therapy easy using blood eosinophils (13); in the DREAM trial, eosinophilic asthma was identified using blood eosinophil counts and/or fractional expired nitric oxide (FeNO) in severe asthma patients and baseline blood eosinophil count predicted treatment effect. However, blood neutrophils might also be a marker of disease severity and as we currently have no treatments to reduce airway neutrophilia (39), our study emphasizes the need for new treatments in asthma.
References

10. Park PA, McDonald VM, Gibson PG. Adjusting prednisone using blood eosinophils reduces exacerbations and improves asthma control in difficult patients with asthma. Respir Med 2015;20:1282–8.