ADULT-ONSET ASTHMA

PREDICTORS OF CLINICAL COURSE AND SEVERITY

Guus Alexander Westerhof

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Introduction

Chapter 1

GENERAL INTRODUCTION AND AIMS

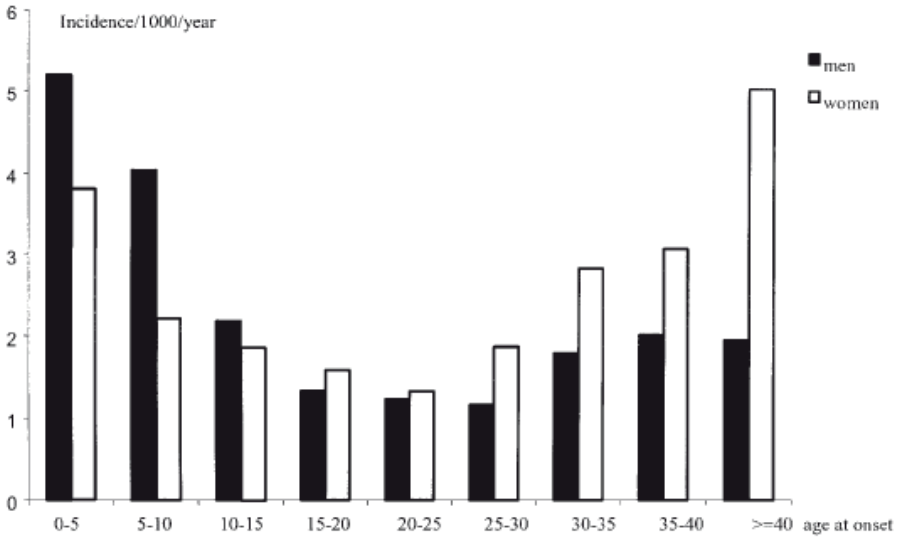
Guus A. Westerhof

EPIDEMIOLOGY AND RISK FACTORS OF ADULT-ONSET ASTHMA

Asthma is a common respiratory disease that affects 334 million people of all ages worldwide.[1] Characteristics of asthma are typical symptoms (wheezing, chest tightness, shortness of breath), reversible airway obstruction, bronchial hyperreactivity and chronic airway inflammation.[2] Asthma has been regarded for a long time as a disease that develops in childhood. Genetic predisposition, atopy and respiratory infections early in life have been established as the major risk factors for developing the disease.[3, 4] The prognosis is generally considered favourable, up to 65% of children has "overgrown" their asthma when they are middle aged.[5] However, since several decades a different asthma type that emerges in adulthood has been recognized, so called adult-onset asthma.[6-9] This type of asthma differs from childhood onset asthma with respect to epidemiology, risk factors, phenotypes, pathophysiology and probably also prognosis.[10]

Childhood asthma has an incidence of 3.7-5.2 per 1000 person-years and the incidence decreases with increasing age.[3, 11] Boys are more prone to develop asthma in childhood than girls (4.5 vs 2.9/1000).[3] On the contrary, the incidence of adult-onset asthma in women was estimated 4.6 per 1000 person-years and 3.6 per 1000 person-years in men in a pooled analysis of several population studies. A trend towards a higher incidence was seen with increasing age (see Figure 1).[11, 12] Consequently the prevalence of asthma rises with age and ranges from 6-10% in older adults.[13] In contrast to the limited number of risk factors for childhood asthma, a wide range of influencing factors for the development of adult-onset asthma have been found. These include genetic factors,[14, 15] exposure to occupational agents like wheat flour and cleaning products,[16, 17] air pollution,[18] cigarette smoke exposure,[15, 19, 20] respiratory infections,[21] NSAID hypersensitivity,[22, 23] female sex hormones,[24] stress[25] and obesity.[26, 27]

Figure 1. Incidence of asthma stratified per age category. Adapted from De Marco *et al.*[11]

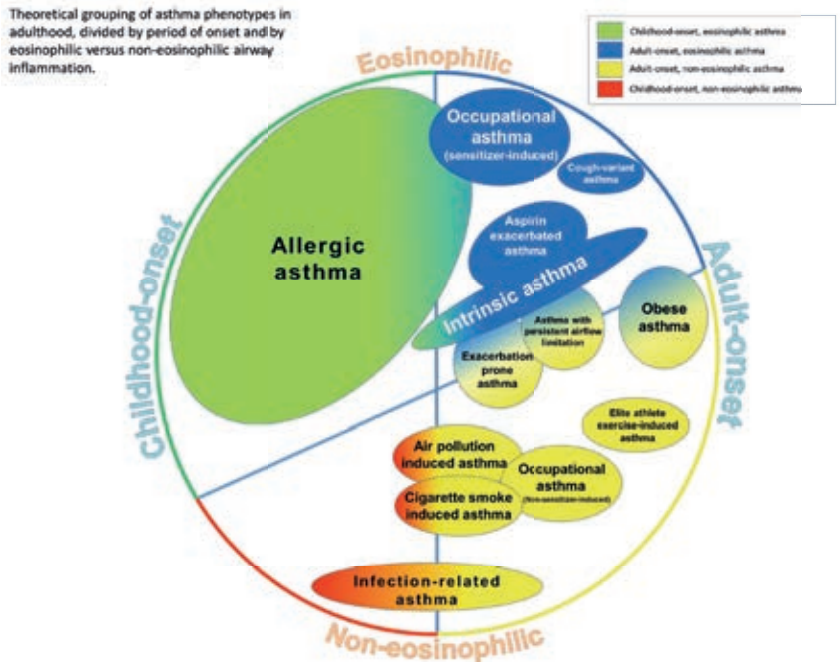


ADULT-ONSET ASTHMA, A HETEROGENEOUS DISEASE

ASTHMA PHENOTYPES

Adult-onset asthma is a heterogeneous disease with a variable clinical picture and a complex pathophysiology.[10, 28] Therefore the term adult-onset asthma can be regarded as an umbrella term for several distinct airway diseases, so called asthma phenotypes.[29] Early day phenotyping of asthma was based on clinical recognition of subtypes[6, 23, 30] and more recently an integrative statistical approach using cluster analysis has shown a wide variety of asthma-phenotypes.[8, 31] These phenotypes can be based on several aspects of the disease, most used are either clinical and physiological features, asthma triggers or airway inflammation type.[28, 32] Well known clinical phenotypes are for instance obesity associated asthma,[31] asthma with chronic sinusitis and nasal polyps,[33] cough-variant asthma,[34] asthma with persistent airflow limitation[35] and asthma with frequent exacerbations[36]; trigger associated phenotypes like occupational asthma,[17] smoking related asthma,[37] atopic or non-atopic asthma[38, 39] and finally inflammatory phenotypes such as eosinophilic or neutrophilic asthma.[40] Asthma phenotypes are not mutually exclusive in a patient; considerable overlap exists between them. Figure 2 gives an overview of the different childhood- and adult-onset asthma phenotypes.[32]

Figure 2. Schematic view of different asthma phenotypes, divided by childhood or adult-onset and eosinophilia. Adapted from Hekking and Bel.[32]



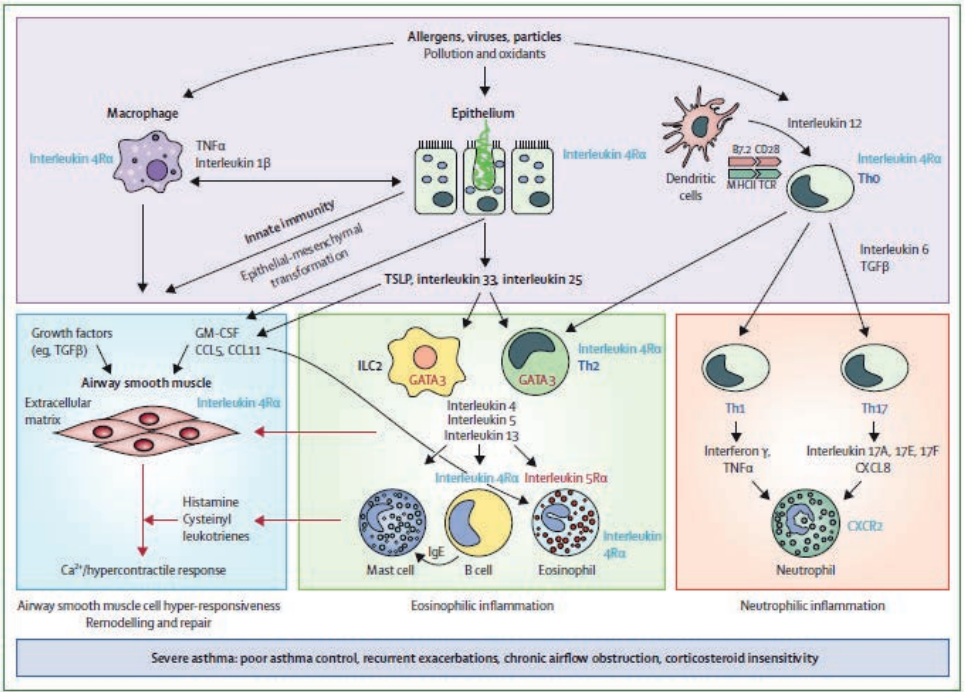
PATHOPHYSIOLOGY

As some phenotypes already suggest, there are several underlying inflammatory and non-inflammatory mechanisms leading to these diseases. The combination of these mechanistic (molecular) pathways and the associated phenotype is called an endotype. In this process an interplay exists between genetic susceptibility, functional elements (like lung function, airway hyper-responsiveness, obesity), environment, inflammation/immunity of the airways and response to treatment which leads to a certain disease manifestation.[41] Inflammation and immunity play a central role in asthma therapy and therefore will be explained into more detail.

The classical asthmatic airway inflammation (as in childhood asthma) is characterized by eosinophils, basophils and mast cells infiltrating the airways.(see Figure 3) This inflammation is initiated via allergens triggering the airway epithelium which leads to activation of dendritic cells and thereafter T-helper-2 (Th2) cells secreting pro-inflammatory cytokines (Interleukin(IL)-4, IL-5, IL-13).[42] The inflammatory response eventually leads to mucus hypersecretion and triggers airway smooth muscle cells resulting in hyper-responsiveness and airway wall remodeling. However, apart from this classic Th-2 pathway, more recently also the innate immune system was found to be able to initiate eosinophilic airway inflammation.[43] Innate lymphoid cells type 2 (ILC-2) are activated via interleukins 1, 25, 33 and thymic stromal lymphopoetin (TSLP) secreted by airway epithelium and macrophages after triggering by pollutants, microbes or allergens. This leads to a more pronounced secretion of type 2 cytokines interleukins 4, 5 and 13 and hence eosinophilic airway inflammation.[44] However, up to two thirds of asthma patients has no signs of eosinophilic inflammation.[40, 45] In these patients neutrophilic airway inflammation may be present, although also pauci-granulocytic (no signs of granulocytic inflammation) asthma has been described.[45] Neutrophilia is mainly initiated by interleukin 17, interleukin 8 and tumor necrosis factor-α (TNF-α) secreted by T-helper-17 (Th17) and T-helper-1(Th1) cells. Environmental substances such as diesel exhaust and cigarette smoke are known to trigger this pathway.[46] Finally, an interplay between Th17 and type 2 activation exists, which might be influenced by asthma treatment.[47]

Knowledge of the airway inflammation type in a patient is important to monitor the disease and tailor asthma therapy. For instance, corticosteroid treatment guided by airway eosinophilia has been shown to reduce asthma exacerbation rate.[48] Novel treatments directed against specific type 2 pathway cytokines like mepolizumab[49] and dupilumab[50] can improve asthma outcomes even further. However the assessment of airway inflammation is quite invasive, unpleasant for patients, time consuming and costly. Therefore several biomarkers of eosinophilic airway inflammation (*e.g.* blood eosinophils, fraction of exhaled nitric oxide (FeNO) and serum IgE) have been investigated in mixed asthma populations. Most studies found moderate accuracy for identifying eosinophilic airway inflammation,[40, 51, 52] without taking asthma phenotype into account.

Figure 3. Pathophysiological pathways underlying different inflammatory types of asthma.



TNF: tumor necrosis factor α, TSLP: thymic stromal lymphopoeitn, Th x: T-helper- type x cell, TGFβ: transforming growth factor β, GM-CSF: granulocyte-macrophage colony-stimulating factor, ILC2: innate lymphoid cell type 2. Adapted from Chung.[53]

PURPOSE OF THIS THESIS

This thesis focuses on the clinical course and severity of adult-onset asthma, which is poorly studied and remains poorly understood. So far, a very limited number of longitudinal studies in adult-onset asthma patients have been performed. The wide variety of clinical phenotypes and underlying pathophysiological mechanisms described above has been observed mainly in cross-sectional studies or studies with childhood-onset asthma patients. The course of adult-onset asthma over time is still largely unknown, although incidence and prevalence of the disease are increasing, with almost half of all asthma patients in a secondary clinic having adult-onset disease. This leaves clinicians with uncertainties about the prognosis in a large proportion of their asthma patients, whereas in the clinic it is important to recognize patients who develop frequent asthma exacerbations or increased asthma severity in an early phase of the disease to allow intensive treatment and strict monitoring. On the contrary, it can be reassuring and might prevent overtreatment to recognize patients who will have asthma remission. Early interventions targeted to the specific type of airway inflammation might improve the patient's prognosis dramatically (preserved lung function, improved quality of life). In order to be able to identify patients who are eligible for targeted treatments (e.g. mepolizumab, dupilumab), biomarkers of airway inflammation pathways are needed. Whether these markers are influenced by asthma phenotype is unknown. Especially for patients with a recent-onset of adult asthma predictors of increased asthma severity, exacerbations and remission are not known. Therefore, research on the prognosis of adult-onset asthma is required. This led to the following research questions for the present thesis:

1. Literature overview of adult-onset asthma prognosis.
2. Differs the diagnostic accuracy of surrogate markers of airway eosinophilia between adult-onset asthma phenotypes?
3. How accurate are surrogate markers of airway eosinophilia in detecting sputum eosinophils in a general asthma population?
4. Which factors predict remission and persistence of adult-onset asthma?
5. Which factors determine an increase in asthma severity?
6. What are the predictors of frequent asthma exacerbations in adult-onset asthma, and do these differ between for example smokers and never smoker?

The content of this thesis is mainly based on data of the Adonis-study (Adult-onset Asthma and Inflammatory Sub-phenotypes). In this prospective observational study, 200 adult patients with a recent diagnosis (<1 year) of asthma were included. These patients were extensively characterized with regard to clinical, functional and inflammatory parameters. Patients were asked to participate in the follow-up phase as well, with yearly visits to take history, spirometry and peripheral blood. After 4-5 year a reassessment of the baseline parameters was performed. In addition, a second database was set-up in which Adonis-study baseline

data was pooled with two other clinical trials with similar methodologies. Together, these three cohorts consisted of 571 patients with adult-onset asthma.

The outline of the thesis is as follows: at first, **Chapter 2** gives an overview of all the studies about prognosis of adult-onset asthma. Thereafter, **Part 1** focusses on the accuracy of biomarkers of airway inflammation and serves as a method check. **Chapter 3** addresses biomarkers of eosinophilic airway inflammation in different adult asthma phenotypes, based on the combined cohort data. **Chapter 4,** describes a systematic review and meta-analysis to identify and pool all studies about biomarkers of airway eosinophilia in asthma patients. Consecutively, **Part 2** of this thesis addresses the clinical course of adult-onset asthma. The data of the Adonis-study was used in **Chapter 5** to describe predictors of asthma remission and persistence and in **Chapter 6** to describe predictors of increased asthma severity. In **Chapter 7** the combined cohort was used to investigate whether predictors of frequent asthma exacerbations are different in smokers and never-smokers.

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Chapter 2

THE PROGNOSIS OF ADULT-ONSET ASTHMA

A LITERATURE REVIEW

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Hanneke Coumou*
Vivian I.V. Gerretsen
Elisabeth H.Bel

ABSTRACT

This narrative review examines the known prognostic factors of adult-onset asthma with respect to lung function decline, increased asthma severity and asthma remission. The number of longitudinal studies investigating the course of adult-onset asthma is limited, which highlights the need for further follow-up studies. Amplified lung function decline is associated with male gender, atopic status and poor baseline lung function. Increased asthma severity is influenced by smoking and low lung function, whereas current uncontrolled asthma and smoking predict having uncontrolled asthma in the future. Asthma exacerbations can be predicted by high symptom scores, low lung function and markers of airway eosinophilia. Remission rate of adult-onset asthma is low and mainly seen in patients with mild asthma and a short disease duration. Smoking has a profound negative effect on asthma remission. The most important factor for prognosis of occupational asthma is ceasing exposure to the causative agent, which has a positive effect on all aspects of the disease.

Conclusion: Two prominent influential factors in all domains of adult-onset asthma prognosis are smoking and baseline lung function. Clinicians treating adult-onset asthma patients should notice these factors and realize these patients are at risk for a poor prognosis. However, more research is needed in order to offer patients more certainty about their prognosis and possible treatable factors.

PROGNOSIS IN CHILDHOOD – AND ADULT – ONSET ASTHMA

One important distinctive variable for asthma phenotyping is age of asthma onset, making a division in childhood- and adult-onset asthma[1-6]. Studies have shown clear differences between childhood-onset asthma and adult-onset asthma [7, 8]. For example, onset of asthma in adulthood is associated with upper airway symptoms [9, 10] and in contrast to childhood asthma, the impaired lung function is independent of disease duration [8].

However, most follow-up studies describe only the clinical course of childhood-onset asthma. These studies have shown that atopic children with a history of wheezing before the age of 3 years are at risk for an impaired lung function at school age[11]. Moreover, children with early lung function impairment combined with environmental exposure are at risk for developing persistent asthma[12]. Childhood asthma might only exist during childhood and adolescence but more than 40% will have asthma as adults[13, 14]. Factors such as atopy, parental asthma, recurrent respiratory infections and the asthma severity in childhood have been shown to predict asthma persistence and severity into adulthood [15]. Childhood-asthma severity is also associated with faster lung function decline in adulthood [15], just as persistent airway hyperresponsiveness and frequent asthma exacerbations [16-18].

On the contrary, the clinical course of adult-onset asthma has been studied to a much lesser extent. The limited available literature indicates that the prognosis tends to be poor with low remission rate and a fast deterioration of lung function [19]. From cross-sectional studies it is known that adult-onset asthma patients are often non-atopic [9, 20] and have severe airflow obstruction [21, 22]. Adult-onset asthma itself is also a heterogeneous disease that consists of different phenotypes such as eosinophilic inflammation-predominant asthma, obese women [5, 23] and occupational asthma[24, 25]. The latter is a specific phenotype provoked by workplace substances that accounts for approximately 15% of all adult-onset asthma patients [26, 27].

As asthma phenotypes differ with regard to asthma triggers, clinical characteristics and inflammation, also the prognosis might be variable. Many unresolved issues exist about the clinical course and factors determining the prognosis. In order to offer patients better-tailored treatment, it is relevant to acknowledge these prognostic factors. Therefore, the aim of this review is to summarize the known determinants of adult-onset asthma prognosis, supplemented with data on adult asthma in general. Results are presented according to factors associated with lung function decline, increased asthma severity and asthma remission.

FACTORS DETERMINING DECLINE IN LUNG FUNCTION IN ADULT ASTHMA

Patients with asthma have a faster decline in lung function compared to healthy controls [28, 29], but an even greater lung function decline is seen with a later age of disease onset [30-32]. Late-onset asthma also increases the risk of persistent airflow limitation [21, 33]. However, little is known about factors influencing lung function of adult-onset asthma patients and only a few longitudinal studies are available.

PREDICTORS OF LUNG FUNCTION DECLINE IN ADULT-ONSET ASTHMA

In adults with asthma different clinical and inflammatory factors influence the decline in lung function. Ulrik et al. showed a difference in annual FEV_1 decline between non-atopic and atopic asthma, respectively 50 ml/y vs 22,5 ml/y ($p<0.0001$) [34]. Whereas Cibella et al,. did not find an effect of atopy on FEV_1 decline [35]. However, they did find a steeper FEV_1 decline in young (≤43y) asthmatics with a baseline FEV_1<80% predicted. In a longitudinal analysis of a Korean asthma cohort, Park *et al.*, found no FEV_1 decline after 12 months in a cluster with late-onset asthma. However, this cluster was predominantly female (72,6%) and had mild asthma with an FEV_1 of almost 100% predicted at baseline [36]. Sakagami *et al.* characterized in a cluster analysis 2 out of 3 clusters as late-onset asthma [37]. One cluster was male dominated, with low FEV_1/FVC at diagnosis and with the highest serum total IgE, but a low incidence of atopy. This group had a more accelerated FEV_1 decline compared to the other clusters. Amelink *et al*. found similar results in a cross-sectional study, where non-atopic males with adult-onset asthma had more often persistent airflow limitation and might be at risk for accelerated lung function decline [22]. Another study reported an accelerated FEV_1 decline in a subgroup with persistent airflow limitation which was correlated to baseline sputum eosinophils ($r = 0.53$, $P <0.05$) and fraction of nitric oxide in exhaled air (FeNO)($r = 0.55$, $P <0.05$) [38]. A 5 year follow up study in difficult-to-treat asthma patients confirmed the latter finding in patients with a baseline FEV_1 ≥80%; FeNO ≥20 ppb was predictive for excess lung function decline (RR of 3.1 (95% CI, 1.7–3.4)) [39]. For persistent airflow limitation sputum eosinophilia (≥2%) was also reported as an independent risk factor with an odds ratio (OR) of 8.9 (95% 1.3–59.0) [21]. Still, the role of eosinophilic inflammation as predictor of lung function decline is not entirely established as inflammatory profiles might change over time[40-42]. In a study with 97 severe asthma patients Newby *et al*., reported high fluctuation in eosinophil percentage as a dependent factor for postbronchodilator FEV_1 decline [43]. Furthermore, infection with Chlamydia pneumonia in non-atopic adult-onset asthma patients was strongly associated to a decreased FEV_1/FVC-ratio related to asthma duration as compared to atopic patients [44]. And finally, a large longitudinal population study found in patients with asthma onset ≥ 25 years, male gender and pack years to be the strongest risk factors of persistent airflow limitation (per 10 pack year a risk ratio (RR) 1.4 95% CI 1.2-1.7) [33]. Similar results were reported in a cross-sectional study with 1017 severe or difficult-to-treat asthma patients. The authors found an association between

persistent airflow limitation and male gender (OR, 4.5; 95% CI, 2.3 to 8.5); older age (OR per 10 years, 1.4; 95% CI, 1.3 to 1.6) and current or past smoking (OR, 3.9; 95% CI, 1.8 to 8.6; and OR, 1.6; 95% CI, 1.2 to 2.3, respectively) [45]. Perret *et al*., also found an association between smoking and persistent airflow limitation, but this was limited to patients with atopy [46].

DETERMINANTS OF LUNG FUNCTION IN OCCUPATIONAL ASTHMA

Studies investigating occupational asthma reported the lung function decline in these patients is mostly influenced by continued exposure. A study by Anees *et al.*, showed no influence of gender or baseline FEV_1 but decline in lung function was associated with continued exposure to the causative agent [47]. Just after removal from exposure, the patients had an uplift in FEV_1, followed by a subsequent decline at lower rate than during exposure [47]. Other studies also showed a steeper decline in lung function with an irregular slope in the groups that continued being exposed compared to the group that avoided exposure [47-49]. The relationship between bronchial hyperresponsiveness (BHR) and removal from exposure is less clear. One study showed a correlation between time of removal and improvement in BHR [50], whereas another study showed persistent hyperresponsiveness after 10 years of removal [51].

FACTORS INFLUENCING ASTHMA SEVERITY IN ADULTS

ASPECTS OF ASTHMA SEVERITY

Classification of asthma severity[52] is based on the minimum level of asthma medication needed to prevent the disease from becoming uncontrolled or which remains uncontrolled despite high dose therapy [53-55]. Uncontrolled asthma can be either: poor symptom control (e.g. asthma control questionnaire (ACQ)-score >1.5 [56]), frequent or severe exacerbations or increased airflow limitation [57]. Compared to childhood asthma, adult-onset asthma tends to be more severe [5, 58]. Studies investigating the prognosis of asthma severity in general and the different aspects of uncontrolled asthma will be addressed here, except for airflow limitation, which was addressed in the previous paragraph.

PREDICTORS OF INCREASE IN ASTHMA SEVERITY

Several predictors of increased asthma severity in adult-onset asthma have been described, for instance smoking, increasing age, high symptom scores and low lung function. After a follow-up of 70 months in a cohort of 250 patients, a low FEV_1 and increasing age were related to increased asthma severity (based on GINA severity grading). Patients with severe asthma at the end of follow-up also showed a significant increase in BMI during the study [59]. This study did not find an association between asthma severity and smoking, whereas others did. Polosa *et al*. found a dose-response relation between pack years and the development of moderate-severe asthma in rhinitis patients, with an odds ratio (OR) 2.9 (95% CI 1.1-7.5) for 11-20 pack year and OR 5.6 (95% CI 1.4-21.7) for >20 pack years [60]. In our own cohort of 200 adults with a recent diagnosis of asthma we found the number of pack years and lower FEV_1/FVC as univariate predictors of an increase in asthma severity after 2 years follow-up. The only independent predictor in the multivariate analysis was pack years, where every ten pack years smoked gave an OR 1.4 (95% CI, 1.02-1.91) for developing more severe asthma [61]. Another study with a mixed population of adults with asthma (both childhood and adult-onset asthma) reported a high symptom score, low FEV_1 and ICS use at baseline to be strongly related to severe asthma after 9 years follow-up. Furthermore the strongest prognostic factors in a multivariate model were chronic cough or worsening of cough, high IgE level or hospitalisation history. The authors found no relation between age of onset and asthma severity, hence results might be extrapolated to adult-onset asthma patients separately [62].

Finally, the predictive ability of phenotypes found by cluster analysis is limited. Cluster analysis has been used to identify different asthma phenotypes in several cross-sectional studies [5, 23, 63-65]. One prospective study investigated the prognostic value of these clusters in a mixed asthma population and showed that severe asthma clusters were not able to predict disease course with regard to asthma control, exacerbations rate or treatment requirements [66]. Whether patients still belong to the same phenotypes after a certain period differs between asthma phenotypes [40, 67]. Patients with adult-onset asthma were more likely to belong to

nonallergic phenotypes and these clusters were more prone to show a phenotype shift with regard to worsening asthma outcomes as compared to allergic clusters [67].

PREDICTORS OF UNCONTROLLED ASTHMA

The strongest predictors of uncontrolled asthma are smoking and uncontrolled asthma at baseline. A higher risk of developing uncontrolled asthma was observed in a 10-year follow-up of allergic rhinitis patients with new-onset asthma who smoked. An increasing number of pack years gave an increased risk of uncontrolled asthma, with OR 13.4 (95% CI 4.6-39.2) for patients who had smoked > 10 pack years [60]. Another important predictor of future asthma control is the current status of asthma control. In an 8-year follow-up study of 214 patients the distribution of asthma control at baseline was significantly associated with asthma control at follow-up. The authors reported for partial asthma control at baseline a relative risk ratio (RRR) 2.7 and for no asthma control at baseline a RRR 7.7 for uncontrolled asthma at follow-up. Furthermore, women had a higher risk of uncontrolled asthma as compared to men (RRR 4.3). Chronic cough and phlegm production gave a RRR 3 for having uncontrolled asthma during follow-up [68]. Several studies in general asthma populations reported similar predictors of poor future asthma control, such as signs of current uncontrolled asthma[69-71] and smoking [70, 71], but also higher BMI [72].

PREDICTING ASTHMA EXACERBATIONS

Predictors of asthma exacerbations have been evaluated in both cross-sectional and follow-up studies, they include markers of inflammation, environmental triggers and asthma control status. In a 1 year follow-up study with strict recording of exacerbations, Kupczyk *et al.* evaluated multivariate predictors of 2 or more exacerbations and found fraction of exhaled nitric oxide >45 ppb (OR 4.3, 95%CI 1.0-18.3) and smoking (OR 2.9, 95% CI 1.1-7.4) as independent predictors. For 3 or more exacerbations only smoking was a significant predictor with an OR 3.6 (95% CI 1.1-12) [73]. Recently, we showed in a cohort of never smoking and (ex) smoking adult-onset asthma patients, different predictors of frequent exacerbations in these two groups. In never smokers higher blood eosinophil counts were associated with frequent exacerbations, whereas in (ex)smokers higher blood neutrophil counts and a higher dose inhaled corticosteroids were associated [74]. This in line with several other studies in general asthma populations that found an association between blood or sputum eosinophils and exacerbations in non-smokers [75-77]. Whereas in patients with severe eosinophilic late-onset asthma apart from FeNO >50 ppb, also air trapping and sinus disease were predictors of frequent exacerbations [78]. Andersen *et al*. evaluated the effect of long-term exposure to air pollution on the risk of asthma hospitalisation in patients 50 years or older in a population cohort study. The risk of hospital admission for asthma exacerbations was positively associated with exposure to increasing concentration of NO_2 at their residences. Results were corrected for age, smoking status, tobacco exposure, occupational exposure, obesity and educational

level [79]. A case-control study with ten year follow-up evaluated risk factors for emergency room visits and found FEV_1 <65%, ex- or current smoking and having more symptoms as independent risk factors.[80] Additionally, several studies have been performed in a mixed population of adult patients with both childhood and adult-onset asthma. Ten Brinke *et al.* investigated the influence of several comorbidities on the occurrence of frequent exacerbations. They found psychological dysfunctioning, recurrent respiratory infections, gastro-oesophageal reflux, severe chronic sinus disease and obstructive sleep apnoea to be associated with frequent exacerbations in difficult-to-treat asthma patients [81]. Others reported patients with uncontrolled asthma were at higher risk for asthma exacerbations on the short term (1-2 weeks) [69, 82]. Whereas for exacerbation risk on the long run besides uncontrolled asthma also higher medication use, low FEV_1, obesity [83] and recent exacerbations[84] are predictors of exacerbations.

DETERMINANTS OF ASTHMA CONTROL IN OCCUPATIONAL ASTHMA

The most important determinant of asthma control and severity in patients with occupational asthma is continuing exposure to causative agents. Exposure to HMW or LMW asthmagens in the past year was associated with uncontrolled asthma in a large European study. Past 10 year occupational exposure was even stronger associated with uncontrolled asthma [85]. Another study (n=25) where half of the patients ceased exposure to the asthmagen investigated asthma symptoms, severity and health expenditure during 1 year follow-up. All subjects that continued to be exposed remained symptomatic and used asthma medication, whereas half of those who ceased exposure had been asymptomatic for at least half a year. Although both groups showed an improvement of asthma severity, the improvement was more marked in the group that ceased exposure [86].

REMISSION OF ADULT-ONSET ASTHMA

HOW OFTEN DOES ASTHMA REMISSION OCCUR IN ADULTS?

The remission rate in adult-onset asthma is much lower [87, 88] compared to childhood-onset asthma where 29-65% of the patients is in remission in early adulthood [89-91]. The incidence of remission in adult-onset asthma patients varies from 0.6% to 2% per year [59, 90, 92-94]. A markedly increased chance of remission has been observed retrospectively in the period shortly (4-7 years) after onset of the disease, after which it decreases quickly [90]. Interpreting and comparing the different incidences found is further complicated by the considerable variability in the definition of asthma remission. The definition varies from just absence of symptoms to complete normalization of bronchial hyperreactivity or no need for asthma treatment anymore. In the current paragraph we did not adhere to one specific definition.

PREDICTING REMISSION IN ADULT ASTHMA

Several cohort studies have been performed to find factors associated with asthma remission, albeit mainly in mixed childhood and adult-onset asthma populations. In most studies gender was not significantly associated with remission [59, 88, 92, 94]. Except in one study where men were more likely to go into remission [90]. Asthma remission occurs more often in younger subjects[92, 93] and in subjects with a short disease duration [90, 93]. Patients with a higher age of asthma-onset were less likely to have asthma remission [88, 90, 95], as were middle aged and elderly patients [92]. In contrast, others observed an increased remission rate in females with a higher age at diagnosis [68].

Asthma remission is most commonly seen in patients with mild asthma. In many studies this was characterized by minimal levels of symptoms or complete asthma control and low dose asthma medication use at baseline [59, 68, 93, 94, 96, 97]. Whereas patients with no or partial asthma control were very unlikely to be in remission at the end of follow-up, this accounts for both early and late-onset asthma [68, 93, 98, 99]. Moreover, patients in remission had a higher FEV_1 at baseline [59, 93, 95] and the highest increase in FEV_1 during follow-up compared to patients with persistent asthma [93]. Comorbid conditions, such as allergic sensitization or rhinitis, are more often absent in patients in remission [59].

An important external influential factor is smoking. One study found the years of smoking prior to the onset of asthma were significantly lower in patients with asthma remission after a follow-up period [88]. Furthermore, remission occurs more frequent in non-smokers or ex-smokers as compared to current smokers [59, 94]. Finally, cessation of smoking during the follow-up period increased the odds of having asthma remission by 6 times [92].

REMISSION IN OCCUPATIONAL ASTHMA

Remission of occupational asthma can only be achieved if exposure to the causative agent is ceased [86, 100, 101]. Even then, the remission rate in occupational asthma varies between 5-46% after up to two years of cessation [86, 100, 101]. None of the subjects who continued being exposed to allergens or irritants were in remission at the end of the follow-up period [86, 100, 101].

SUMMARY

In this review we summarized the known prognostic factors of adult-onset asthma with respect to lung function decline, increased asthma severity and asthma remission. The number of longitudinal studies investigating the course of adult-onset asthma is limited, which highlights the need for further follow-up studies. Lung function decline is steeper in non-atopic adult-onset asthma patients; they are at risk for developing persistent airflow limitation. Furthermore, male gender and smoking are predictors of poorer lung function and poor baseline lung function is associated with a lower lung function at follow up. Increased asthma severity is influenced by smoking and low lung function, whereas current uncontrolled asthma and smoking predict having uncontrolled asthma in the future. Asthma exacerbations can be predicted by high symptom scores, low lung function and markers of airway eosinophilia. Remission rate of adult-onset asthma is low and mainly seen in patients with mild asthma and short disease duration. Smoking has a profound negative effect on asthma remission. The most important factor for prognosis of occupational asthma is ceasing exposure to the causative agent, which has a positive effect on all aspects of the disease. Taken together, two prominent influential factors in all domains of adult-onset asthma prognosis are smoking and baseline lung function. Clinicians treating adult-onset asthma patients should notice these factors and realize these patients are at risk for a poor prognosis. However, more research is needed in order to offer patients more certainty about their prognosis and possible treatable factors.

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Part 1

MARKERS OF DISEASE ACTIVITY; AIRWAY INFLAMMATION

Chapter 3

BIOMARKERS TO IDENTIFY SPUTUM EOSINOPHILIA IN DIFFERENT ADULT ASTHMA PHENOTYPES

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ABSTRACT

BACKGROUND: Several biomarkers have been used to assess sputum eosinophilia in asthma. It has been suggested that the diagnostic accuracy of these biomarkers might differ between asthma phenotypes. We investigated the accuracy of biomarkers in detecting sputum eosinophilia (≥3%) in different adult asthma phenotypes.

METHODS: Levels of eosinophils in blood and sputum, FeNO and total IgE from 336 adult patients, enrolled in 3 prospective observational clinical trials and recruited at five pulmonology outpatient departments, were analyzed. Area's under the receiver operating characteristics curves (AUC) for detecting sputum eosinophilia were calculated, and compared between severe and mild, obese and non-obese, atopic and non-atopic, and (ex-)smoking and never-smoking asthma patients.

RESULTS: Sputum eosinophilia was present in 116 patients (35%). In the total group the AUC was 0.83 (95%CI 0.78-0.87) for blood eosinophils, 0.82 for FeNO (0.77-0.87) and 0.69 (0.63-0.75) for total IgE. AUC's were similar for blood eosinophils and FeNO between different phenotypes. Total IgE was less accurate to detect sputum eosinophilia in atopic and obese patients than in non-atopic and non-obese patients.

CONCLUSION: Blood eosinophils and FeNO had comparable diagnostic accuracy (superior to total IgE) to identify sputum eosinophilia in adult asthma patients irrespective of asthma phenotype such as severe, non-atopic, obese and smoking-related asthma.

INTRODUCTION

Eosinophilic airway inflammation is an important distinguishing characteristic of specific adult asthma phenotypes [1]. To assess this type of airway inflammation, sputum eosinophil counts are generally considered as the gold standard [2]. Treatment guided by sputum eosinophils reduces the frequency of asthma exacerbations [3] and patients with sputum eosinophilia have a better response to inhaled corticosteroids with respect to reducing airway hyperresponsiveness, decreasing asthma symptoms and improving quality of life compared to those without [4, 5]. Not surprisingly, the recent ATS/ERS guidelines on severe asthma recommend sputum eosinophils combined with clinical criteria to guide asthma therapy [6]. Unfortunately, sputum induction and differential sputum cell counts are only feasible in specialized clinics, are not always successful and do not give immediate results [7].

Several alternative methods to assess airway eosinophilia have been proposed in the literature, including non-invasive biomarkers such as the fraction of exhaled nitric oxide (FeNO) [8-10], peripheral blood eosinophil counts [10, 11] and total immunoglobulin E (IgE) [10], with varying diagnostic accuracy. However, specific patient characteristics that distinguish between different adult asthma phenotypes such as asthma severity [12], obesity [13], atopy [14], and (ex-)smoking status [15] may influence both airway and systemic inflammation. Therefore, the accuracy of biomarkers to assess sputum eosinophilia may vary between these different asthma phenotypes.

The aim of the present study was to evaluate the diagnostic accuracy of FeNO, blood eosinophils and total IgE for detecting sputum eosinophilia, defined as ≥3% [16, 17] in a large heterogeneous group of adult asthma patients, as well as in patients with different asthma phenotypes.

METHODS

PATIENTS

We collected data from 571 patients with adult-onset asthma (onset of asthma after the age of 18) who had been included in three separate observational clinical trials (Netherlands Trial Register numbers: NTR2217, NTR1846 and NTR1838) [18, 19] between 2009 and 2012. These prospective trials aimed at phenotyping patients with adult-onset asthma based on an extensive set of clinical, functional and inflammatory parameters. Patients aged 18 years or older were eligible if they had a confirmed diagnosis of asthma based on international guidelines (history of variable respiratory symptoms and documented variable expiratory airflow limitation) [20]. Patients with other pulmonary diseases, non-related major co-morbidities, pregnancy or a smoking history of > 10 pack years combined with fixed airflow obstruction/reduced diffusion capacity were excluded. Detailed in- and exclusion criteria have been reported elsewhere ([18, 19] and NTR2217). All eligible patients visiting the pulmonology outpatient department of four secondary and one tertiary referral clinic in the Netherlands were invited to participate. All three trials were reviewed and approved by medical ethical boards before their initiation. All patients gave informed consent. The present additional analysis was registered in the Netherlands Trial Register under number NTR 4589.

ASSESSMENT OF SPECIFIC PHENOTYPIC CHARACTERISTICS

a. **Asthma severity**
   Asthma severity was assessed according to the IMI-criteria, 21 based on medication use and degree of asthma control. Severe asthma was defined by the use of ≥1000 µg/day fluticasone equivalent and/or daily oral corticosteroids plus a second controller, combined with an asthma control score >1.5 [22] on the Juniper Asthma Control Questionnaire (ACQ)[23] or at least 2 exacerbations in the past 12 months. Patients who did not fulfill these criteria were considered as having mild-moderate asthma.
b. **Obesity**
   Obesity was defined as a body mass index (BMI) ≥30 kg/m2.
c. **Atopy**
   Specific IgE to common aeroallergens was measured by ImmunoCAP; atopy was defined as specific IgE >0.35 Ku/L for at least one allergen.
d. **Smoking status**
   Smoking status was recorded during history taking. (Ex-)smokers were either current or previous smokers. Non-smokers were patients that had never smoked.

REFERENCE STANDARD: SPUTUM EOSINOPHILS

Sputum induction was performed according to internationally accepted standards by trained lung function analysts [24]. All patients inhaled a nebulized saline solution for 5 minutes, if possible repeated up to 3 times. Sputum processing was performed according to full sample method and differential cell counts were analyzed on cytospin preparations. Results for different sputum cell types are presented as percentage of total non-squamous cell count. Laboratory analyses were performed blinded to patient characteristics and index test results.

INDEX TESTS: FENO, BLOOD EOSINOPHILS AND TOTAL IGE

FeNO (index test 1) was measured with a portable rapid-response chemoluminescent analyser (flow rate 50mL/s; NIOX System, Aerocrine, Sweden). FeNO results are reported as parts per billion (ppb) [25].

Venous blood was collected and differential white blood cells counts were performed. Absolute blood eosinophil numbers (index test 2) are reported as 10^9 cells per liter. Total IgE (index test 3) was measured by ImmunoCAP and reported as Ku/L. All measurements in blood samples were performed by the general laboratories of the participating hospitals and blinded to the outcome of other tests.

All data were collected in 1-2 visits less than 2 weeks apart.

STATISTICAL ANALYSIS

Adequate sputum samples from 336 patients were available (Figure E1, Study flowchart) and these patients were included in the analyses of diagnostic accuracy. Baseline characteristics between patients with and without adequate sputum were compared . Patients with missing data on blood esoinophils, FeNO or total IgE were excluded for the analysis of that index test.

Receiver operator characteristics curve (ROC) analysis was used to evaluate the diagnostic accuracy of FeNO, blood eosinophils, total IgE and their combinations to identify sputum eosinophilia ≥3%. This was done first in the complete group and thereafter in subgroups with specific phenotypic patient characteristics as described above. Analysis included the following: a) area under the ROC curve (AUC) (95% confidence interval (95%CI)) for the different biomarkers (FeNO, blood eosinophils, total IgE), b) sensitivity (95%CI) and corresponding threshold of each biomarker at a specificity of ≥95%, and c) specificity (95%CI) and corresponding threshold of each biomarker at a sensitivity of ≥95%. McNemar test was used to compare sensitivities and specificities between biomarkers. DeLong tests were used to compare AUC's between different asthma phenotypes and to evaluate whether combinations of any of the three biomarkers improved the diagnostic accuracy of each single biomarker.

We also developed a multivariate logistic regression model for the prediction of sputum eosinophilia ≥3% based on phenotypic features and the three markers. First, we evaluated whether patient characteristics (age, sex, BMI, asthma duration, race, smoking status, FEV_1 post bronchodilator, FEV_1/FVC post bronchodilator, atopy status, medication use (high dose

vs. low dose) were significantly associated with sputum eosinophilia in a univariate analysis ($p<0.20$). With the significant characteristics we then built a multivariable logistic model. We then used a stepwise procedure to arrive at a parsimonious model by removing in each step the variable with the smallest Wald statistic, until further removal would lead to a significant loss in goodness-of-fit ($p<0.05$; likelihood-ratio test). Then, the three markers were added to the resulting multivariable model, and the stepwise procedure was repeated.

Data were analyzed using SPSS version 22 and R version 3.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Table I shows baseline characteristics of the 336 patients who were included in the analyses. Compared to these patients, the excluded patients (n=235) were younger, more often female and had slightly lower blood eosinophils (Table E1). Sputum eosinophilia was present in 116 patients (35%). FeNO, blood eosinophils and total IgE were missing in 10, 5 and 4 included patients, respectively. Correlations of the three biomarkers with sputum eosinophils are shown in Figure E2.

DIAGNOSTIC ACCURACY OF BIOMARKERS

In the complete group as well as in the 8 subgroups, FeNO and blood eosinophils had similar diagnostic accuracy, whereas the AUC for total IgE was significantly lower (Tables 2 and 3). Combining FeNO and blood eosinophils significantly improved diagnostic accuracy compared to FeNO alone (p=0.001) or blood eosinophils alone (p=0.027) (AUC 0.87 (95%CI 0.83-0.91),Table 2-3, Figures 1-2). Adding total IgE to the combination of FeNO and blood eosinophils did not significantly improve the AUC (0.87, p=0.732). Total IgE performed significantly better in obese than in non-obese patients, and in non-atopic compared to atopic patients, respectively (Table 3, Figure 2).

A multivariable logistic model was created and reduced using stepwise backward selection. The final model included age, sex, FEV_1/FVC, pulmonary medication (high or low ICS dose), FeNO and blood eosinophils (Table E2). This model further improved the diagnostic accuracy to a minimal extend compared to FeNO and blood eosinophils combined (AUC 0.89 (95%CI 0.85-0.93), p=0.041).

Sensitivity, specificity and biomarker thresholds

Table 2 shows the sensitivity and specificity for each biomarker at either a high specificity or sensitivity and the associated threshold of this marker; Figure E3 shows the formula to calculate the probability of sputum eosinophilia for the combined model of FeNO and blood eosinophils.

At a sensitivity of ≥95% (i.e. low number of false negatives) FeNO, blood eosinophils and total IgE had a comparable specificity, whereas the specificity of FeNO and blood eosinophils combined was significantly higher. Negative predictive values ranged between 0.92 and 0.94 for biomarker values below the corresponding thresholds.

At a specificity of ≥95% (i.e. low number of false positives) sensitivities for FeNO, blood eosinophils and their combination did not significantly differ, but the sensitivity of total IgE was significantly lower compared to the other biomarkers. The positive predictive values of FeNO, blood eosinophils and their combination ranged from 0.79 to 0.84, but was only 0.47 for total IgE.

With these thresholds (Table 2), the biomarkers can be used in up to half of the patients,

as they had test results below the lower threshold or above the upper threshold: 47% for FeNO and blood eosinophils combined (150/322), 36% for FeNO (117/326), 34% for blood eosinophils (113/331) and 25% for total IgE (83/332).

Thresholds for the separate biomarkers in different phenotypes are summarized in Table 3, details are shown in the appendix (Table E3-E10). Across subgroups, thresholds were relatively stable for FeNO and the FeNO/blood eosinophils combination model, but varied considerably for the upper levels of blood eosinophils and IgE.

Table 1. Baseline characteristics of patients who provided an adequate sputum sample (n=336).

Age, years	53	± 13
Sex, female (%)	55	
BMI, kg/m^2	28	± 5
Age of asthma onset	45	± 15
Asthma duration (years)	3	(0-10)
Current or ex-smoker (%)	54	
Pack years	1	(0-13)
ICS, fluticasone equivalent µg	500	(250-500)
ACQ-score	1.3	± 0.8
Atopy (%)	32	
Nasal polyposis (%)	19	
pb FEV1 % predicted	97	± 18
pb FEV1/FVC % predicted	93	± 12
FeNO, ppb	23	(13-42)
Total IgE, Ku/L	56	(18-216)
Blood neutrophils, $x10^9$/l	4.3	± 1.7
Blood eosinophils, $x10^9$/l	0.2	(0.1-0.3)
Sputum neutrophils, %	66.3	(45.4-82.3)
Sputum eosinophils, %	0.8	(0.1-6.6)

Data are presented as mean ± SD, percentage or median (interquartile range).
BMI, body mass index; ICS, inhaled corticosteroid; ACQ, asthma control questionnaire, FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; FeNO, fraction of exhaled nitric oxide; ppb, part per billion.

Table 2. Diagnostic accuracy of the biomarkers in the complete group (n=336).

Test	AUC	Positivity threshold	Sensitivity	Specificity	PPV	NPV	TP	FP	FN	TN
FeNO	0.82 (0.77-0.87)	≥12.2 ppb	0.96 [0.90, 0.99]	0.28 [0.22, 0.34]	0.41 [0.35, 0.47]	0.92 [0.82, 0.97]	108	154	5	59
		≥64.5 ppb	0.39 [0.30, 0.49]	0.95 [0.92, 0.98]	0.81 [0.68, 0.90]	0.75 [0.69, 0.80]	44	10	69	203
Blood eosinophils	0.83 (0.78-0.87)	≥0.09x10^9/L	0.96 [0.90, 0.99]	0.26 [0.20, 0.33]	0.40 [0.34, 0.46]	0.92 [0.81, 0.97]	108	161	5	57
		≥0.41x10^9/L	0.36 [0.27, 0.46]	0.95 [0.91, 0.97]	0.79 [0.65, 0.88]	0.74 [0.69, 0.79]	41	11	72	207
Total IgE	0.69 (0.63-0.75)	≥13.5 kU/L	0.96 [0.90, 0.99]	0.28 [0.22, 0.34]	0.41 [0.35, 0.47]	0.92 [0.82, 0.97]	110	157	5	60
		≥763.5 kU/L	0.08 [0.04, 0.14]	0.95 [0.92, 0.98]	0.47 [0.25, 0.71]	0.66 [0.61, 0.71]	9	10	106	207
FeNO+blood eosinophils	0.87 (0.83-0.91)	≥0.095*	0.95 [0.90, 0.99]	0.39 [0.32, 0.46]	0.45 [0.39, 0.52]	0.94 [0.86, 0.98]	106	129	5	82
		≥0.70*	0.46 [0.36, 0.56]	0.95 [0.91, 0.98]	0.84 [0.71, 0.91]	0.77 [0.71, 0.82]	51	10	60	201

Table 3. Distribution of marker thresholds at 95% sensitivity and specificity in different asthma phenotypes.

	Range lower threshold, sensitivity ≥95%	Range upper threshold, specificity ≥95%
FeNO, ppb	8.6-15.1	48.5-69.5
Blood eosinophils, x10^9/L	0.06-0.095	0.34-0.73
Total IgE, kU/L	8.5-25.5	389-2181
FeNO+blood eosinophils	0.086-0.138*	0.656-0.75*

* All test combinations were log transformed; these values correspond to an individual's probability of sputum eosinophilia, as determined by the formula provided in Figure E2.
AUC, area under the curve; FeNO, fraction of exhaled nitric oxide; FN, false negative; FP, false positive; TN, true negative; TP, true positive.

Table 4. Diagnostic accuracy in in patients with different asthma phenotypes.

Obesity	Non-obese	Obese	p-value
Eosinophilia (<3%/≥3%)	154/82	66/34	0.90
FeNO	0.83 (0.77-0.88)	0.78 (0.68-0.89)	0.46
Blood eosinophils	0.83 (0.77-0.89)	0.82 (0.73-0.91)	0.82
IgE	0.73 (0.67-0.80)	0.59 (0.47-0.70)	**0.03**
FeNO + Blood eosinophils	0.88 (0.83-0.93)	0.85 (0.76-0.93)	0.55

Atopy	Non-atopic	Atopic	
Eosinophilia (<3%/≥3%)	153/74	67/42	0.28
FeNO	0.83 (0.77-0.89)	0.78 (0.69-0.88)	0.40
Blood eosinophils	0.83 (0.77-0.89)	0.83 (0.74-0.91)	0.99
IgE	0.75 (0.68-0.82)	0.57 (0.46-0.68)	**<0.01**
FeNO + Blood eosinophils	0.88 (0.82-0.92)	0.85 (0.77-0.93)	0.63

Asthma severity	Mild-moderate	Severe asthma	
Eosinophilia (<3%/≥3%)	161/58	58/57	**<0.01**
FeNO	0.81 (0.74-0.88)	0.83 (0.75-0.91)	0.67
Blood eosinophils	0.82 (0.76-0.89)	0.80 (0.72-0.89)	0.73
IgE	0.68 (0.61-0.76)	0.66 (0.56-0.76)	0.70
FeNO + Blood eosinophils	0.86 (0.81-0.92)	0.85 (0.78-0.92)	0.81

Smoking status	Never smoker	Ex- or current smoker	
Eosinophilia (<3%/≥3%)	103/51	117/65	0.62
FeNO	0.84 (0.77-0.90)	0.81 (0.73-0.88)	0.52
Blood eosinophils	0.86 (0.79-0.93)	0.80 (0.73-0.87)	0.23
IgE	0.64 (0.55-0.73)	0.74 (0.66-0.81)	0.13
FeNO + Blood eosinophils	0.88 (0.82-0.94)	0.86 (0.80-0.92)	0.64

AUC and 95% confidence interval are given per biomarker in every subgroup. The difference between the AUC's for every biomarker is compared within the separate subgroups, result depicted as p-value. AUC, area under the curve; FeNO, fraction of exhaled nitric oxide.

Figure 1. ROC curves for FeNO, blood eosinophils, total IgE and a combined model (n=336).

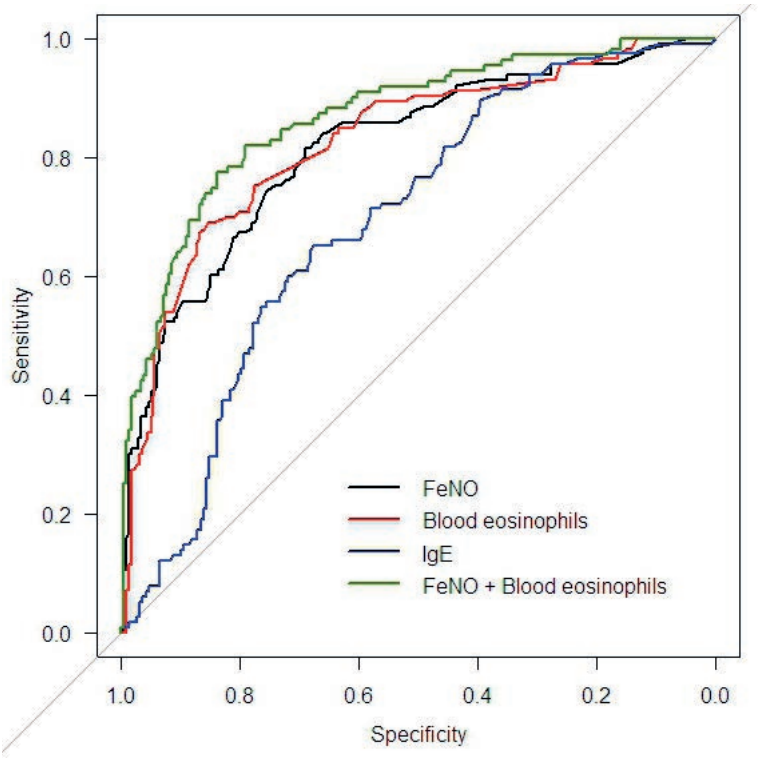
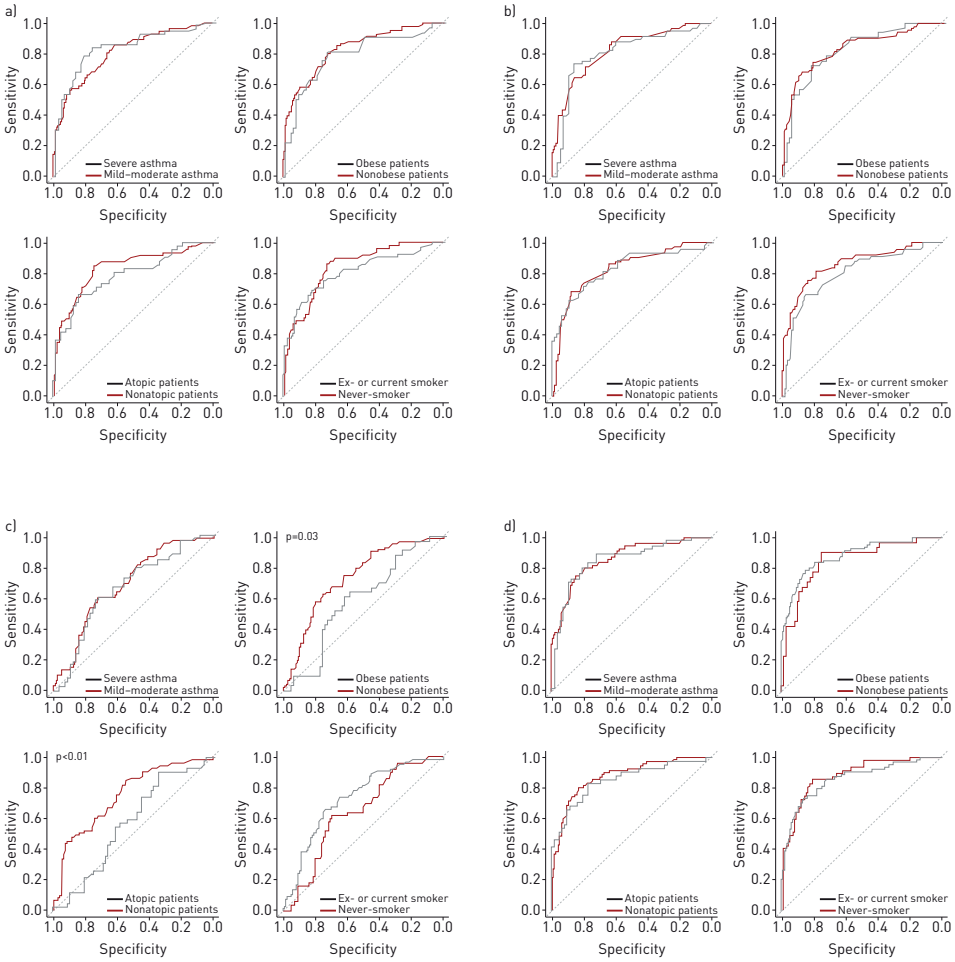


Figure 2. ROC curves for FeNO (a), blood eosinophils (b), total IgE (c) and a combined model of FeNO and blood eosinophils (d) in patients with different asthma phenotypes.



DISCUSSION

This study shows that the diagnostic accuracy of FeNO and blood eosinophils to detect sputum eosinophilia did not significantly differ between obese and non-obese, atopic and non-atopic, (ex-)smoking and never-smoking, and severe and mild-moderate asthma patients. Total IgE was less accurate in atopic and obese patients than in non-atopic and non-obese patients. In unselected adult-onset asthma patients the diagnostic accuracy of FeNO and blood eosinophils is superior to that of total IgE, whilst combining FeNO and blood eosinophils into one model improves the overall diagnostic accuracy. The results suggest that FeNO and blood eosinophils (but not total IgE) can be used to confirm or exclude sputum eosinophilia with high certainty in up to half of adult asthma patients irrespective of asthma phenotype.

The present study is the first to compare the diagnostic accuracy of FeNO, blood eosinophils, total IgE and their combinations between different adult asthma phenotypes. Previous studies have mainly investigated the diagnostic accuracy of these biomarkers in general asthma populations. Our findings in the total study group of asthma patients on FeNO, blood eosinophils and total IgE are in line with the results of these previous studies, which we recently summarized in a systematic review [26], in which we found a pooled AUC of 0.75 for FeNO, 0.78 for blood eosinophils and 0.65 for total IgE. Our findings on FeNO and blood eosinophils are more promising than those of two other recent reports [10, 27] in which the authors concluded that FeNO and blood eosinophils lack sufficient sensitivity or specificity to be useful as markers of sputum eosinophilia. In addition, we developed a combination model of FeNO and blood eosinophils, which increased the diagnostic accuracy significantly compared to the separate markers. Adding four clinical variables to the model further increased the AUC, although only to a very minimal extend. For clinical purposes the use of two variables is obviously more practical.

The diagnostic accuracy of FeNO and blood eosinophils in detecting sputum eosinophilia was similar in the different asthma phenotypes. This may be surprising, since remarkable differences in airway eosinophilia and its associated cytokines and markers have been described in specific asthma subgroups, for example, between obese and non-obese asthma patients [28]. One study showed more eosinophils in the airway submucosa than in the airway lumen of obese patients with asthma, and also higher levels of interleukin(IL)-5 in BAL fluid [13]. Apparently, only a subset of obese asthma patients with eosinophilic airway inflammation shows sputum eosinophilia. In our study, total IgE was relatively more accurate to predict sputum eosinophilia in non-obese patients as compared to obese patients, but had lower diagnostic accuracy than the other two biomarkers. Discordance between different biomarkers for airway eosinophilia has been reported previously.[17, 29] More interestingly, discordance between various biomarkers to effects of anti-inflammatory therapy or ability to predict

asthma attacks have also been noted for various biomarkers.[7, 29, 30] These data suggest that discordance between biomarkers in different asthma phenotypes may point towards different underlying mechanisms.

There was no significant difference in diagnostic accuracy of FeNO and blood eosinophils between atopic and non-atopic patients. One previous study showed lower diagnostic accuracy for FeNO in non-atopic patients than in atopic patients [8]. The discrepancy between these results and ours could be due to differences in patients' characteristics or the devices used to measure FeNO. The higher diagnostic accuracy of total IgE in non-atopic patients as compared to atopic patients might be related to different underlying mechanisms. While eosinophilia in classical atopic asthma is likely to be T-helper cell (T_H)-2 driven and includes higher basal IgE production, in non-atopic asthma, there is accumulating evidence that activation of eosinophils might be mediated by alternative pathways [14].

Patients with severe asthma often show discrepancies between airway and blood eosinophilia, which is probably explained by their high doses of inhaled or oral corticosteroid treatment. We did not find a difference in the diagnostic accuracy of blood eosinophils or FeNO between mild-moderate and severe asthma patients, but previous studies have found conflicting results. One study found and AUC of blood eosinophils of 0.55 in corticosteroid-treated patients, and of 0.73 in untreated patients [31], whereas these numbers were 0.75 and 0.62, respectively, in another study [10]. Three previous studies evaluated the accuracy of FeNO among severe/treated and mild/untreated asthma patients [8, 10, 32]. None of them found considerable differences in the differences in the AUC's. Remarkably, despite comparable AUC's for FeNO and blood eosinophils in our study, the upper threshold range for blood eosinophils was relatively wide due to the higher threshold in patients with severe asthma as compared to the other asthma phenotypes (Table 3 and E10). Apparently, a subset of patients with severe asthma shows elevated levels of blood eosinophils without evidence of airway eosinophilia, which confirms previous findings[12]. Circulating eosinophils might serve as a reservoir in these patients, thereby maintaining airway inflammation, which cannot be adequately suppressed by inhaled corticosteroids.

Smoking in asthma has often been associated with neutrophilic airway inflammation [15], and enhancement of T_H2 mediated inflammation[33], and has also been shown to be associated with reduced FeNO levels [34]. Therefore, (ex)smoking could have had an effect on the diagnostic accuracy of FeNO to detect sputum eosinophilia [8, 9]. A previous study found a lower AUC for FeNO among smokers compared to non-smokers (0.63 vs. 0.77) [8], but this was obviously not the case in our study. This suggests that even in smokers and ex-smokers FeNO can be used as a biomarker for sputum eosinophilia.

The major strength of our study is the large number and the extensive characterization of the patients, which enabled us to investigate clinical (sub)phenotypes of adult-onset asthma. Another strength is that we reported biomarker thresholds at either high sensitivity or high specificity. These cut-off points are more useful for practicing physicians to confirm or exclude airway eosinophilia with high certainty. A limitation of this approach, however, is that this method only gives a clear outcome in up to half of the patients; the remainder of the patients still needs to undergo sputum induction to confirm or exclude sputum eosinophilia. Another possible limitation of our study is the number of missing sputum samples, in particular in patients with mild-moderate asthma. This limits the extrapolation of our results to all patients with adult asthma. However, unsuccessful sputum induction in mild-moderate asthma might be indicative of a low level of sputum eosinophils, which fits in with the observed lower level of blood eosinophils in this group.

Our study has clinical implications. First, it shows that in a large subset of adult patients airway eosinophilia can be identified with high certainty by using FeNO and blood eosinophils instead of induced sputum. Second, it shows that the accuracy of these biomarkers is similar in various subtypes and severities of asthma. Currently, FeNO and blood eosinophils are mainly used in clinical trials to identify patients with eosinophilic asthma who are eligible for treatment with novel targeted therapies. For example for mepolizumab, a blood eosinophil cutoff >0,15*10^9/L was introduced to detect eosinophilic asthma and predict reduction of asthma exacerbations [35]. Our data show that this is an adequate threshold to detect eosinophilia, since an eosinophil count <0.09*10^9/L is associated with absence of airway eosinophilia in 92% of the patients. Still, consensus about the respective biomarker thresholds is needed, as well as an algorithm and external validation that incorporates a combination of biomarkers.

In conclusion, we showed that FeNO and blood eosinophils have a comparable diagnostic accuracy to identify airway eosinophilia in adult asthma patients irrespective of phenotypic characteristics such as asthma severity, atopy, obesity and smoking status, and, possibly, irrespective of underlying pathways leading to airway eosinophilia. In future clinical trials and day-to-day practice both markers, preferably in combination, may become the preferred method to assess eosinophilic airway inflammation and to guide targeted treatment in adult asthma patients with different phenotypes.

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SUPPLEMENTARY MATERIAL

Table E1. Comparison of characteristics of all adult-onset asthma patients who provided an adequate sputum sample and who did not.

	No sputum available (n=235)	Sputum available (n=336)	p-value
Age, years	49 ± 14	53 ± 13	**0.002**
Sex, female (%)	69	55	**0.002**
BMI, kg/m^2	28 ± 5	28 ± 5	0.586
Age of asthma onset	43 ± 14	45 ± 15	0.101
Asthma duration in years	2 (0-7)	3 (0-10)	**0.038**
Current smoker/ex-smoker (%)	52	54	0.896
Pack years (med)	9 (5-18)	11 (4-21)	0.841
ICS, fluticasone equivalent µg	250 (250-750)	500 (250-500)	0.208
ACQ-score	1.4 ± 1.0	1.3 ± 0.8	0.233
Atopy (%)	33	32	0.935
Nasal polyposis (%)	19	19	0.964
pb FEV1 % predicted	97 ± 19	97 ± 18	0.664
pb FEV1/FVC % predicted	94 ± 12	93 ± 12	0.397
Fraction of exhaled NO, ppb	21 (13-38)	23 (13-42)	0.124
Total IgE, Ku/L	55 (20-143)	56 (18-216)	0.332
Blood neutrophils, $x10^9$/l	4.2 ± 1.8	4.3 ± 1.7	0.335
Blood eosinophils, $x10^9$/l	0.15 (0.08-0.28)	0.20 (0.10-0.30)	**0.014**

Data are presented as mean ± SD, percentage or median (range).

Table E2. Multivariate logistic regression models

FeNO + Blood Eosinophils	B	Exp(B)	95% C.I.for EXP(B)	
			Lower	Upper
Log FeNO (ppb)	1.47	4.36	2.81	6.78
Log Blood Eosinophils ($x10^9$/l)	1.07	2.90	1.96	4.31
Constant	-3.92	0.02		

Baseline characteristics + FeNO + Blood Eosinophils	B	Exp(B)	95% C.I.for EXP(B)	
			Lower	Upper
Age (years)	0.01	1.01	0.98	1.04
Female gender	0.17	1.18	0.61	2.30
FEV/VC post ≥70%	-1.38	0.25	0.13	0.50
High medication use	0.80	2.23	1.17	4.26
Log FeNO (ppb)	1.47	4.33	2.67	7.04
Log Blood Eosinophils ($x10^9$/l)	0.97	2.63	1.76	3.93
Constant	-4.23	0.02		

Table E3. Threshold per marker at either 95% sensitivity or specificity in atopic patients.

Test	Threshold	Sensitivity	Specificity
FeNO	≥15.1 ppb	0.95	0.25
	≥64.3 ppb	0.42	0.95
Blood Eosinophils	≥0.085 x10^9/L	0.95	0.20
	≥0.46 x10^9/L	0.45	0.96
Total IgE	≥25.5 kU/L	0.95	0.06
	≥2181 kU/L	0.02	0.96
FeNO+blood eosinophils	≥0.13*	0.95	0.29
	≥0.714*	0.51	0.95

Table E4. Threshold per marker at either 95% sensitivity or specificity in non-atopic patients.

Test	Threshold	Sensitivity	Specificity
FeNO	≥9.9 ppb	0.96	0.16
	≥48.5 ppb	0.44	0.95
Blood Eosinophils	≥0.085 x10^9/L	0.96	0.29
	≥0.41 x10^9/L	0.31	0.95
Total IgE	≥8.5 kU/L	0.96	0.26
	≥389 kU/L	0.95	0.10
FeNO+blood eosinophils	≥0.086*	0.96	0.43
	≥0.656*	0.47	0.95

Table E5. Threshold per marker at either 95% sensitivity or specificity in non-obese patients.

Test	Threshold	Sensitivity	Specificity
FeNO	≥13.1 ppb	0.95	0.33
	≥58.5 ppb	0.42	0.95
Blood Eosinophils	≥0.06 x10^9/L	0.95	0.22
	≥0.41 x10^9/L	0.37	0.95
Total IgE	≥13.5 kU/L	0.95	0.33
	≥586 kU/L	0.13	0.95
FeNO+blood eosinophils	≥0.12*	0.95	0.46
	≥0.68*	0.50	0.95

Table E6. Threshold per marker at either 95% sensitivity or specificity in obese patients.

Test	Threshold	Sensitivity	Specificity
FeNO	≥8.6 ppb	0.97	0.08
	≥69.5 ppb	0.28	0.95
Blood Eosinophils	≥0.095 x10^9/L	0.97	0.25
	≥0.49 x10^9/L	0.25	0.95
Total IgE	≥15.5 kU/L	0.97	0.18
	≥1081.5 kU/L	0.03	0.96
FeNO+blood eosinophils	≥0.086*	0.97	0.37
	≥0.75*	0.42	0.95

Table E7. Threshold per marker at either 95% sensitivity or specificity in never smokers.

Test	Threshold	Sensitivity	Specificity
FeNO	≥15.1 ppb	0.96	0.41
	≥55.5 ppb	0.43	0.95
Blood Eosinophils	≥0.085 x10^9/L	0.96	0.29
	≥0.375 x10^9/L	0.54	0.95
Total IgE	≥15.5 kU/L	0.95	0.29
	≥1081.5 kU/L	0.04	0.95
FeNO+blood eosinophils	≥0.13*	0.96	0.49
	≥0.709*	0.49	0.95

Table E8. Threshold per marker at either 95% sensitivity or specificity in (ex)smokers.

Test	Threshold	Sensitivity	Specificity
FeNO	≥9.6 ppb	0.95	0.14
	≥63.5 ppb	0.39	0.95
Blood Eosinophils	≥0.085 x10^9/L	0.95	0.24
	≥0.41 x10^9/L	0.28	0.95
Total IgE	≥13.5 kU/L	0.95	0.28
	≥496.5 kU/L	0.11	0.95
FeNO+blood eosinophils	≥0.086*	0.95	0.33
	≥0.658*	0.53	0.95

Table E9. Threshold per marker at either 95% sensitivity or specificity in mild-moderate asthma patients.

Test	Threshold	Sensitivity	Specificity
FeNO	≥12.2 ppb	0.96	0.27
	≥63.5 ppb	0.36	0.96
Blood Eosinophils	≥0.085 x10^9/L	0.96	0.25
	≥0.34 x10^9/L	0.39	0.95
Total IgE	≥13.5 kU/L	0.95	0.31
	≥586 kU/L	0.12	0.95
FeNO+blood eosinophils	≥0.138*	0.96	0.48
	≥0.68*	0.42	0.95

Table E10. Threshold per marker at either 95% sensitivity or specificity in severe asthma patients.

Test	Threshold	Sensitivity	Specificity
FeNO	≥12.4 ppb	0.95	0.30
	≥65 ppb	0.38	0.95
Blood Eosinophils	≥0.085 x10^9/L	0.95	0.28
	≥0.73 x10^9/L	0.18	0.95
Total IgE	≥22 kU/L	0.97	0.21
	≥1815 kU/L	0.02	0.95
FeNO+blood eosinophils	≥0.095*	0.95	0.37
	≥0.75*	0.46	0.95

* Table E3-E10: All test combinations were log transformed; these values correspond to an individual's probability of sputum eosinophilia, as determined by the formula provided in Figure E2.

Figure E1. Flowchart of patient selection, combination of 3 prospective cohort studies.

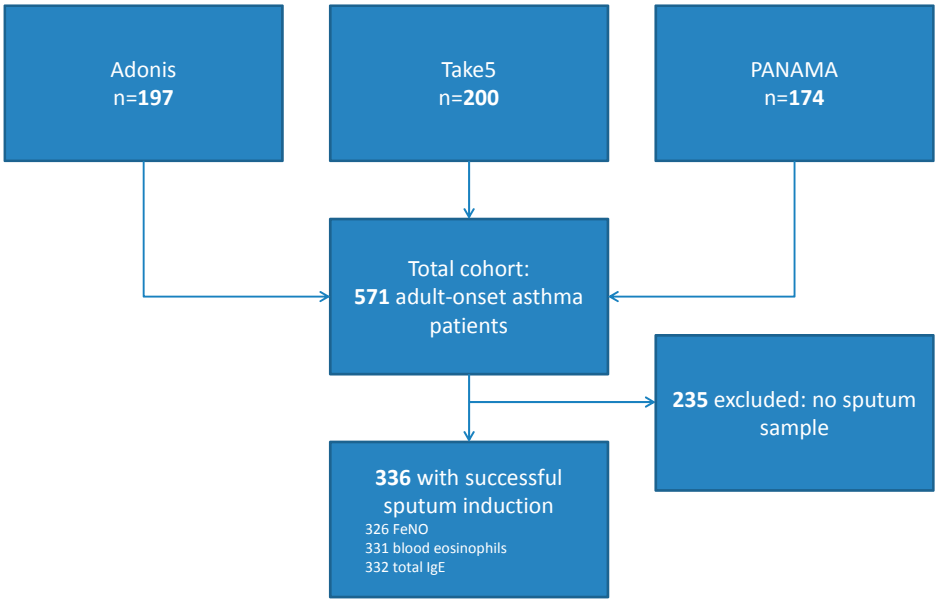
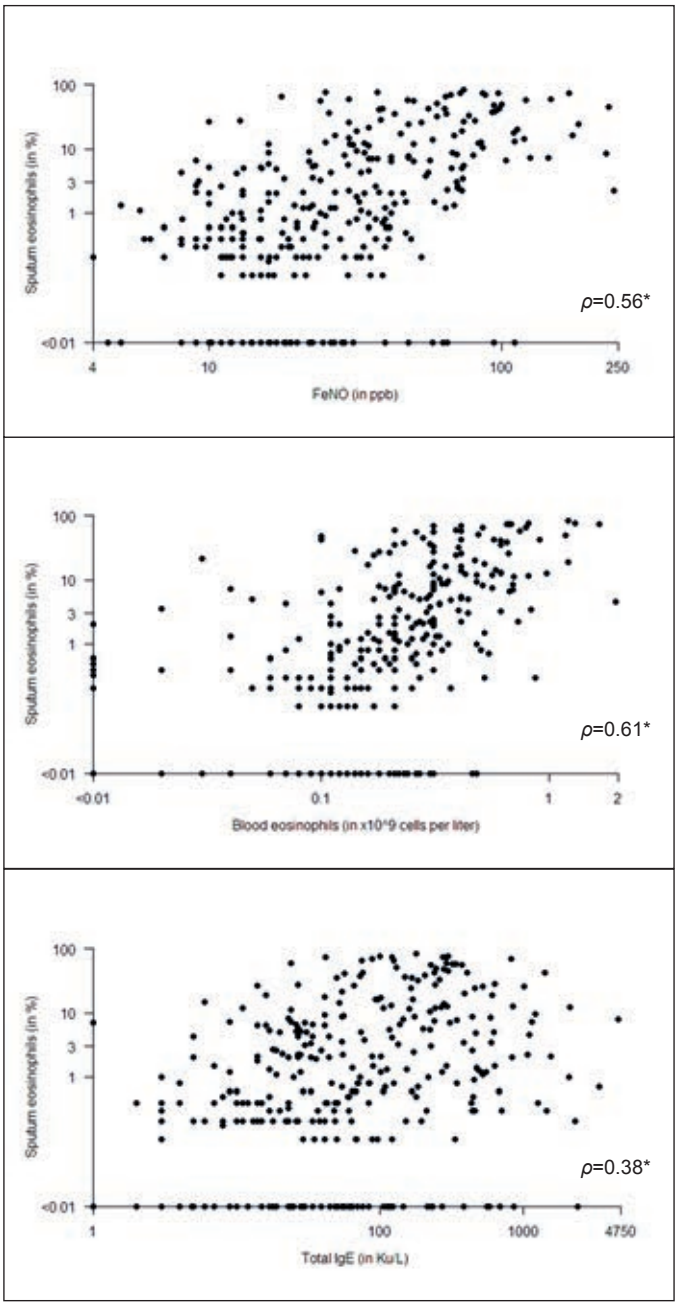


Figure E2. Correlation of FeNO, blood eosinophils and total IgE with sputum eosinophils.



* Spearman's rho (ρ) significant at p<0.01

Figure E3. Formula to calculate probability of sputum eosinophilia based on a model combining FeNO (ppb) and blood eosinophils (10^9 cells/l).

1 / (1 + (e^-(-3,920 + (LN(FeNO) x 1,473) + (LN(blood eos+0,01) x 1,066))))

Chapter 4

DIAGNOSTIC ACCURACY OF MINIMALLY INVASIVE MARKERS FOR DETECTION OF AIRWAY EOSINOPHILIA IN ASTHMA

SYSTEMATIC REVIEW AND META-ANALYSIS

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ABSTRACT

BACKGROUND: Eosinophilic airway inflammation is associated with increased corticosteroid responsiveness in asthma, but direct airway sampling methods are invasive and/or laborious. Minimally invasive markers for airway eosinophilia could present an alternative, but estimates of their accuracy vary.

METHODS: We performed a systematic review and searched MEDLINE, Embase and PubMed for studies evaluating the diagnostic accuracy of markers against a reference standard of induced sputum, bronchoalveolar lavage and/or endobronchial biopsy in patients with (suspected) asthma (inception-August 2014). Unpublished results were obtained by contacting authors of studies that did not report on diagnostic accuracy, but had data from which estimates could be calculated. Risk of bias was evaluated using QUADAS-2. Meta-analysis was used to produce summary estimates of accuracy.

RESULTS: We included 32 studies: 24 in adults and eight in children. Of these, 28 (84%) showed risk of bias in at least one domain. In adults, three markers had extensively been investigated: Fraction of Exhaled Nitric Oxide (FeNO) (17 studies; 3,216 patients; summary area under the receiver operator curve (AUC) 0.75 (95%CI 0.72-0.78)); blood eosinophils (14 studies; 2,405 patients; 0.78 (0.74-0.82)); total Immunoglobulin E (IgE) (7 studies; 942 patients; 0.65 (0.61-0.69)). In children, only FeNO (6 studies; 349 patients; summary AUC 0.81 (0.72-0.89)) and blood eosinophils (3 studies; 192 patients; 0.78 (0.71-0.85)) had been investigated in more than one study. Induced sputum was most frequently used as the reference standard. Summary estimates of sensitivity and specificity in detecting sputum eosinophils ≥3% in adults were: 0.66 (0.57-0.75) and 0.76 (0.64-0.85) for FeNO; 0.71 (0.65-0.76) and 0.77 (0.70-0.83) for blood eosinophils; 0.64 (0.42-0.81) and 0.71 (0.42-0.89) for IgE.

INTERPRETATION: FeNO, blood eosinophils and IgE have moderate diagnostic accuracy. Their use as a single surrogate marker for airway eosinophilia in asthmatic patients will lead to a substantial number of false positives and/or negatives.

INTRODUCTION

Historically, asthma control has been pursued by means of symptom and lung function monitoring[1]. Although the currently available asthma medications are effective in controlling the disease in most patients, a minority deteriorates despite maximal treatment. Non-eosinophilic asthma responds poorly to corticosteroid therapy, the current standard treatment for suppressing airway inflammation. Approximately half of the asthmatic patients seem to be persistently non-eosinophilic[2].

Bronchoalveolar lavage (BAL) and endobronchial biopsy (EBB) are the reference standards for identifying the extent of eosinophilic airway inflammation, but these tests are invasive and expensive. Another option is induced sputum, which has shown to be clinically useful in guiding asthma treatment[3].

A Cochrane review showed that the frequency of asthma exacerbations is significantly lower in patients in whom inhaled corticosteroids are tailored based on sputum eosinophila, compared to those in whom management is based on traditional methods of asthma monitoring[3]. Recent guidelines recommend guiding treatment in severe asthma by sputum eosinophil counts in addition to clinical criteria in centers experienced in using this technique[1;4]. Sputum eosinophilia may also have prognostic value as a marker for persistent airflow limitation[5], deteriorating asthma over time[6], and responsiveness to future therapies specifically targeting eosinophilic inflammation, such as Mepolizumab[7].

Unfortunately, sputum induction is time-consuming, requires experienced laboratory personnel, and many patients are unable to produce adequate samples. Several minimally invasive markers of eosinophilic airway inflammation, such as Fraction of exhaled Nitric Oxide (FeNO), blood eosinophils and serum periostin, may have potential as a surrogate to replace sputum induction, but their accuracy to distinguish between patients with and without airway eosinophilia remains controversial.

We performed a systematic review and meta-analysis with the purpose of obtaining summary estimates of the diagnostic accuracy[8] of markers for airway eosinophilia in asthmatic patients.

METHODS

ELIGIBILITY CRITERIA

Studies were included if they had evaluated the diagnostic accuracy of one or more blood, serum, nasal lavage, or exhaled breath markers[9] (index test) in detecting airway eosinophilia (target condition) in patients with (suspected) asthma. Direct airway sampling methods (induced sputum, BAL, and/or EBB) were considered as acceptable reference standards, independent of the threshold for positivity used. We excluded review articles.

SEARCH AND SELECTION

A medical information specialist (RS) developed searches in MEDLINE, Embase, and PubMed without date or language restrictions (Appendix 1). The searches were updated in August 2014. Two independent reviewers (DAK, GAW) examined titles and abstracts of all search results. Full reports of studies that were considered potentially eligible by at least one of them were obtained and independently assessed for inclusion. Disagreements were resolved by consensus. One reviewer (DAK) also scanned reference lists of included articles, and searched trial registries (ClinicalTrials.gov, Current Controlled Trials, Netherlands Trial Register, Australian New Zealand Clinical Trials Registry) for unpublished or ongoing studies.

DATA EXTRACTION AND QUALITY ASSESSMENT

One reviewer (DAK) performed data extraction, which was verified by a second reviewer (GAW). We identified the first author, country, journal, year of publication, recruitment setting, sample size and characteristics of included patients (age, gender, body mass index, atopy status, asthma severity, FEV_1 % predicted, smoking status, corticosteroid treatment status). We also extracted the index test(s), reference standard(s), test positivity thresholds, disease prevalence, accuracy estimates, and data for 2x2 tables presenting index test results by reference standard results for each reported threshold. If 2x2 tables were not reported, we attempted to reconstruct them from summary estimates or by contacting corresponding authors through email. If it appeared from an article that multiple markers had been assessed, but diagnostic accuracy data were not reported for all of them, we contacted authors to obtain these data. Two authors (DAK, GAW) independently assessed risk of bias and applicability concerns using QUADAS-2[10].

ENRICHMENT SAMPLE

To enrich the number of included studies, we tried to identify unpublished data by contacting authors of published studies that had not reported on the diagnostic accuracy of a marker to detect airway eosinophilia, but seemed to have data from which accuracy estimates could be calculated. Studies were selected if they had performed at least one index test and one reference standard, as defined above. Such studies were only eligible if they explicitly

distinguished eosinophilic from non-eosinophilic patients, included at least an arbitrary number of 50 asthmatic patients, and were published before January 2014. We contacted corresponding authors through email, and asked whether they were willing to calculate and share estimates of accuracy or to send their (blinded) dataset.

Whenever we obtained datasets, we evaluated diagnostic accuracy as follows. First, we estimated the ability of each index test to discriminate between patients with and without airway eosinophilia by calculating the Area Under the Receiver Operating Characteristic Curve (AUC-ROC). Then we selected the "optimal cutpoint" of sensitivity and specificity on the ROC curve using the Youden index, as had been done by almost all included diagnostic accuracy studies. Depending on the reference standard available, we repeated this analysis for each definition of airway eosinophilia used in the included studies. Patients with missing data on the index test or reference standard were excluded from the analysis for that specific marker. Datasets were analysed using R v3.0.

META-ANALYSIS

We analysed studies in children and adults separately. To get a view of the overall diagnostic performance of each marker, we performed random effects meta-analysis of AUC estimates[11], independent of which reference standard or definition of airway eosinophilia had been used. Whenever a study reported more than one AUC estimate for one marker in the same group of patients, for example because the study relied on multiple definitions of airway eosinophilia, we included the highest AUC reported. If a study reported an AUC estimate in the total study group as well as in subgroups, we only included the estimate for the total study group. However, if a study reported on these estimates in subgroups only and not in the total study group, we included the AUCs of all subgroups. If sufficient data were available (≥3 studies), we repeated this meta-analysis for studies that had used the same reference standard and airway eosinophilia definition. We assessed statistical heterogeneity using the I^2 statistic[12].

From each collected or reconstructed 2x2 table, we calculated estimates of sensitivity and specificity and 95% CIs. We used a hierarchical random effects model[8] to obtain summary estimates of sensitivity and specificity for studies that had used the same reference standard and airway eosinophilia definition. We did so whenever four or more tables were available. If articles provided data on direct, head-to-head comparisons of two or more markers, we evaluated whether there were significant differences in accuracy between markers. Such direct comparisons ensure that differences in accuracy are not caused by heterogeneity across study populations. We used Deeks' funnel plot asymmetry test to assess risk of publication bias[13]. SAS v9.2 was used to fit the models.

RESULTS

SEARCH AND SELECTION

The searches retrieved 2,919 unique records, all of them providing titles and/or abstracts in English language. Among these, we found 21 eligible diagnostic accuracy studies (Figure 1). Another 18 studies fulfilled the eligibility criteria for the enrichment sample. Contacting their authors led to eight additional inclusions. We could also include data from two studies from our own department, and identified one more through a conference poster. No additional studies were identified by scanning reference lists and searching trial registries. Overall, we included 24 studies performed in adults, and eight in children (Appendix 2).

STUDY CHARACTERISTICS

Detailed characteristics of included studies are provided in Appendix 3. In summary, all studies used a single set of inclusion criteria (cohort studies) and the number of patients included in the analysis of diagnostic accuracy varied from 24 to 566 in adults, and from 27 to 150 in children. The mean/median age ranged from 27.0 to 59.8 in adults, and from 6.8 to 13.0 in children.

In all cases, study participants had been recruited in secondary or tertiary care facilities and both males and females had been included. Studies in adults included asthma patients with varying severity: mild-moderate (n=4; 17%), mild-severe (n=4; 17%), moderate-severe (n=4; 17%), severe (n=5; 21%), or not reported (n=7; 29%). In children, asthma severity was mild (n=1; 13%), mild-severe (n=1; 13%), moderate-severe (n=1; 13%), severe (n=2; 25%), or not reported (n=3; 38%). In adults, 12 studies (50%) included current non-smokers only, one study (4%) current smokers only, and 11 studies (46%) included both.

Two studies in adults (8%) evaluated corticosteroid (inhaled and/or oral) untreated patients only, 11 studies (46%) evaluated corticosteroid-treated patients only, and 11 studies (46%) included both treated and untreated patients. In children, these numbers were one (13%), three (38%), and four (50%), respectively. There were large between-study differences in atopy and asthma severity status.

In adults, 21 studies (88%) used only sputum as the reference standard, whereas two studies (8%) used sputum and EBB, and one study (4%) used BAL and EBB. In children, sputum was the reference standard in four studies (50%), BAL in two studies (25%), BAL and EBB in one study (13%), and sputum, BAL and EBB in one study (13%).

RISK OF BIAS

Detailed results of the QUADAS-2 assessment are provided in Appendix 4. All but five studies (84%) showed risk of bias in at least one domain, often because thresholds for index test positivity had not been predefined (n=21; 66%), or because more than 10% of the patients had been excluded because of missing reference standard results (n=14; 44%). In addition,

methods for patient sampling (n=22; 69%) and/or blinding of the index test (n=20; 63%) and/or blinding of the reference standard (n=18; 56%) were often unclear.

META-ANALYSIS: AUCS

All extracted and obtained diagnostic accuracy data for markers and reference standards are summarized in Appendix 5. Results of meta-analyses of AUC estimates are presented in Table 1, with detailed results in Appendix 6.

Adults

Five different definitions of airway eosinophilia had been used across studies, most often based on sputum eosinophils ≥2% or ≥3%. The prevalence of eosinophilia ranged from 20% to 88%.

We obtained diagnostic accuracy data for nine markers, but only FeNO, blood eosinophils, total Immunoglobulin E (IgE), serum periostin, serum Eosinophil Cationic Protein and Exhaled Breath Condensate pH had been investigated in more than one study (Table 1). When we pooled data, independent of which reference standard or airway eosinophilia definition had been used, the summary AUC of these markers never exceeded 0.78. We found substantial heterogeneity in most analyses (Appendix 6).

FeNO (17 studies; 3,216 patients), blood eosinophils (14 studies; 2,405 patients) and IgE (7 studies; 942 patients) have been investigated in more than two studies, with pooled AUC estimates of 0.75 (range 0.59-0.88), 0.78 (0.63-0.91) and 0.65 (0.56-0.69), respectively. We repeated these meta-analyses for studies that had used sputum eosinophils ≥3% and eosinophils ≥2% as the definition of airway eosinophilia (Appendix 7), but the summary AUCs were barely affected: 0.74 (range 0.52-0.88) and 0.73 (0.53-0.81), respectively, for FeNO; 0.78 (0.63-0.91) and 0.78 (0.66-0.84) for blood eosinophils; and 0.63 (0.56-0.69) and 0.66 (0.61-0.68) for IgE.

Periostin showed promising performance in one study (AUC 0.84), but these results were not replicated in a second study (AUC 0.55)[14]. Nasal lavage eosinophils (AUC 0.88) and a model based on exhaled Volatile Organic Compounds (VOCs; AUC 0.98) showed high accuracy, but were only investigated in single studies.

Three studies reported combinations of markers, but none of these showed a significant improvement in the diagnostic accuracy compared to single markers (data not shown).

Comparisons between published and unpublished diagnostic accuracy data for FeNO, blood eosinophils and IgE are provided in Appendix 8. Adding unpublished data led to a considerable increase in precision, but did not affect summary estimates of accuracy.

Children

Five different definitions of airway eosinophilia had been used across studies, most often based on sputum eosinophils ≥2.5% (Appendix 5). The prevalence of eosinophilia ranged

from 21% to 81%. The diagnostic accuracy was evaluated for three markers; two of them in more than one study (Table 1): FeNO (6 studies; 349 patients) and blood eosinophils (3 studies; 192 patients) had pooled AUC estimates of 0.81 (range 0.56-0.89) and 0.78 (0.74-0.81), respectively.

META-ANALYSIS: SENSITIVITIES AND SPECIFICITIES

Adults

Sufficient data (≥4 studies) to perform meta-analysis were only available for induced sputum as the reference standard. Forest plots of FeNO, blood eosinophils and IgE for detecting sputum eosinophils ≥3% and ≥2% are presented in Figure 2a-b, with summary ROC curves in Figure 3. Almost all studies had used the "optimal cutpoint" of sensitivity and specificity on the ROC curve to define the positivity threshold of the markers. These thresholds varied widely. For example, the "optimal" threshold for FeNO to detect sputum eosinophils ≥3% ranged from 10ppb to 41ppb.

Summary estimates of sensitivity and specificity of FeNO, blood eosinophils, and IgE for detecting sputum eosinophils ≥3% and ≥2%, obtained by meta-analysis, are presented in Table 2. They ranged from 0.63 to 0.76 for sensitivity, and from 0.59 to 0.83 for specificity.

When pooling direct comparisons, FeNO was found to be significantly more accurate than IgE in detecting sputum eosinophils ≥2% (4 studies; p=0.025), but not in detecting sputum eosinophils ≥3% (5 studies; p=0.34). Pooling of other direct comparisons (FeNO vs. blood eosinophils and IgE vs. blood eosinophils) revealed no significant differences.

Statistical testing for funnel plot asymmetry revealed no evidence of publication bias (Appendix 9). Forest plots of sensitivity and specificity of FeNO, blood eosinophils, and IgE for detecting sputum eosinophilia in subgroups based on smoking, treatment, and asthma severity status are provided in Appendix 10.

Children

The forest plot and summary ROC curve of FeNO for detecting sputum eosinophils ≥2.5-3% are presented in Figure 2c and 3. Summary estimates of accuracy based on five studies (318 patients) were 0.72 (95%CI 0.24-0.95) for sensitivity and 0.77 (0.20-0.98) for specificity, again without evidence of publication bias (Appendix 9).

Table 1: Overall diagnostic performance of markers for detecting any airway eosinophilia.

	Studies in adults[1]				Studies in children[1]			
Index test	**Studies evaluating marker**	**AUCs Included**	**Sum of patients**	**AUC[2]**	**Studies evaluating marker**	**AUCs Included**	**Sum of patients**	**AUC[2]**
	N	**N**	**N**	**Pooled (95%CI)**	**N**	**N**	**N**	**Pooled (95%CI)**
FeNO	17	19	3216	0.75 (0.72-0.78)	6	5	349	0.81 (0.72-0.89)
Blood eosinophils	14	14	2405	0.78 (0.74-0.82)	3	3	192	0.78 (0.71-0.85)
Serum IgE	7	7	942	0.65 (0.61-0.69)	0	-	-	-
Serum Periostin	2	3	204	0.65 (0.49-0.81)	0	-	-	-
Serum ECP	2	2	174	0.72 (0.64-0.81)	1	1	77	0.75*
EBC pH	2	2	96	0.76 (0.63-0.90)	0	-	-	-
Exhaled VOCs	1	1	18	0.98*	0	-	-	-
EBC model	1	1	53	0.69*	0	-	-	-
Nasal lavage eosinophils	1	1	130	0.88*	0	-	-	-

[1]Five different definitions of airway eosinophilia were used across studies, based on different thresholds for induced sputum, bronchoalveolar lavage and/or endobronchial biopsy.

[2]Results based on random effects meta-analysis. Detailed information on diagnostic accuracy data for individual studies can be found in Appendix 5.

*Meta-analysis not possible as only one study reported on AUC.

List of abbreviations: AUC: Area under the receiver operator curve; EBC: Exhaled Breath Condensate; ECP: Eosinophil Cationic Protein; FeNO: Fraction of exhaled Nitric Oxide; IgE: Immunoglobulin E; VOCs: Volatile Organic Compounds.

Table 2: Summary estimates of sensitivity and specificity for detecting sputum eosinophilia in adults.

	Sputum eosinophils ≥3%				Sputum eosinophils ≥2%			
Index test	**Studies**	**Patients**	**Sensitivity**	**Specificity**	**Studies**	**Patients**	**Sensitivity**	**Specificity**
	N	**N**	**(95%CI)**	**(95%CI)**	**N**	**N**	**(95%CI)**	**(95%CI)**
FeNO	12	1720	0.66 (0.57-0.75)	0.76 (0.65-0.85)	9	1667	0.65 (0.55-0.74)	0.75 (0.62-0.84)
Blood eosinophils (in /µL)	12	1967	0.71 (0.65-0.76)	0.77 (0.70-0.83)	6	1180	0.66 (0.56-0.75)	0.83 (0.62-0.94)
Blood eosinophils (in %)	5	920	0.76 (0.52-0.90)	0.74 (0.67-0.80)	2	171	-	-
Serum IgE	6	699	0.64 (0.42-0.81)	0.71 (0.42-0.89)	4	754	0.63 (0.36-0.84)	0.59 (0.37-0.79)

List of abbreviations: AUC: Area under the receiver operator curve; FeNO: Fraction of exhaled Nitric Oxide; IgE: Immunoglobulin E.

Figure 1: Study selection.

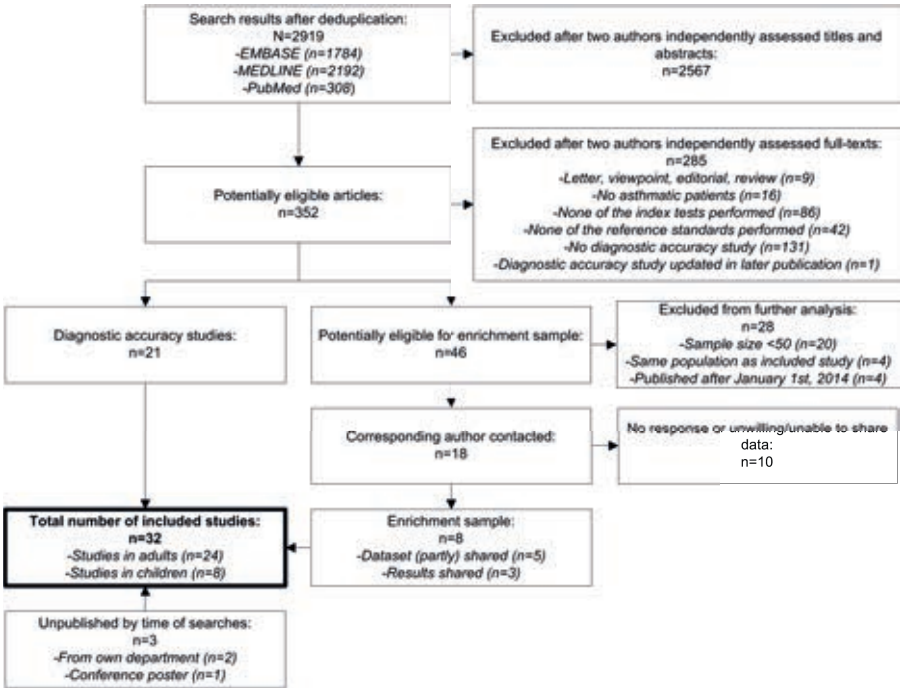
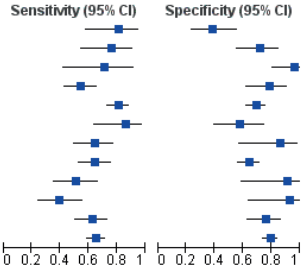


Figure 2: Forest plots for detection of sputum eosinophilia.

Figure 2a: FeNO, blood eosinophils and IgE for detection of sputum eosinophils ≥3% in adults

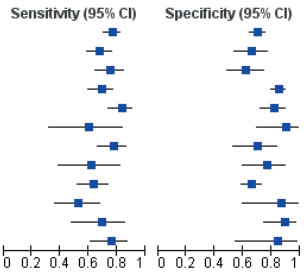
FeNO (in ppb) vs. induced sputum eosinophils ≥3%

Study	TP	FP	FN	TN	Threshold	Sensitivity (95% CI)	Specificity (95% CI)
Lemiere 2006*	17	24	4	15	10.45	0.81 [0.58, 0.95]	0.38 [0.23, 0.55]
ten Brinke 2001*	19	11	6	28	12.1	0.76 [0.55, 0.91]	0.72 [0.55, 0.85]
Hillas 2011*	10	1	4	25	14.0	0.71 [0.42, 0.92]	0.96 [0.80, 1.00]
Meijer 2002*	43	8	36	29	15.45	0.54 [0.43, 0.66]	0.78 [0.62, 0.90]
Westerhof 2014*	92	66	21	147	23.95	0.81 [0.73, 0.88]	0.69 [0.62, 0.75]
Yap 2011*	18	14	3	19	24.5	0.86 [0.64, 0.97]	0.58 [0.39, 0.75]
Carvalho Pinto 2012*	34	2	19	12	26.7	0.64 [0.50, 0.77]	0.86 [0.57, 0.98]
Hastie 2013*	49	58	27	104	30.0	0.64 [0.53, 0.75]	0.64 [0.56, 0.72]
Tseliou 2010*	23	1	22	10	31.0	0.51 [0.36, 0.66]	0.91 [0.59, 1.00]
Jia 2012**	17	1	26	12	35.0	0.40 [0.25, 0.56]	0.92 [0.64, 1.00]
Greulich 2012*	48	14	29	44	35.0	0.62 [0.51, 0.73]	0.76 [0.63, 0.86]
Schleich 2013*	147	59	78	224	41.0	0.65 [0.59, 0.72]	0.79 [0.74, 0.84]



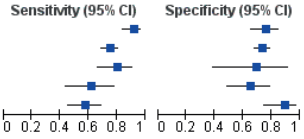
Blood Eosinophils (in /µL) vs. induced sputum eosinophils ≥3%

Study	TP	FP	FN	TN	Threshold	Sensitivity (95% CI)	Specificity (95% CI)
Schleich 2013*	173	85	52	198	220.0	0.77 [0.71, 0.82]	0.70 [0.64, 0.75]
Liang 2012*	84	23	40	45	220.0	0.68 [0.59, 0.76]	0.66 [0.54, 0.77]
Greulich 2012*	58	22	19	36	230.0	0.75 [0.64, 0.84]	0.62 [0.48, 0.74]
Westerhof 2014*	78	32	35	186	260.0	0.69 [0.60, 0.77]	0.85 [0.80, 0.90]
Zhang 2014*	71	14	14	65	260.0	0.84 [0.74, 0.91]	0.82 [0.72, 0.90]
Wagener 2014**	9	2	6	19	270.0	0.60 [0.32, 0.84]	0.90 [0.70, 0.99]
Meijer 2002*	58	11	17	26	285.0	0.77 [0.66, 0.86]	0.70 [0.53, 0.84]
Yap 2011*	13	8	8	27	300.0	0.62 [0.38, 0.82]	0.77 [0.60, 0.90]
Hastie 2013*	52	58	30	114	300.0	0.63 [0.52, 0.74]	0.66 [0.59, 0.73]
Jia 2012**	22	2	20	13	300.0	0.52 [0.36, 0.68]	0.87 [0.60, 0.98]
ten Brinke 2001*	18	4	8	33	315.0	0.69 [0.48, 0.86]	0.89 [0.75, 0.97]
Bacci 2006*	35	2	11	11	320.0	0.76 [0.61, 0.87]	0.85 [0.55, 0.98]



Blood Eosinophils (in %) vs. induced sputum eosinophils ≥3%

Study	TP	FP	FN	TN	Threshold	Sensitivity (95% CI)	Specificity (95% CI)
Zhang 2014*	78	19	7	60	2.7	0.92 [0.84, 0.97]	0.76 [0.65, 0.85]
Schleich 2013*	169	75	56	208	3.0	0.75 [0.69, 0.81]	0.73 [0.68, 0.79]
Bacci 2006*	37	4	9	9	3.45	0.80 [0.66, 0.91]	0.69 [0.39, 0.91]
Choi 2012*	21	15	13	28	4.55	0.62 [0.44, 0.78]	0.65 [0.49, 0.79]
Meijer 2002*	43	4	32	33	5.8	0.57 [0.45, 0.69]	0.89 [0.75, 0.97]



IgE (in IU/mL) vs. induced sputum eosinophils ≥3%

Study	TP	FP	FN	TN	Threshold	Sensitivity (95% CI)	Specificity (95% CI)
Westerhof 2014*	75	70	40	147	72.0	0.65 [0.56, 0.74]	0.68 [0.61, 0.74]
Jia 2012**	23	2	19	13	100.0	0.55 [0.39, 0.70]	0.87 [0.60, 0.98]
Meijer 2002*	65	23	13	14	103.0	0.83 [0.73, 0.91]	0.38 [0.22, 0.55]
ten Brinke 2001*	18	15	8	23	112.5	0.69 [0.48, 0.86]	0.61 [0.43, 0.76]
Choi 2012*	23	20	11	23	146.5	0.68 [0.49, 0.83]	0.53 [0.38, 0.69]
Yap 2011*	6	2	15	31	900.0	0.29 [0.11, 0.52]	0.94 [0.80, 0.99]

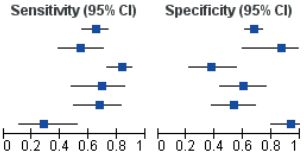
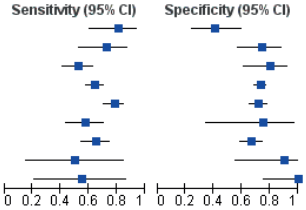


Figure 2b: FeNO, blood eosinophils and IgE for detection of sputum eosinophils ≥2% in adults

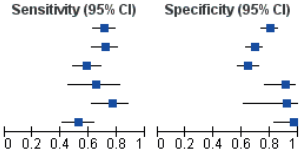
FeNO (in ppb) vs. induced sputum eosinophils ≥2%

Study	TP	FP	FN	TN	Threshold	Sensitivity (95% CI)	Specificity (95% CI)
Lemiere 2006*	21	20	5	14	10.45	0.81 [0.61, 0.93]	0.41 [0.25, 0.59]
ten Brinke 2001*	21	9	8	26	12.1	0.72 [0.53, 0.87]	0.74 [0.57, 0.88]
Meijer 2002*	45	6	41	24	15.45	0.52 [0.41, 0.63]	0.80 [0.61, 0.92]
McGrath 2012*	165	134	92	365	20.0	0.64 [0.58, 0.70]	0.73 [0.69, 0.77]
Westerhof 2014*	104	54	29	139	23.95	0.78 [0.70, 0.85]	0.72 [0.65, 0.78]
Carvalho Pinto 2012*	34	2	25	6	26.7	0.58 [0.44, 0.70]	0.75 [0.35, 0.97]
Hastie 2013*	58	49	31	100	30.0	0.65 [0.54, 0.75]	0.67 [0.59, 0.75]
Ibrahim 2011*	4	1	4	9	49.1	0.50 [0.16, 0.84]	0.90 [0.55, 1.00]
Silkoff 2005*	5	0	4	13	72.9	0.56 [0.21, 0.86]	1.00 [0.75, 1.00]



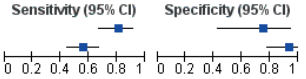
Blood Eosinophils (in /µL) vs. induced sputum eosinophils ≥2%

Study	TP	FP	FN	TN	Threshold	Sensitivity (95% CI)	Specificity (95% CI)
Westerhof 2014*	95	39	38	159	210.0	0.71 [0.63, 0.79]	0.80 [0.74, 0.86]
McGrath 2012*	74	80	29	178	220.0	0.72 [0.62, 0.80]	0.69 [0.63, 0.75]
Hastie 2013*	56	56	39	103	300.0	0.59 [0.48, 0.69]	0.65 [0.57, 0.72]
ten Brinke 2001*	19	3	10	31	315.0	0.66 [0.46, 0.82]	0.91 [0.76, 0.98]
Bacci 2006*	36	1	11	11	320.0	0.77 [0.62, 0.88]	0.92 [0.62, 1.00]
Meijer 2002*	43	1	39	29	415.0	0.52 [0.41, 0.64]	0.97 [0.83, 1.00]



Blood Eosinophils (in %) vs. induced sputum eosinophils ≥2%

Study	TP	FP	FN	TN	Threshold	Sensitivity (95% CI)	Specificity (95% CI)
Bacci 2006*	38	3	9	9	3.45	0.81 [0.67, 0.91]	0.75 [0.43, 0.95]
Meijer 2002*	46	2	36	28	5.76	0.56 [0.45, 0.67]	0.93 [0.78, 0.99]



IgE (in IU/mL) vs. induced sputum eosinophils ≥2%

Study	TP	FP	FN	TN	Threshold	Sensitivity (95% CI)	Specificity (95% CI)
Westerhof 2014*	82	63	53	134	72.0	0.61 [0.52, 0.69]	0.68 [0.61, 0.74]
Meijer 2002*	71	17	14	13	103.0	0.84 [0.74, 0.91]	0.43 [0.25, 0.63]
ten Brinke 2001*	19	14	10	21	112.5	0.66 [0.46, 0.82]	0.60 [0.42, 0.76]
Hastie 2013*	61	59	31	92	120.0	0.66 [0.56, 0.76]	0.61 [0.53, 0.69]

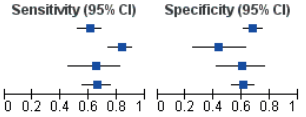
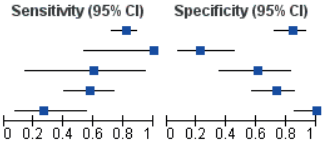


Figure 2c: FeNO for detection of sputum eosinophils ≥2.5-3% in children

Study	TP	FP	FN	TN	Threshold	Sensitivity (95% CI)	Specificity (95% CI)
Sivan 2009*	76	9	17	48	18.0	0.82 [0.72, 0.89]	0.84 [0.72, 0.93]
Lex 2005**	6	17	0	5	23.0	1.00 [0.54, 1.00]	0.23 [0.08, 0.45]
Lex 2006**	3	7	2	11	23.0	0.60 [0.15, 0.95]	0.61 [0.36, 0.83]
Fleming 2013**	22	11	16	30	35.0	0.58 [0.41, 0.74]	0.73 [0.57, 0.86]
Toyran 2014*	4	0	11	23	49.8	0.27 [0.08, 0.55]	1.00 [0.85, 1.00]



Studies are ordered by threshold.

* Threshold based on "optimal cutpoint" between sensitivity and specificity on receiver operating characteristics curve.

**Threshold selection arbitrary, based on results from previous studies, or unknown.

List of abbreviations: TP = True Positive; FP = False Positive; FN = False Negative; TN = True Negative.

Figure 3: Summary receiver operating characteristics curve for detecting sputum eosinophils ≥3% in adults, or ≥2.5-3% in children.

Figure 3a: FeNO (in ppb) in adults

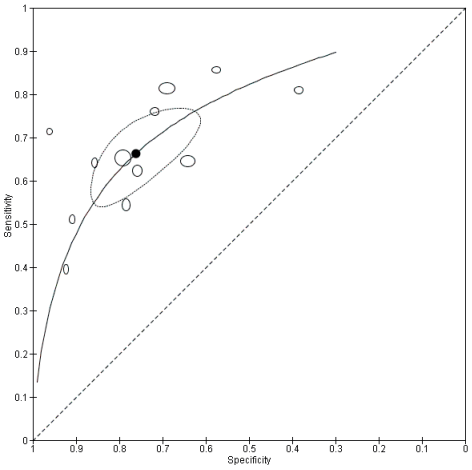


Figure 3b: Blood eosinophils (in /µL) in adults

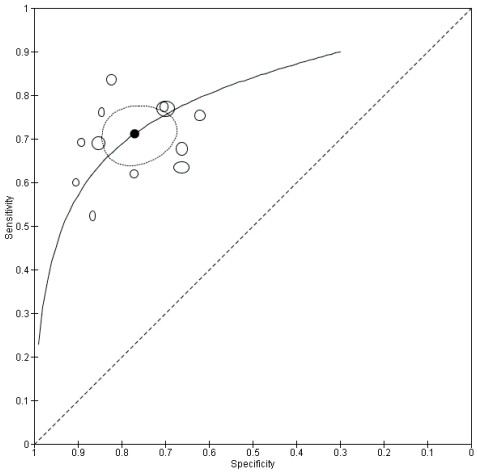


Figure 3c: IgE (in UL/mL) in adults

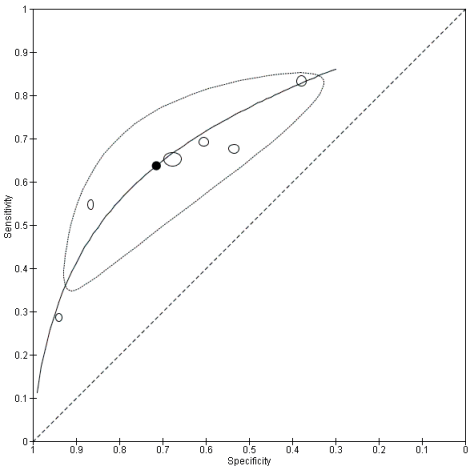
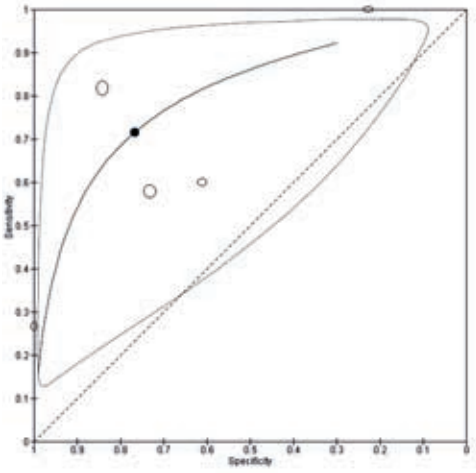


Figure 3d: FeNO (in ppb) in children



Each open circle represents the results from a singlestudy. Closed circles represent summary estimates. Dotted ellipses represent 95% confidence regions around summary estimates.

DISCUSSION

We systematically reviewed studies on the diagnostic accuracy of minimally invasive markers for detecting airway eosinophilia in asthma. In adults, FeNO, blood eosinophils, and total IgE have been extensively investigated, but their ability to distinguish between patients with and without airway eosinophilia seems limited, with summary estimates of AUC, sensitivity, and specificity never exceeding 0.8. Other markers, such as VOC-analysis, were reported to be more accurate in single studies, but these results have not yet been replicated. Studies in children are scarce, but findings for FeNO and blood eosinophils are comparable to those in adults.

Several considerations deserve attention. Almost all studies showed risk of bias. These sources of bias are likely to overestimate diagnostic accuracy[10], which would mean that the extracted accuracy estimates, although usually moderate, may be even too optimistic. Suboptimal reporting, a common phenomenon for diagnostic accuracy studies[15], often withheld us from a proper evaluation of risk of bias.

Failure to publish is a common phenomenon in diagnostic accuracy studies[16]. We aimed to reduce the risk of publication bias by searching trial registries, and by contacting authors of published studies that seemed to have data from which accuracy estimates could be calculated. This approach was successful. More than one third of the included results were unpublished by the time of our searches. However, this approach also has its limitations. First, only a minority of diagnostic accuracy studies is currently registered[17]. Second, most of the included unpublished data came from studies that were, at least partially, reported and had included at least 50 patients. These may differ from smaller studies, or those that do not get published at all. Though we did not observe any differences between accuracy estimates obtained from published and unpublished data (Appendix 8) and we observed no funnel plot asymmetry (Appendix 9), we cannot completely exclude the possibility of reporting bias. Drivers of non-publication are unknown in diagnostic research, but it is likely that studies with lower accuracy estimates have lower chances of getting published. Should this be the case, this may have led to further overestimations of accuracy.

Overall, nine different definitions of airway eosinophilia were used across studies, based on different thresholds for eosinophilia in induced sputum, BAL and/or EBB. These three airway compartments do not exhibit strong correlations with regard to eosinophil counts[18]. Although the diagnostic accuracy of markers may vary across different eosinophilia definitions, we observed that the summary AUCs were stable when comparing studies using any definition of airway eosinophilia, sputum eosinophils ≥3%, or sputum eosinophils ≥2%. There was also considerable heterogeneity regarding the study population and test methods. Some studies only included smokers, for example, while others only included non-smokers, and at least four different FeNO devices were used. Many studies analysed both patients with childhood- and adult-onset asthma. In the latter group, distinguishing asthma from COPD and ACOS (Asthma-

COPD Overlap Syndrome) can be problematic. The accuracy of markers may vary across these different subgroups. The prevalence of airway eosinophilia also differed considerably across studies. Diagnostic accuracy typically varies with clinical setting, context, and prevalence. Although the results from the individual studies reflect considerable heterogeneity, we felt safe to draw conclusions because AUCs for FeNO, blood eosinophils and IgE consistently reflected moderate accuracy.

Combining markers with other clinical features in a prediction model is likely to improve diagnostic accuracy compared to single markers, but this has not been sufficiently investigated yet. All but three studies only reported on accuracy estimates of single markers. Since we did not have individual patient data (IPD), we were unable to further analyse the incremental value of combining markers.

The most robust evidence for the clinical value of detecting airway eosinophilia comes from a Cochrane review that demonstrated that the frequency of asthma exacerbations can be significantly reduced when tailoring inhaled corticosteroids on sputum eosinophilia[3]. For a marker to be able to replace induced sputum in this context, sensitivity, specificity and AUC should probably be at least above 90%, so that at most 10% of all patients will be misclassified and, potentially, subjected to inappropriate clinical decisions. Our review shows that there are currently no single markers available with a large enough documented accuracy to fulfill these criteria. It must be noted, though, that recent guidelines recommending the use of sputum eosinophil counts in severe asthma, acknowledge that the quality of evidence is "very low"[1;4]. In addition, they do not recommend sputum-guided treatment in the general asthma population. Some of the markers evaluated in this review on their own may have better potential in managing asthma than sputum eosinophil counts. This is illustrated by a recent study in which VOC-analysis predicted corticosteroid responsiveness with greater accuracy than sputum eosinophils[19], and by another study which showed good response to Mepolizumab among patients with severe eosinophilic asthma as determined by blood eosinophils[20]. The latter study illustrates the accumulating evidence for the potential role of blood eosinophils as a predictor of responsiveness to novel targeted therapies against eosinophilic airway inflammation[7].

Moderate accuracy does not necessarily make the investigated markers useless. Markers can also be applied in a triage setting, for example, for ruling-out (high sensitivity required) or ruling-in (high specificity required) airway eosinophilia. In case of a high specificity, those with a positive test result would be considered as eosinophilic. With a limited sensitivity, those with a negative test result would need to undergo further testing (e.g. sputum induction). Most included studies only reported on the "optimal cutpoint" between sensitivity and specificity, based on the Youden index. When a marker is not sufficiently accurate to replace the existing test, this optimal cutpoint is clinically not very practical because both sensitivity and specificity are typically suboptimal at this cutpoint. Therefore, it does not inform the reader about the ability of the marker to rule-in or rule-out airway eosinophilia. Furthermore,

data-driven selection of an optimal cutpoint leads to over-optimistic estimates of sensitivity and specificity[10]. It could be more informative to report on sensitivity at a fixed high specificity (e.g. 95%), or the other way around.

An ATS guideline on the Interpretation of FeNO for Clinical Applications strongly "recommends the use of FeNO in the diagnosis of eosinophilic airway inflammation"[21]. It also strongly "recommends that low FeNO less than 25ppb (20ppb in children) be used to indicate that eosinophilic inflammation and responsiveness to corticosteroids are less likely", "that FeNO greater than 50ppb (35ppb in children) be used to indicate that eosinophilic inflammation and, in symptomatic patients, responsiveness to corticosteroids are likely" and "that FeNO values between 25ppb and 50ppb (20-35ppb in children) should be interpreted cautiously and with reference to the clinical context". Our results are challenging this concept. Appendix 5 shows that, at FeNO thresholds below 25ppb, sensitivity ranges from 0.52 to 0.86 in adults. This means that of every 100 asthmatic patients with airway eosinophilia tested by FeNO, up to 48 would be falsely considered as not having airway eosinophilia, and effective treatment may be withheld from them. In children, sensitivity for FeNO thresholds below 20ppb ranges from 0.75 to 0.82, indicating that up to 25 patients would be false negatives. Although these thresholds may be relevant in specific subgroups of asthma, these findings indicate that FeNO results should be interpreted with much more caution in the general asthma population than recommended by the ATS.

It is probably not surprising that the markers evaluated in our review generally were moderately accurate. The underlying biological mechanisms determining airway eosinophil counts are considerably different from those of some of the investigated markers[22]. Several studies also showed significant variability in blood eosinophils[23] and IgE[24] in the same asthmatic subjects in short periods of time. Some asthmatic patients were shown to have persistently elevated FeNO levels, not suppressed by corticosteroid treatment and not reflecting raised sputum eosinophils[25]. Corticosteroid treatment significantly influences FeNO, blood eosinophils, IgE and sputum eosinophils[26], but the relative magnitude of this effect could vary across markers. Diagnostic accuracy may therefore be influenced by treatment status. Also many other factors, such as age, gender, reflux disease, smoking, and atopy, have been shown to influence FeNO levels[27]. This may also be the case with other markers and further compromises the identification of an accurate minimally invasive test for airway eosinophilia.

Similar reproducibility problems could apply to the reference standard and target condition. Although some studies showed that a threshold of 3% for sputum eosinophils is reproducible over time[28], others found the phenotypic classification of asthma to change frequently, both spontaneously and in response to treatment[29]. Longitudinal studies examining sputum cell counts in successive exacerbations found considerable heterogeneity in the type of inflammation within the same individuals[30]. Consequently, a diagnosis of eosinophilic asthma based on a single sputum sample may be questionable.

Based on our findings, we discourage the use of FeNO, blood eosinophils, or IgE as single

surrogate tests for detecting airway eosinophilia in asthma. Our meta-analyses show that, at the optimal cutpoint, sensitivities and specificities of these markers for detecting sputum eosinophilia are moderate, and their use would lead to large numbers of false positives and/or false negatives. Future research will mainly need to focus on whether these markers can be applied as rule-in or rule-out tests, whether markers that were poorly investigated or clinical prediction models incorporating multiple markers along with other clinical data are more accurate, perhaps in specific settings or subgroups, and whether these markers on their own merits have potential in managing asthma[26]. A next step could be an extensive IPD project, combining existing datasets from observational asthma studies in which both clinical features, minimally invasive markers, and one or more reference standards for airway eosinophilia were assessed. Thresholds for ruling-in and ruling-out airway eosinophilia based on individual markers can then be reliably determined, and an optimal multivariable clinical prediction model can be developed. The clinical value of these findings can subsequently be investigated in terms of, for example, response to therapy or the reduction of exacerbations.

RESEARCH IN CONTEXT

SYSTEMATIC REVIEW

MEDLINE, Embase and PubMed were searched from inception to August 2014 for studies evaluating the diagnostic accuracy of minimally invasive markers against a reference standard of induced sputum, bronchoalvealar lavage and/or endobronchial biopsy in patients with (suspected) asthma. A detailed search strategy can be found in Appendix 1. Twenty-one studies were eligible. We also included 11 studies with unpublished diagnostic accuracy data. To put our research in context, we used the same search strategy to look for studies that investigated a potential clinical role of airway eosinophilia or the investigated minimally invasive markers in asthma, especially focusing on systematic reviews and clinical guidelines that aim to include all relevant information on a specific topic.

INTERPRETATIONS

There is evidence for a clinical role of monitoring airway eosinophilia in asthma. A Cochrane review demonstrated that the frequency of asthma exacerbations can be significantly reduced when tailoring inhaled corticosteroids on sputum eosinophilia. Recent ERS/ATS and GINA guidelines recommend guiding treatment in severe asthma by sputum eosinophil counts in addition to clinical criteria in centers experienced in using this technique, although they acknowledge that quality of evidence is “very low”. Since existing reference standards are invasive, expensive and/or often not feasible, a minimally invasive marker would be clinically useful. FeNO is often referred to as a surrogate for airway eosinophila in the medical literature, and an ATS guideline on the Interpretation of FeNO for Clinical Applications strongly recommends specific cutoffs of FeNO to rule-in and rule-out eosinophilic inflammation. This systematic review found that the performance of FeNO, blood eosinophils, and total IgE in detecting airway eosinophilia in asthma is limited. There are currently no single markers available with a large enough documented accuracy to replace induced sputum.

SUPPLEMENTARY MATERIAL

Appendix 1-10 can be viewed online at thelancet.com

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Chapter 5

BIOMARKERS FOR DIAGNOSING ASTHMA: NOT QUITE A SMOKING GUN?

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BIOMARKERS FOR DIAGNOSING ASTHMA: NOT QUITE A SMOKING GUN?

Up to 35% of asthma patients are smokers, which is comparable to the proportion of smokers in the general population [1]. This large subgroup of asthma patients has particularly poor clinical outcomes, with increased morbidity and mortality rates [1-3]. Over the past decades, evidence has accumulated that smoking induces considerable alterations in airway inflammatory processes in asthma patients [1, 2]. Not surprisingly, exhaled nitric oxide (FeNO) [4] and blood eosinophils [5], both biomarkers of eosinophilic airway inflammation, are substantially different in asthma patients who smoke, compared to those who do not. Smoking also induces resistance to corticosteroid treatment [6], which is the mainstay of asthma therapy. This raises the question whether the optimal strategy to diagnose and manage asthma patients is the same for smokers and non-smokers.

In this issue of *Clinical and Experimental Allergy,* Giovannelli and colleagues report on an evaluation of the association between FeNO or blood eosinophils and allergic asthma [7]. This study addresses an important, yet so far insufficiently investigated topic: whether or not the association between these biomarkers and allergic asthma is similar in the smoking and non-smoking population.

In a large cross-sectional survey including 1,607 patients in the age range of 40 to 64 years selected from the general population, Giovannelli and colleagues found that FeNO and blood eosinophils were associated with a diagnosis of allergic asthma in never and former smokers, but not in current smokers. In a multivariate model, non-smoking patients with allergic asthma had much higher FeNO levels and blood eosinophil counts, compared to non-smoking individuals without allergic asthma. However, in the subgroup of current smokers no such differences were observed.

The authors made an attempt to quantify the potential clinical value of FeNO and blood eosinophils in distinguishing between patients with and without allergic asthma. For the group as a whole the diagnostic accuracy appeared to be far from perfect, whereas it seemed non-existing in the subgroup of smokers.

Interestingly, these findings differ from those in a previous study that evaluated the ability of FeNO to diagnose asthma in 282 patients with asthma-like symptoms. Overall, the diagnostic accuracy was equally mediocre, but not significantly different between never-smokers, ex-smokers and current smokers [8]. Whether these findings differ due to chance, heterogeneity in the populations studied, or varying methods between the studies, is open for debate.

Despite the careful design and impressive sample size, the study by Giovannelli and colleagues has a few limitations. Only 14 patients out of 294 current smokers (5%) were diagnosed with allergic asthma. This small number has certainly limited the power to detect significant associations with FeNO levels and blood eosinophil counts in this subgroup. In addition, the authors relied on a reference standard of self-reported physician-diagnosed asthma. Yet, serious concerns exist about the accuracy of self-reported asthma [9]. Nevertheless,

these results add important data to the growing evidence of FeNO and blood eosinophils as biomarkers for diagnosing allergic asthma.

The findings of Giovannelli and colleagues have important clinical and scientific implications. Firstly, they extend the results from previous studies, indicating that FeNO and blood eosinophils may act differently in smokers and in non-smokers [4, 5]. Physicians should take this into account when using FeNO and blood eosinophils in the clinical work-up of asthma patients.

Secondly, the results highlight the urgent need for more research in asthma patients with a smoking history. In clinical asthma trials, smokers and ex-smokers are usually excluded, which implies that about one third of the asthma population is not taken into account. Therefore, results of these trials may not necessarily be extrapolated to smoking patients. Accumulating evidence suggests that smoking asthma patients represent a separate asthma phenotype, in particular with respect to the clinical value of biomarkers of airway inflammation in predicting response to treatment [10]. The findings by Giovannelli and colleagues further amplify this message.

Thirdly, the results confirm that FeNO and blood eosinophils have only moderate accuracy for diagnosing asthma, both in smokers and in non-smokers, as has been reported in previously published smaller evaluations [11]. Physicians should realize that the use of these biomarkers as single diagnostic tests in clinical practice can easily lead to false positive or false negative test results.

This does not necessarily mean that these biomarkers have no value at all. Single markers that are insufficiently accurate to serve as stand-alone diagnostic tests may have important value as rule-in or rule-out tests [12]. A test that is very sensitive but less specific may be useful to rule-out a disease, as the number of false negatives is low. Vice versa, a test that is very specific but less sensitive may be useful to rule-in a disease. Markers can also be combined to develop a prediction model with improved diagnostic accuracy [13]. Unfortunately, there are currently no well-defined and reproducible thresholds for FeNO and blood eosinophils for ruling-in or ruling-out allergic asthma, and efforts to build such prediction models have so far been limited [11]. Future studies should focus on the identification and validation of such thresholds and models, because if there is a role for FeNO and blood eosinophils in the diagnosis of asthma, it will probably lie there. Such studies should take into account that thresholds may be different between smokers and non-smokers, as the current study once again illustrates [11].

Giovannelli and colleagues conclude: “These findings raise questions about the clinical value of FeNO and blood eosinophils in smokers”. Although this is certainly true for diagnosing asthma, it should be noted that these biomarkers may have other clinical applications as well. For example, they may be used for identifying specific inflammatory asthma phenotypes [14, 15], for disease monitoring and assessing the risk of asthma exacerbations [16-18], or for selecting patients that are likely to respond to treatment [11, 19].

Numerous studies have evaluated the ability of FeNO and blood eosinophils to detect specific asthma phenotypes. Identification of asthma patients with eosinophilic airway inflammation is clinically valuable, as this subgroup of patients is more likely to respond to corticosteroid treatment [19]. However, in a recent systematic review, the diagnostic accuracy of FeNO and blood eosinophils for detecting airway eosinophilia turned out to be only moderate [14]. Interestingly, one recent study in 336 asthma patients compared the accuracy of these two biomarkers in detecting airway eosinophilia between never-smokers and former- or current-smokers, and no significant differences were observed between these groups of patients [15].

The role of FeNO and blood eosinophils in selecting patients for specific treatment strategies could be more promising. Asthma is a heterogeneous disease, and the response to treatment is highly variable [20]. This is especially true for smoking or ex-smoking asthma patients, in whom the type of airway inflammation is altered due to smoking effects [2]. Markers that are able to identify who will respond to a specific treatment can therefore be of great clinical value. Although controversial and inconclusive, evidence suggests that FeNO and blood eosinophils may have such value, not only for selecting responders to traditional corticosteroid treatment, but also to novel targeted therapeutics [11, 21, 22]. Whether this equally applies to smoking and non-smoking patients remains to be investigated in future clinical trials.

In conclusion, the study by Giovannelli and colleagues shows that in the general population, FeNO and blood eosinophils are associated with allergic asthma, yet only in non-smokers. These findings do not necessarily question the clinical value of these biomarkers in smoking asthma patients, but suggest that their potential value in these patients may be different. In an era where clinical practice focuses more and more towards personalized medicine, phenotyping and subphenotyping patients has become increasingly important in the clinical workup of asthma patients; smoking status seems to be an important determinant in this differentiation.

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Part 2

CLINICAL COURSE OF ADULT-ONSET ASTHMA

Chapter 6

CLINICAL PREDICTORS OF REMISSION AND PERSISTENCE OF ADULT-ONSET ASTHMA

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ABSTRACT

BACKGROUND: Adult-onset asthma is an important but relatively understudied asthma phenotype and little is known about its natural course and prognosis. The remission rate is believed to be low, and it is still obscure which factors predict remission or persistence of the disease.

OBJECTIVE: To determine the remission rate, and identify predictors of persistence and remission of adult-onset asthma.

METHODS: Two hundred adult patients with recently diagnosed (<1 year) asthma were recruited from secondary and tertiary pulmonary clinics and prospectively followed for 5 years. Clinical, functional and inflammatory parameters were assessed at baseline and at yearly visits. Asthma remission was defined as absence of asthma symptoms for ≥1 year and no asthma medication use for ≥1 year. Descriptive statistics and logistic regression analysis were performed.

RESULTS: Five-year follow-up data of 170 patients (85%) was available. Of these, 27 patients (15.9%) experienced asthma remission. Patients with asthma persistence were older, had worse asthma control, required higher doses of inhaled corticosteroids, had more severe airway hyperresponsiveness, more often nasal polyps and higher levels of blood neutrophils as compared to patients who experienced clinical remission.

In a multivariable logistic regression analysis, only moderate-severe bronchial hyperresponsiveness and nasal polyps were independent predictors of asthma persistence. Patients with these two characteristics had less than 1% chance of asthma remission.

CONCLUSION: One in six patients with adult-onset asthma experiences remission within the first 5 years of the disease. In patients with moderate to severe bronchial hyperresponsiveness and nasal polyposis the chance of remission is close to zero.

INTRODUCTION

Adult-onset asthma is a clinically important, but relatively understudied phenotype of asthma.[1] In contrast to childhood atopic asthma, adult-onset asthma had not been investigated systematically until 2004.[2] Most available data about adult-onset asthma come from cross-sectional studies and have shown that it is a heterogeneous condition with at least three distinct phenotypes.[3] Adult-onset asthma is suggested to be more severe than childhood onset asthma, less responsive to treatment and associated with accelerated decline in lung function.[4] Remission of childhood-onset asthma occurs in up to two-thirds of the patients,[5-7] whereas adult-onset asthma has been reported to be more chronic with a much lower remission rate.[6, 8-13] However, most of these studies are difficult to interpret because they are based on "self-reported asthma" or "self-reported physician-diagnosed asthma" or database data in which a mixed childhood- and adult-onset population is studied. Only two studies prospectively followed patients with adult-onset asthma, but unfortunately these studies lacked power to test predictors of remission by multivariable analysis.[9, 13]

Knowledge about the clinical course and outcome of adult-onset asthma and its determinants may be important for several reasons. For patients, it is important to know the chance of disease remission or persistence so that they can adjust plans for their future life. For physicians, knowing risk factors of asthma persistence might serve to identify treatable factors with a potential beneficial effect on the course of the disease.[14] For researchers, identification of determinants of asthma outcome might help to better understand the aetiology of the disease. Finally, for policy makers and health care payers, knowledge of asthma remission and persistence rates will allow more accurate estimates of healthcare expenditure.

The aim of the present study was to identify clinical, functional or inflammatory predictors of asthma persistence and remission in a prospectively followed cohort of adults with newly diagnosed, well-defined asthma.

METHODS

PATIENTS

Two hundred adults with new-onset asthma were recruited from one academic and two non-academic pulmonary outpatient clinics between 2009 and 2011 and were prospectively followed for 5 years. All patients had a recent (<1 year) doctor's diagnosis of new-onset asthma. Adult-onset asthma was defined as asthma with an onset of the disease at >18 years of age. Asthma diagnosis was based on typical asthma symptoms and documented reversibility in FEV_1 of > 12% of predicted value and/or a positive inhaled methacholine provocation test (PC_{20} < 8mg/ml).[15] Patients were excluded if they had a self-reported history of childhood asthma or other chronic respiratory diseases in childhood, frequent episodes of dyspnea as a child, or use of bronchodilator or other asthma medication in childhood.

Current smoking and ex-smoking were allowed. Patients with a smoking history of >10 pack years were included only if they showed reversibility in FEV_1 ≥12% of the predicted value, and had a normal CO diffusion capacity (DLCO >80%).

The study was approved by the AMC Medical Ethics Board and registered in the Dutch trial register (NTR1846). All participants were informed and gave written informed consent.

STUDY DESIGN

This prospective longitudinal cohort study was part of the ADONIS-study (Adult-onset asthma and inflammatory subphenotypes).[16] At baseline, patients underwent a comprehensive assessment of clinical, functional and inflammatory parameters (Figure 1). Thereafter, they were treated in regular healthcare facilities. Patients were contacted and invited for a limited reassessment at yearly intervals. Between 4 to 5 years post diagnosis an extensive reassessment of baseline measurements was done. If patients could not be reached by phone, email or postal mail, basic information about asthma symptoms and medication was collected via their general practitioner.

STUDY MEASUREMENTS

Clinical measurements – A comprehensive history was taken, including questions about asthma symptoms, medication use and healthcare utilization in the past year. Patients completed questionnaires including the asthma control questionnaire (ACQ-6; uncontrolled asthma defined as ACQ-score ≥1.5),[17] asthma quality of life questionnaire (AQLQ), and the Sino-nasal Outcome Test (SNOT-22).[18] Obesity was defined as a BMI ≥ 30kg/m^2.

Lung function measurements – Pre- and post-bronchodilator spirometry (FEV_1 and forced vital capacity (FVC)) was performed according to international standards.[19] Diffusion capacity of the lungs for carbon monoxide divided by alveolar volume (DLCOc/VA) was measured with single breath measurement.[20] Total lung capacity (TLC) and residual volume (RV) were

measured by body plethysmography. Bronchial challenge test was performed with inhaled methacholine to establish the concentration causing a 20% fall in FEV_1 (PC_{20}-methacholine). In case patients did not reach a ≥20% fall in FEV_1, a level of 32 mg/ml methacholine was taken as default value. Bronchial hyperresponsiveness can be divided into: mild 1-4mg/ml, moderate to severe <1.0 mg/ml.[21]

Inflammatory parameters – Fraction of exhaled nitric oxide (FeNO) was measured at a flow rate of 50mL/s (NIOX System, Aerocrine, Sweden).[22] Venous blood was collected and differential white blood cell count was performed. Total and specific IgE to common aeroallergens were measured by ImmunoCAP; atopy was defined as IgE >0.35 Ku/L for at least one allergen.

Sputum induction was performed according to international standards.[23] Sputum processing was done according to full sample method and differential cell counts were stained and analyzed on cytospin preparations.

Sinonasal imaging – The presence of nasal polyps was evaluated based on sinus CT-scanning and nasal endoscopy.[24]

ASSESSMENT OF ASTHMA REMISSION AND PERSISTENCE

Clinical asthma remission[8-10, 25] was the primary outcome of the study and defined as: no asthma symptoms for ≥1 year and no asthma medication use for ≥1 year at the 5-year follow-up visit. Asthma persistence was defined as presence of asthma symptoms in the last year or use of any asthma medication (beta-2-agonists or inhaled corticosteroids) in the last year. Secondary outcomes included pathophysiological confirmation of clinical remission by means of change in lung function, bronchial hyperresponsiveness and inflammatory markers.[9]

STATISTICAL ANALYSIS

Comparisons of baseline variables between patients in remission and patients with persistent asthma were done by Student t-test, Mann Withney-U test or chi-square. Wilcoxon signed rank test and paired t-tests were used to analyze the changes of variables over time. Univariate logistic regression was used to select significant baseline variables for the multivariable logistic regression model. The significant predictor variables were used in a multivariable binary logistic regression model and selected by backward selection of the model. Results are expressed as beta with standard error (SE). Statistical significance was set at a P value of less than .05.

Analyses were performed in SPSS version 23.0 (IBM SPSS, Chicago, Ill) and R-studio V0.99.467, Package logistf (Integrated development environment for R, Boston, MA).

RESULTS

Clinical data of 170 patients (85% of initial cohort, see Figure 1) were available at 5-year follow-up (mean follow-up duration 4.8±0.6 years); of which 108 (64%) patients underwent an evaluation in the lung function laboratory, 27 (16%) patients were contacted by telephone, and from 35 (21%) patients data were obtained from the primary care database. Additional data collected at yearly intervals were obtained from 75% of the patients. Patients who were lost to follow-up differed slightly from the rest of the cohort with respect to a younger age and lower incidence of nasal polyposis, there was no difference in smoking status (data not shown).

INCIDENCE AND CHARACTERISTICS OF PATIENTS WITH ASTHMA REMISSION OR PERSISTENCE

Clinical remission of asthma occurred in 27 out of 170 patients (15.9%). Remission was observed in the course of time, the median duration from baseline to remission was 45 months (range 9-45 months).

At baseline, patients with persistent asthma were older, had worse asthma control (asthma control questionnaire (ACQ)-score 1.34 *vs* 0.89, p=0.026), required higher doses of inhaled corticosteroids (median fluticasone equivalent 313 µg *vs* 250 µg, p=0.007), had more severe bronchial hyperresponsiveness (PC_{20}-methacholine 2.7 *vs* 5.8 mg/ml, p=0.003), were more often diagnosed with nasal polyps (25% *vs* 0%, p=0.004) and had higher levels of blood neutrophils as compared to patients who experienced clinical remission after 5 years. There was no difference between the two groups in lung function, percentage of eosinophils in blood or sputum, or level of exhaled nitric oxide (Table 1).

At 5-year follow-up, patients in remission showed a significant reduction in ICS dose and ACQ-score. These patients also showed a reduction in PC_{20}-methacholine over time, resulting in the absence of bronchial hyperresponsiveness in the majority of patients. Patients with asthma persistence did not show a significant change in PC_{20}-methacholine, but showed an increase in persistent airway obstruction as reflected by a lower post-bronchodilator FEV_1/FVC. Neither group showed significant changes in markers of eosinophilic inflammation, however patients with persistent asthma showed an increase in sputum neutrophils (Table 2). In a post hoc analysis, patients with asthma persistence with and without nasal polyps were examined in more detail (Table 3). Furthermore, the observed increase of sputum neutrophil levels in patients with asthma persistence appeared to be only present in patients without nasal polyps (p=0.004, n=30) and not in those with nasal polyps (p=0.122, n=15).

PREDICTORS OF ASTHMA REMISSION AND PERSISTENCE

Univariate logistic regression analysis showed that age, ICS-dose, ACQ-score, PC_{20}-methacholine and nasal polyps were significant predictors of asthma outcome. Multivariable logistic regression showed that more severe bronchial hyperresponsiveness (lower PC_{20}-methacholine,

per step decrease in dose: Beta(SE)=0.99(0.39), p=0.005) and the presence of nasal polyps (Beta(SE)=2.96(1.47), p=0.001) were independent predictors of asthma persistence. The probability of asthma remission can be calculated by the following formula: x/(1+x), where x = e^(-4.75791+(nasal polyps*2.9419)+((Log10(PC_{20}-Metacholine))*0.93938)). Thus, when applying this model to an imaginary patient with nasal polyps and PC_{20}-methacholine of 1 mg/ml at the time of asthma diagnosis this would give a chance of less than 1% for asthma remission within the next 5 years (Figure 2).

Table 1. Baseline Characteristics of Patients With Persistent Asthma and Clinical Remission.

	Persistent asthma (n=143)		Clinical remission (n=27)		P-value
Female, %	57		44		0.243
Age, years	50	±14	44	±15	**0.039**
BMI, kg/m^2	28.2	±5.2	26.5	±5.0	0.132
Ex- or current smoker, %	58		48		0.342
Pack years	4	(0-15)	1	(0-12)	0.266
ACQ-6 score	1.34	±0.92	0.89	±0.67	**0.026**
Inhaled corticosteroid (ICS) use, %	81		70		0.212
ICS and second controlller, %	64		52		0.248
Oral corticosteroid use, %	3		0		0.389
ICS dose (fluticasone equivalent)	313	(250-500)	250	(0-250)	**0.007**
Asthma medication use, %	92		89		0.561
pre FEV_1 % predicted	93	±17	95	±16	0.709
pre FVC% predicted	106	±16	102	±16	0.291
post FEV_1, % predicted	100	±17	99	±14	0.785
post FVC% predicted	108	±16	103	±17	0.107
FEV_1 % reversibility	5	(2-9)	4	(2-6)	0.134
post FEV_1/FVC, % predicted	95	±11	98	±10	0.158
post DLCOcVA, % predicted	98	±15	96	±17	0.562
post RV/TLC ratio, % predicted	88	±20	86	±13	0.527
PC_{20}-Methacholine mg/ml	2.7	(0.8-6.6)	5.8	(2.9-32)	**0.003**
Nasal polyps, %	25		0		**0.004**
GERD, %	39		42		0.763
Atopy, %	44		46		0.667
Obesity, %	33		19		0.138
Total IgE, kU/L	68	(26-236)	59	(30-115)	0.497
FeNO, ppb	21	(13-45)	29	(12-44)	0.698
Blood neutrophils, 10^9/L	3.7	(3.0-4.6)	3.0	(2.7-4.0)	**0.038**
Blood eosinophils, 10^9/L	0.17	(0.1-0.28)	0.15	(0.08-0.26)	0.601
Sputum eosinophils, %	0.5	(0.1-3.8)	0.6	(0.2-1.5)	0.688
Sputum neutrophils, %	71	(50-84)	82	(71-87)	0.143

Pre: pre-bronchodilator. Post: post-bronchodilator. GERD: gastroesophageal reflux disease. Data are presented as mean (±SD), median (interquartile range) or percentage.

Table 2. Comparison Between Baseline and 5-year Follow-up Data.

	Asthma persistence (n=143)				Remission (n=27)			
	Baseline	5 years	p-value		Baseline	5 years	p-value	
BMI, kg/m^2	28.6 ±5.5	28.7 ±5.6	0.761	n=99	25.8 ±3.4	26.5 ±3.1	0.071	n=12
ACQ-6 score	1.32 ±0.93	1.08 ±0.86	**0.008**	n=114	0.8 ±0.71	0.23 ±0.34	**0.002**	n=18
Inhaled steroid dose (fluticasone equiv)	313 (250-500)	250 (0-500)	0.296	n=134	250 (0-250)	0 NA	**<0.001**	n=27
pre FEV_1 % predicted	94 ±17	95 ±19	0.379	n=92	93 ±14	93 ±13	0.852	n=12
post FEV_1 % predicted	100 ±17	101 ±19	0.262	n=96	97 ±12	98 ±12	0.678	n=12
FEV_1 % reversibility	5 (2-9)	5 (2-9)	0.581	n=95	4 (2-6)	5 (2-8)	0.438	n=12
post FEV_1/VC% predicted	95 ±11	92 ±12	**0.002**	n=96	97 ±8	95 ±8	0.463	n=12
post DLCOcVA, % predicted	100 ±15	100 ±16	0.785	n=96	98 ±18	100 ±15	0.373	n=12
post RV/TLC ratio, % predicted	87 ±17	85 ±14	0.075	n=91	85 ±16	89 ±24	0.713	n=12
PC_{20}-Methacholine, mg/ml	2.7 (0.8-6.6)	2.7 (1.2-16)	0.522	n=76	5.8 (2.9-32)	32 (5-32)	**0.021**	n=11
Total IgE, kU/L	68 (26-236)	58 (21-177)	0.268	n=95	59 (30-115)	98 (33-168)	0.091	n=11
FeNO, ppb	21 (13-45)	20 (12-35)	0.371	n=94	29 (18-44)	26 (14-31)	0.158	n=12
Blood neutrophils, 10^9/L	3.7 (3-4.6)	3.4 (2.7-4.2)	0.369	n=96	3.0 (2.7-4)	3.5 (2.5-4.5)	0.388	n=12
Blood eosinophils, 10^9/L	0.17 (0.1-0.28)	0.17 (0.12-0.27)	0.489	n=97	0.15 (0.08-0.26)	0.18 (0.1-0.27)	0.875	n=12
Sputum eosinophils, %	0.8 (0.2-4.2)	0.6 (0.2-2.8)	0.200	n=45	0.6 (0.4-0.6)	0.6 (0.4-1.6)	0.180	n=2
Sputum neutrophils, %	67 (50-77)	79 (66-91)	**0.001**	n=45	63 (49-63)	54 (34-70)	0.655	n=2

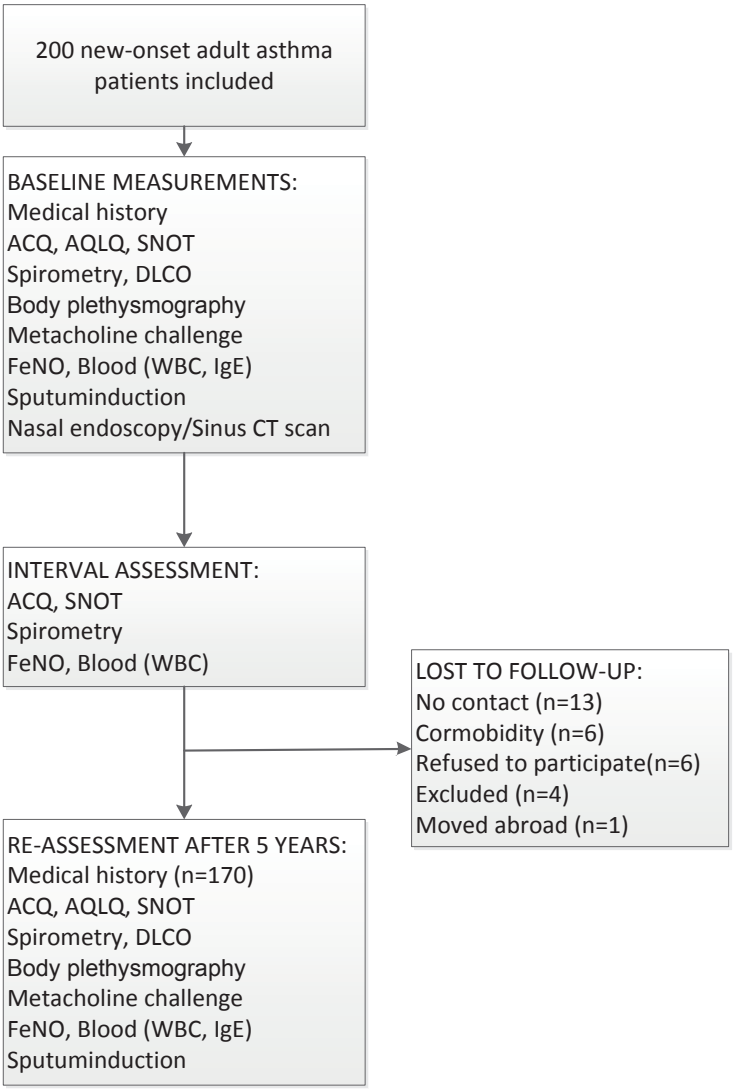
Pre: pre-bronchodilator. Post: post-bronchodilator. Data are presented as mean (±SD) or median (interquartile range).

Table 3. Patients with Asthma Persistence, with and without Nasal Polyps.

	Absence of nasal polyps (n=107)			Nasal polyps (n=35)			p-value
			n			n	
Gender, % female	65		107	29		35	**<0.001**
Age, years	48	±14	107	56	±14	35	**0.004**
BMI, kg/m^2	28.5	±5.4	107	27.2	±4.9	35	0.225
(Ex)smoker, %	53		107	71		35	**0.059**
Pack years	2	(0-14)	106	8	(0-18)	35	0.132
ACQ-6 score	1.40	±0.94	100	1.12	±0.81	33	0.133
Inhaled corticosteroid (ICS) use, %	79		107	85		34	0.450
ICS and second controlller, %	62		107	69		35	0.463
Oral corticosteroid use, %	4		105	0		32	0.262
ICS dose (fluticasone equivalent)	250	(125-500)	107	500	(250-500)	34	0.489
Any asthma medication baseline, %	92		107	94		34	0.632
pre FEV_1 % predicted	94	±16	104	89	±20	31	0.152
pre FVC% predicted	105	±16	102	106	±18	29	0.985
post FEV_1 % predicted	100	±16	107	99	±19	35	0.707
post FVC% predicted	108	±16	107	109	±17	35	0.726
FEV_1 % reversibility	5	(2-8)	104	5	(3-11)	34	0.143
post FEV_1/FVC% predicted	96	±11	107	92	±12	35	**0.056**
Post DLCOcVA % predicted	96	±15	107	105	±15	35	**0.004**
post RV/TLC ratio % predicted	87	±19	101	93	±21	32	0.137
PC_{20}-Metacholine, mg/ml	2.8	(0.9-6.4)	98	2.5	(0.5-10.6)	32	0.944
Nasal polyp surgery, %	NA		NA	66		35	NA
CRS, %	44		99	100		35	**<0.001**
GERD, %	42		107	29		35	0.155
Atopy, %	42		106	49		35	0.465
Obesity, %	37		107	20		35	**0.058**
Total IgE, kU/L	60	(23-229)	101	79	(30-240)	34	0.396
FeNO, ppb	16	(13-31)	101	39	(19-68)	34	**<0.001**
Blood neutrophils, 10^9/L	3.7	(2.9-4.8)	103	3.8	(3.3-4.3)	35	0.994
Blood eosinophils, 10^9/L	0.16	(0.1-0.24)	103	0.26	(0.2-0.35)	35	**<0.001**
Sputum eosinophils, %	0.4	(0.1-1.6)	65	2.3	(0.9-8.9)	21	**0.001**
Sputum neutrophils, %	72	(52-86)	65	68	(33-79)	21	0.188

Pre: pre-bronchodilator. Post: post-bronchodilator. GERD: gastroesophageal reflux disease. Data are presented as mean (±SD), median (interquartile range) or percentage.

Figure 1. Consort diagram.



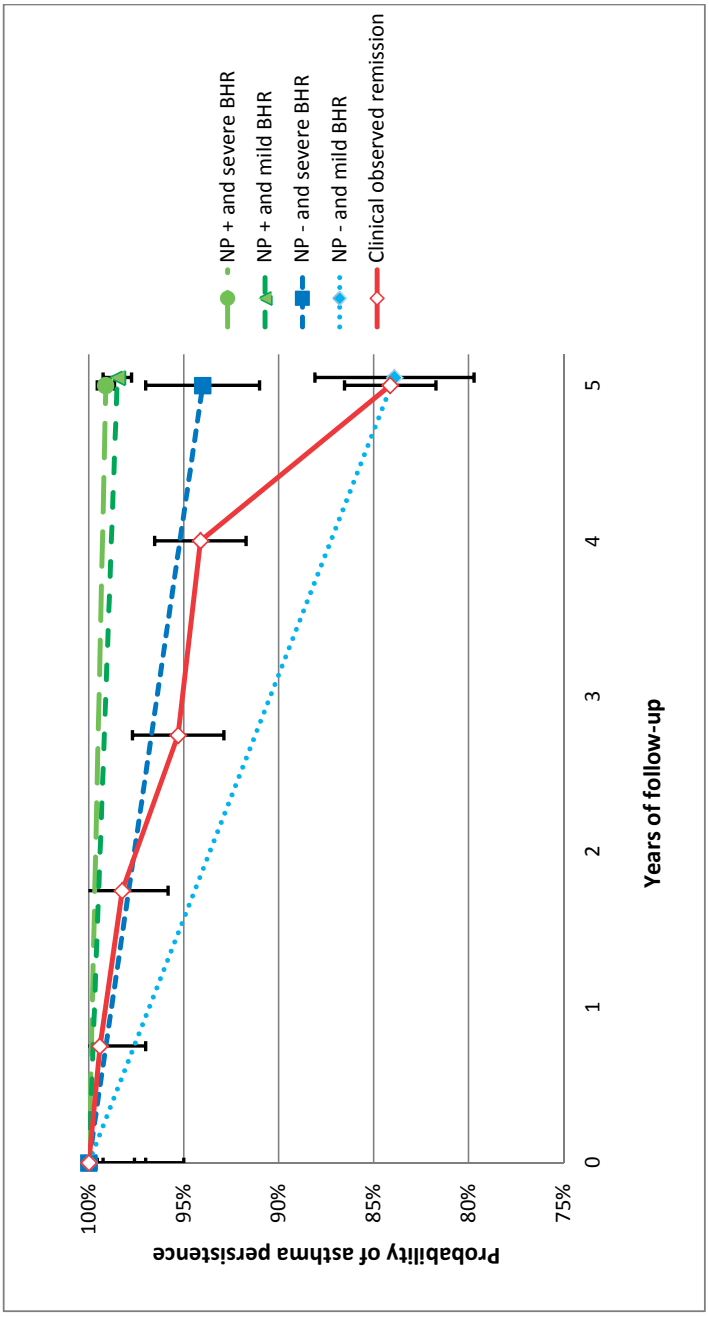
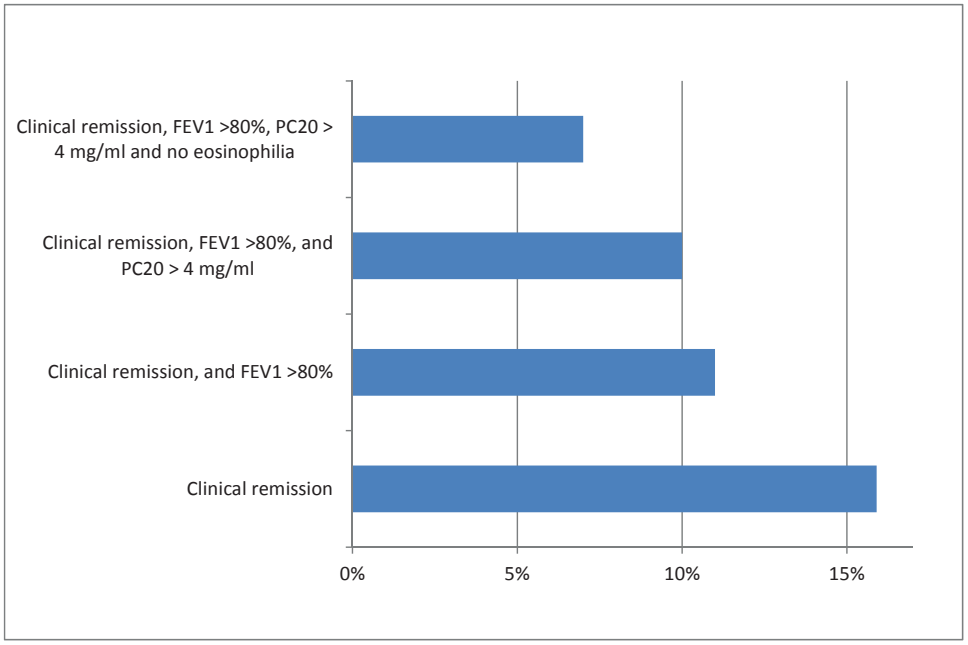


Figure 2. Probability of Asthma Persistence.

NP +: presence of nasal polyps. NP -: absence of nasal polyps. BHR: bronchial hyperresponsiveness. Dashed lines represent the probability of asthma persistence calculated by the prediction model based on baseline characteristics (NP and BHR). The continuous line shows the clinically observed percentage of patients experiencing asthma remission in the cohort. The error bars represent standard error.

Figure 3. Asthma Remission Incidence Based on Different Definitions of Remission.



No eosinophilia: sputum eosinophils < 3%.

DISCUSSION

This study shows that one in six patients with adult-onset asthma experiences clinical remission within the first 5 years of the disease, which is more than previously reported. Independent risk factors of asthma persistence include moderate to severe bronchial hyperresponsiveness and nasal polyposis, which together reduce the chance of asthma remission to less than 1%. These results demonstrate that asthma severity - defined by BHR - and upper airways involvement at the time of asthma diagnosis are major outcome determinants of newly diagnosed asthma in adults.

In the present study we found an asthma remission rate of 3.2% per year, which is relatively high. In the literature, a wide range of remission rates in adults with asthma has been reported varying from 0.6 to 3% per year.[6, 8, 10, 25, 26] An important problem when comparing these figures with those from our study is the lack of a common definition of asthma remission. Furthermore, most studies have not differentiated between childhood and adult-onset asthma, which most likely caused an overestimation of the remission rate.

Few studies specifically addressed remission rates in adult-onset asthma and only one study exclusively included patients with new-onset adult asthma.[9] The latter study found a clinical remission rate of 0.8% per year, which is considerably lower than in our study. Even after applying more strict rules for remission, by including normal lung function and absence of airway hyperresponsiveness, the remission rate was still higher than previously reported (Figure 3).[9] This discrepancy might be due to differences in asthma severity of the patients, although this is not likely. All our patients were recruited from secondary or tertiary care centres, and diagnosed with asthma by pulmonologists, which was confirmed by spirometry or airway hyperresponsiveness tests. One retrospective study suggested that remission rates were relatively high early after disease onset, and decreased after 4-7 years,[6] which is in line with our findings, and might explain the relatively high remission rates after the first 5 years of the disease.

Our study was set up to identify predictors of remission and persistence of new onset asthma in adults. Two previously published studies on this topic[9, 13] reported that there were differences between patients who remitted and not remitted (e.g. with respect to baseline FEV_1), but the number of patients with remitted asthma in those two studies was too low to properly analyze that point. Our study had enough remitted patients for more detailed statistical analysis and we found that airway hyperresponsiveness and nasal polyposis were strongly associated with asthma persistence but not lung function at baseline. In fact, all patients with nasal polyps showed asthma persistence. The importance of nasal polyposis in determining the persistence of asthma is a novel finding. Previous studies have shown a close association between upper airways involvement and asthma.[27, 28] Allergic and non-

allergic rhinitis have been shown to predict asthma onset,[29, 30] and chronic rhinosinusitis is an important comorbid condition in patients with asthma.[31] More importantly, chronic rhinosinusitis with nasal polyposis is associated with greater asthma severity and frequent exacerbations.[32] Nasal polyposis as a predictor of chronicity and persistence of new onset asthma has not been reported before, although it is conceivable that asthma will persist as long as nasal polyps persist, since both are likely manifestations of the same underlying pathophysiological process.[33]

Our study shows that apart from comorbid nasal polyps, the persistence of asthma is associated with the severity of the disease itself as reflected by increased bronchial responsiveness, although this association was six times weaker than for nasal polyps. Previous studies already reported an important role for bronchial hyperresponsiveness in the prognosis of asthma patients, in particular for predicting accelerated decline in FEV_1.[34] Our findings also fit in with data obtained in childhood asthma, showing that asthma persistence is strongly linked to greater frequency and severity of asthma symptoms, and more severe airway hyperresponsiveness.[35] However, unlike in children, atopic sensitization was not a predictor of persistence in our study of adults with new onset asthma.[36]

The association of nasal polyposis with asthma persistence in our study was strong, yet, only a quarter of the patients with adult-onset asthma showed this condition. For patients without nasal polyposis, predictors of asthma persistence were less obvious. In a post-hoc evaluation of these patients, they appeared to be more frequently female and more often obese than patients with nasal polyps and to have poor asthma control (data shown in Table 3). In addition, these patients showed an increase in neutrophils over the years. This set of clinical characteristics fits in with a specific phenotype of adult-onset asthma, the so-called "obese female asthma phenotype" which has been identified in several large cluster analysis studies.[3, 37, 38] and have been shown to have neutrophilic inflammation.[39, 40] Apparently, patients with obesity associated asthma are not likely to achieve asthma remission, despite positive effects of weight loss in other studies.[41]

An alternative explanation might be the presence of chronic rhinosinusitis without nasal polyposis, which was observed in almost half of patients, and could have led to airway neutrophilia. This increase in non-type 2 inflammation suggests activation of Th17 and Th1 pathways, caused for instance by recurrent respiratory infections or exposure to environmental pollutants and thereby leading to persistent asthma.[42]

What could be mechanisms leading to persistent asthma? Given the strong association between asthma persistence and nasal polyposis, it is likely that common causal mechanisms play a role in the development and persistence of both diseases. These include immune responses against fungi, bacteria, or bacterial enterotoxins leading to type 2 inflammation in both upper and lower airways.[43] Another factor that might induce and maintain both diseases

is cigarette smoke. Cigarette smoking has been shown to be a risk factor for development of chronic airways disease.[44] In our study, the group of patients with persistent asthma and nasal polyps consisted of smokers and ex-smokers for more than 70%. A common mechanism could include cigarette smoke-induced inflammation in the airways, associated with nasal polyp formation and insensitivity to corticosteroids,[45] leading to an increased severity and persistence of nasal polyps and asthma.[16]

Almost two-thirds of the patients with polyps in our cohort had already undergone surgical treatment for nasal polyposis before the onset of asthma, suggesting that in these patients nasal polyposis had preceded the onset of asthma. However, despite this treatment, progression to persistent asthma had still occurred. Apparently, early treatment of nasal polyposis does not prevent asthma to develop or become chronic. Although adequate medical treatment of rhinitis has been shown to improve asthma control[46] our data suggest that surgical treatment of nasal polyposis is insufficient to prevent the development of persistent asthma.

The strength of our study was the prospective follow-up design and the extensive baseline characterisation of the patients. In addition, stringent criteria for asthma were used, consisting of physician's diagnosis confirmed by lung function measurements.[15]
A potential limitation of the study could be our definition of asthma remission. Clinical remission might not reflect pathophysiological remission, which includes normal airway responsiveness, and absence of airway inflammation. Previous studies have demonstrated ongoing eosinophilic airway inflammation, airway hyperresponsiveness and airway remodelling in adolescents in clinical remission of atopic asthma.[47] Thus, subclinical asthma might have overestimated remission rates in our patients with adult-onset asthma. Another potential limitation of our study is that data from only 170 of 200 patients were available after 5 years. This could have introduced a selection bias, for example if the patients who were lost to follow-up had been the ones with an extensive smoking history. (Ex)smoking has been shown to worsen the prognosis of adult onset asthma,[16] and might thereby also influence the chance of asthma remission. However, by comparing patients who were lost to follow-up with the rest of the cohort, we did not observe differences in smoking history.

Finally, the majority of patients were assessed at yearly intervals, which might have influenced the results of our study. However, we do not believe that this was the case, because these interval assessments were done by study investigators who were not linked to the health care providers of the patients. The results of these assessments were not communicated to the health care providers, and did not lead to changes in treatment.

Our study has several clinical implications. Firstly, it provides useful indicators to identify patients with newly diagnosed asthma who are at risk of persistent disease. This new insight

into the prognosis of asthma patients should lead to closer monitoring of these patients in order to improve asthma outcomes. Secondly, treatable traits in these patients including eosinophilic airway inflammation and nasal polyps should be addressed and treated.[14] Biologicals targeting type 2 inflammation that have been shown to be effective in both conditions might be promising, although the effect of such treatment on the prognosis and persistence of asthma remains to be confirmed. Finally, all adults with newly diagnosed asthma should be evaluated for nasal polyposis, preferably by means of nasal endoscopy, because of their strong prognostic value. This advice should also be included in the GINA guidelines, where an examination of the upper airway is recommended in patients with severe asthma only.[15]

In conclusion, this study shows a relatively high remission rate in adult-onset asthma within the first 5 years of the disease, which is reassuring. However, a combination of nasal polyposis and moderate to severe bronchial hyperresponsiveness (PC_{20}-methacholine ≤ 1.0 mg/ml) at the time of asthma diagnosis reduces the chance of remission to almost zero. Recognition of these at risk patients at an early stage is necessary because both conditions are associated with impaired quality of life, reduced workplace productivity, and substantial medical treatment costs. Since eosinophilic airway inflammation seems to play a role in the persistence and chronicity of both asthma and nasal polyposis, this might offer options for early intervention with biologicals targeting key drivers of type 2 inflammation.[48, 49]

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Chapter 7.1

PREDICTORS FOR THE DEVELOPMENT OF PROGRESSIVE SEVERITY IN NEW-ONSET ADULT ASTHMA

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ABSTRACT

BACKGROUND: A proportion of patients with adult-onset asthma develops severe disease. Risk factors for an increase in asthma severity are poorly known.

OBJECTIVE: To identify predictors for the development of severe asthma in adults.

METHODS: A cohort of 200 adults with new-onset asthma was prospectively followed for 2 years. At baseline, patients underwent a comprehensive assessment of clinical, functional, and inflammatory parameters. After 2 years, change in asthma severity was assessed by using the Global Initiative of Asthma (GINA) score (1-4), which is based on asthma control (ACQ), lung function (FEV_1) and inhaled corticosteroid requirement. ANOVA and multiple regression equations were used in the analysis.

RESULTS: 128 patients completed two years of follow-up. 17 patients (13.3%) showed an increase in asthma severity, whereas 53 patients (41.4%) showed a decrease. Lower post-bronchodilator FEV_1/forced vital capacity ratio and a higher number of cigarette pack years smoked at baseline were significantly associated with an increase in asthma severity at follow-up. Multiple regression equations showed that only the number of cigarette pack years smoked was independently associated with an increase in asthma severity with an OR 1.4 (95% CI 1.02-1.91) for every 10 pack year smoked.

CONCLUSION: A history of cigarette smoking in patients with new-onset adult asthma predicts an increase in asthma severity during the first 2 years of the disease in a dose-dependent way.

CLINICAL IMPLICATIONS: Adults with new-onset asthma and a positive smoking history are at risk of developing severe disease and might be candidates for early targeted interventions.

INTRODUCTION

Adult-onset asthma is a heterogeneous disease with a variable clinical course. The disease ranges from very mild to very severe.[1] The majority of these patients have mild or intermittent asthma.[1;2] However, a proportion of patients develops more severe asthma over time.[1] Characteristics of patients with severe, adult-onset asthma are now well described in several cross-sectional studies. We previously reported that these patients are more often non-atopic, and have persistent sputum eosinophilia, higher blood neutrophil counts and more nasal polyps.[3] Other authors also concluded that patients with the adult-onset asthma phenotype are less often atopic[4] and have more sputum eosinophilia[5] compared to patients with the childhood-onset asthma phenotype. Adult-onset asthma patients also suffer from respiratory infections[4] more often and many have persistent airflow limitation.[4;5] Particularly non-atopic males with adult-onset asthma seem to be at risk for persistent airflow limitation,[6] which is possibly related to persistent infection with Chlamydia pneumonia.[7] This suggest that severe adult-onset asthma is a distinct phenotype.[3]

The natural history of disease severity after the diagnosis of adult-onset asthma has never been prospectively evaluated, and predictors for the development of severe asthma are still unknown. Identification of patients at risk for severe asthma at disease onset might be important for early therapeutic intervention.[8]

In the present study we hypothesized that specific predictors for deterioration of adult-onset asthma can be identified at asthma onset. Therefore, the aim of this study was to prospectively identify the predictors of increase in asthma severity in a cohort of patients with new-onset adult asthma.

METHODS

PATIENTS AND DEFINITIONS

Two hundred patients with adult-onset asthma were recruited from the outpatient pulmonary clinics of one academic hospital (AMC, Amsterdam) and two secondary care hospitals in the Netherlands. One in Amsterdam (St Lucas-Andreas Hospital) and one in Hoofddorp, a municipality 20 kilometres from Amsterdam (Spaarne Hospital). All the study measurements were conducted at the AMC. All patients had to have a recent (<1 year) diagnosis of asthma. Adult-onset asthma was defined as asthma with an onset of the disease at >18 years of age. Asthma diagnosis was based on typical symptoms and reversibility in FEV_1 of > 12% of predicted value and/or a positive inhaled methacholine provocation test (PC_{20} < 8mg/ml).[9]

Patients were excluded if they had a self-reported history of childhood asthma or other chronic respiratory diseases in childhood, frequent episodes of dyspnea as a child, or use of bronchodilator or other asthma medication in childhood.

Current smoking and ex-smoking were allowed. Patients with a smoking history of >10 pack years were included only if they showed reversibility in FEV_1 ≥12% of the predicted value, and had a normal diffusion capacity (DLCO >80%).

The study was approved by the AMC Medical Ethics Board. All participants were informed and gave written informed consent. The study was registered in the Dutch trial register (NTR1846).

STUDY DESIGN

This study was part of the ADONIS-study (Adult-onset asthma and inflammatory subphenotypes), a longitudinal prospective cohort study in adults with new-onset asthma. At baseline, patients underwent comprehensive assessment of clinical symptoms, lung function, markers of inflammation (exhaled nitric oxide, blood and sputum eosinophils), atopy, sinus CT scan and nasal endoscopy. Thereafter, they were monitored by their own treating physician at regular intervals. After two years patients were reassessed in our laboratory, with regard to clinical symptoms, lung function, medication requirement and markers of inflammation (fraction exhaled nitric oxide and blood eosinophils).

CLINICAL MEASUREMENTS

A comprehensive history was taken and physical examination was performed. In addition, patients completed self-reported questionnaires including the Asthma Control Questionnaire (ACQ-6),[10] and Sino-nasal Outcome Test (SNOT-22).[11]

LUNG FUNCTION MEASUREMENTS

Lung function measurements were performed in the lung function laboratory according to international recommendations with a daily calibrated spirometer (MasterScreen PFT, Jaeger,

Care Fusion, Hoechberg, Germany) and constituted FEV_1 and FVC.[12] Post-bronchodilator measurements were done 10-15 minutes after inhalation of 400 µg salbutamol.

INFLAMMATORY PARAMETERS

Fraction of exhaled nitric oxide (FeNO) was measured with a portable rapid-response chemoluminescent analyser (flow rate 50mL/s; NIOX System, Aerocrine, Sweden).[13]

Venous blood was collected and white blood cells were counted. Total and specific IgE to common aeroallergens were measured by ImmunoCAP; atopy was defined as IgE >0.35 Ku/L for at least one allergen.

Sputum induction was performed according to international standards.[14] All patients were nebulised 3 times for 5 minutes with a 4.5% saline solution. Sputum processing was done according to full sample method and differential cell counts were analysed on cytospin preparations.

ASSESSMENT OF CHRONIC RHINOSINUSITIS (CRS) AND NASAL POLYPOSIS

CRS was diagnosed according to the European Position Paper on Rhinosinusitis and Nasal Polyps (EP3OS).[15] In short, this diagnosis is based on symptoms, assessed by SNOT-22 questionnaire, combined with sinus CT-scan abnormalities (Lund-Mackay score >11)[16] and/or endoscopic signs of CRS/nasal polyps.[15]

ASTHMA SEVERITY SCORE

Assessment of asthma severity was based on the GINA 2002 guidelines.[17] The GINA score is a composite score based on treatment and clinical measures.[17-20] Patients are divided into four asthma severity categories: intermittent, mild persistent, moderate persistent and severe persistent, that are reported as score 1 to 4. (see Figure E1 in the Online Repository)

Three parameters (inhaled corticosteroid dose, FEV_1 and symptom score) for calculation of GINA severity score were available in all patients at baseline. At follow-up, 20 patients (10%) were lacking a FEV_1 or ACQ-score due to lack of time or practical difficulties in completing study visits. Using the two available parameters, a "least GINA score" was calculated, corresponding with their mildest GINA classification (see Figure E1 in the Online Repository). This modified GINA score for these 20 patients was used for further analysis.

To assess the change in asthma severity; the difference in GINA score at 2 year follow-up with respect to baseline was determined. A negative number represented deterioration of asthma severity, "zero" represented no change and a positive number represented improvement of asthma severity. Patients were divided into one of these three categories.

STATISTICAL ANALYSIS

Comparison of baseline mean values between included and lost-to-follow-up patients were made by Student's t-test or Mann-Whitney rank sum test (for non-normally distributed data).

For nominal variables, the Chi-square test was used.

Differences in baseline variables between the three groups ("deterioration", "no change" and "improvement") were compared using one-way analysis of variance (ANOVA) or Kruskal-Wallis test (for non-normally distributed data). Changes in the three groups over time were analyzed with a paired t-test. Multinomial logistic regression ("no change" group was used as reference) and binary logistic regression ("no change" and "improvement" group together were used as reference) were performed. Because their results were similar, only binary logistic regression outcomes are reported. The significant predictor variables were used in a multivariate binary logistic regression model. Results are expressed as odds ratio (OR).

SPSS Statistics version 20 (SPSS Inc., IBM, Chicago, Ill) were used for all statistical analyses. Statistical significance was set at a p-value of <0.05.

RESULTS

BASELINE CHARACTERISTICS

Amongst the 200 patients initially included in the study, 72 subjects (36%) did no longer participate after 2 years of follow-up. Most patients were lost-to-follow-up due to a lack of time (n=33), co-morbidities (n=9), distance (n=5), language barrier (n=1) or unknown reasons (n=24). Baseline characteristics of included patients and lost-to-follow-up patients are shown in Table 1. Included patients were more often male (p=0.03), used higher doses of inhaled corticosteroids (p=0.05) and were more often (ex-)smokers (p=0.03), compared to lost-to-follow-up patients.

ASTHMA SEVERITY

Seventeen patients (13.3%) showed an increase in asthma severity (increase in GINA score), whereas 53 (41.4%) of patients showed improvement (decrease in GINA score) after two years as compared to baseline. Baseline characteristics of these different groups are shown in Table 2. The patients whose asthma deteriorated used lower doses of inhaled corticosteroids, had milder asthma at baseline (p<0.01) and had smoked more pack years (p=0.02). These patients also tended to be more often current smokers (p=0.078). There were no other significant differences in baseline characteristics including lung function and inflammatory parameters. Table 3 presents the within-subject changes in the same variables after two year follow-up, showing a significant drop in ACQ (p=0.049) despite increased ICS dose (p<0.001) in the deterioration group.

PREDICTORS OF INCREASE IN ASTHMA SEVERITY

At first, univariate logistic regression with multiple variables was performed. Table 4 shows that FEV_1/FVC and smoked pack years were significant predictors of an increase in asthma severity (increase in GINA score). An odds ratio (OR) of 1.5 (95% confidence interval (CI) 1.10-2.02) was found for every 10 pack years a patient had smoked. When using a cut-off point of more than 20 pack years, an OR of 3.6 (95% CI 1.22-10.76) was found. Having a lower FEV_1/FVC at baseline was predictive for deterioration as well. Every 10% lower FEV_1/FVC was associated with an OR of 1.6 (95% CI 1.03-2.56). A cut-off point of FEV_1/FVC <95% of the predicted value (mean of population) was associated with an OR of 3.9 (95% CI 1.30-11.98).

In order to calculate adjusted OR, the significant variables of the univariate analysis were fitted into a logistic regression model. This showed that the only independent predictor for deterioration of asthma severity was the number of smoked pack years with an OR of 1.4 (95% CI 1.02-1.91) for every 10 pack year smoked. A low FEV_1/FVC at baseline was no longer significantly associated with asthma deterioration (see Table 5).

Table 1. Baseline characteristics of included and lost-to-follow-up patients (n=200)

Co-variate	Included (n=128)		Lost-to-follow-up (n=72)		p-value
Age, years	48	(15)	48	(15)	0.925
Female, %	50.8		66.7		**0.030**
BMI, kg/m^2	28.3	(5.2)	26.8	(5.4)	0.062
(ex)-smoker, %	62.2		45.7		**0.026**
Pack year	11	(14)	8	(14)	0.205
Fluticasone equivalent µg	375	(250-500)	250	(0-500)	**0.049**
ACQ score	1.29	(0.92)	1.39	(0.96)	0.478
GINA score	3	(2-4)	3	(2-4)	0.422
Atopy, %	46.1		42.3		0.602
CRS, %	55.2		52.5		0.736
Nasal polyposis, %	19.2		20.3		0.856
pb FEV_1 % predicted	100	(17)	100	(17)	0.975
pb FEV_1/FVC % predicted	95	(11)	96	(11)	0.664
Exhaled NO, ppb	20	(13-45)	22	(13-40)	0.653
Total IgE, Ku/L	61	(26-199)	60	(26-239)	0.872
Blood neutrophils, x10^9/L	3.8	(1.4)	3.7	(1.5)	0.685
Blood eosinophils, x10^9/L	0.16	(0.09-0.25)	0.17	(0.11-0.29)	0.255
Sputum neutrophils percentage	70.8	(48-85)	74.7	(54-83)	0.474
Sputum eosinophils percentage	0.45	(0.1-2.6)	0.90	(0.28-4.3)	0.367

Mean (SD) or median (interquartile range) values are given. Significant p-values are printed in boldface. pb: post-bronchodilator. ppb: parts per billion.

Table 2. Baseline characteristics of patients included in the analysis (n=128).

Co-variate	Deterioration (n=17)		No change (n=58)		Improvement (n=53)		p-value
Age, years	50	(13)	50	(16)	46	(14)	0.375
Sex, female, %	41.2		48.3		56.6		0.474
BMI, kg/m^2	28.7	(4.4)	27.2	(4.5)	29.4	(5.8)	0.086
Smoking status							0.078
Never smoker, %	35.3		32.8		45.3		
Ex-smoker, %	47.1		55.2		50.9		
Current smoker, %	17.6		12.1		3.8		
Smoking history, py	20	(21)	10	(13)	8	(12)	**0.014**
Fluticasone equivalent µg	250	(62-250)	250	(0-500)	500	(250-500)	**<0.001**
ACQ score	1.15	(0.77)	1.13	(0.98)	1.52	(0.86)	0.066
GINA score	2	(2-3)	2.5	(2-3)	3	(3-4)	**<0.001**
Atopy,%	41.2		48.3		45.3		0.865
CRS, %	58.8		48.3		62.0		0.341
Nasal polyposis, %	23.5		20.7		16.0		0.734
pb FEV_1 % predicted	93	(18)	101	(18)	102	(16)	0.145
pb FEV_1/FVC % predicted	90	(9)	96	(11)	97	(10)	0.086
Exhaled NO, ppb	17.4	(14-44)	19.6	(13-43)	22.2	(13-45)	0.862
Total IgE, Ku/L	125	(19-497)	60	(25-170)	51	(27-168)	0.285
Blood neutrophils, x10^9/L	4.2	(1.7)	3.8	(1.3)	3.7	(1.3)	0.369
Blood eosinophils, x10^9/L	0.17	(0.07-0.21)	0.16	(0.09-0.25)	0.16	(0.09-0.29)	0.800
Sputum neutrophils percentage	77.3	(59-89)	71,0	(48-84)	62.7	(47-80)	0.302
Sputum eosinophils percentage	0.4	(0.1-1.3)	0.4	(0.1-2.3)	0.6	(0.2-6.2)	0.426

Mean values (SD) or median (interquartile range) are given. Significant p-values are printed in boldface. pb: post-bronchodilator. ppb: parts per billion.

Table 3. Longitudinal changes; comparison between baseline and 2 years.

	Deterioration (n=17)			No change (n=58)			Improvement (n=53)		
	Baseline	2 years	p-value	Baseline	2 years	p-value	Baseline	2 years	p-value
BMI, kg/m^2	28.7	28.9	0.492	27.2	27.3	0.716	29.4	29.7	0.974
Fluticasone equivalent µg	250	500	**<0.001**	250	250	0.181	500	250	**<0.001**
ACQ score	1.15	1.55	**0.049**	1.13	1.05	0.217	1.52	1.05	**<0.001**
GINA score	2	4	**<0.001**	2.5	2.5	0.322	3	2	**<0.001**
pb FEV_1 % predicted	93	95	0.302	101	103	0.149	102	105	**0.007**
pb FEV_1/FVC % predicted	90	87	0.245	96	94	0.084	97	96	0.100
Exhaled NO, ppb	17.4	25.6	0.326	19.6	21.3	0.106	22.2	23.0	0.513
Blood neutrophils, x10^9/L	4.2	4.6	0.256	3.8	3.7	0.842	3.7	3.5	0.363
Blood eosinophils, x10^9/L	0.17	0.15	0.222	0.16	0.17	0.350	0.16	0.17	0.181

Significant p-values are printed in boldface. pb: post-bronchodilator. ppb: parts per billion.

Table 4. Odds ratios for factors potentially associated with a deterioration in GINA score.

Covariate	Odds ratio	95% CI
FEV_1/FVC <95%	**3.9**	1.30 - 11.98
> 20 pack year	**3.6**	1.22 - 10.76
Sputum neutrophils >64%	2.5	0.60 - 9.98
FEV_1/FVC per 10% lower	**1.6**	1.03 - 2.56
BMI > 30	1.6	0.56 - 4.52
Every 10 pack year smoked	**1.5**	1.10 - 2.02
Nasal polyposis, yes	1.4	0.40 - 4.59
Age > 50 yr	1.3	0.48 - 3.68
CRS, yes	1.2	0.42 - 3.35
Atopy, yes	0.8	0.28 - 2.24
Female	0.6	0.23 - 1.80
Exhaled NO	0.6	0.19 - 1.79
Sputum eosinophils >2%	0.4	0.08 - 2.00

Univariate odds ratios with 95% confidence intervals are given. Significant odds ratios are printed in boldface.

Table 5. Multivariate odds ratios for factors associated with a deterioration in GINA score.

Covariate	Odds ratio	95% C.I.
10 pack year	1.4	1.02 - 1.91
FEV_1/FVC per 10% lower	1.4	0.88 - 2.36

	Odds ratio	95% C.I.
FEV_1/FVC <95%	3.1	0.99 - 9.92
10 pack year	1.4	1.01 - 1.89

Odds ratios with 95% confidence intervals are given. Two models are selected with significant variables from Table 4.

DISCUSSION

This prospective cohort study shows that in adults with new-onset asthma the only independent predictor for an increase in asthma severity within 2 years after diagnosis is a positive smoking history. Even a limited number of pack years of cigarette smoking is associated with increased risk for asthma deterioration, whereas inflammatory parameters, lung function, sex, BMI and sinonasal symptoms are not predictive for worsening of early asthma. These results suggest that previous smoking is the most important factor influencing asthma severity in adult-onset asthma.

To our knowledge this is the first prospective longitudinal cohort study that evaluated predictors for asthma deterioration in adults with new-onset asthma. One previous longitudinal study investigating risk factors of asthma severity was performed in a mixed population of patients with longstanding childhood- and adult-onset asthma.[21] This study showed that patients with severe disease at baseline were at risk for severe disease nine years later. Comparison of this study with our study is difficult due to lack of assessment of longitudinal change in asthma severity in the latter study and differences in patient selection criteria. Our observation that smoking history predicts deterioration extends the findings by Polosa et al.[20] They retrospectively studied patients with allergic rhinitis who developed asthma within a period of 10 years[20] and found that patients with more severe asthma had smoked more pack years than those with milder asthma. Our results also extend the results from other longitudinal studies in mixed asthma populations looking at components of asthma severity, including asthma exacerbations and accelerated decline in FEV_1.[22-26] These studies showed that recent exacerbation history was a strong predictor of future asthma exacerbations,[22] whereas active smoking,[23;26] high levels of exhaled nitric oxide[24] and infiltration of CD8+ cells in the airway wall[25] were shown to be predictors of accelerated decline in FEV_1. All these results provide evidence that a positive smoking history in adult patients with new-onset asthma is a strong and dose-dependent predictor of an increase in asthma severity over the next 2 years.

The strength of our study is its prospective design with extensive baseline assessments to fully characterize the patients. Also, the study population is unique since all patients were recently diagnosed with adult-onset asthma. Smokers and ex-smokers were purposely not excluded from participation into the study as long as they met the stringent diagnostic criteria for asthma. This is important, because most asthma studies exclude smokers or subjects who have smoked >10 pack years, whereas in a large proportion (20-30%) of the actual population of asthma patients are current smokers.[27-29] We avoided including patients with “pure” COPD by excluding (ex-)smokers with persistent airflow limitation or reduced diffusion capacity, but cannot exclude the inclusion of patients with overlap between these diseases.[30] Unfortunately, we did not reassess bronchodilator reversibility and diffusion capacity at the 2 year follow-

up visit. Therefore, we were not able to determine whether asthma in smokers might have evolved phenotypically into COPD. However, there was a trend for a lower postbronchodilator FEV_1/FVC ratio in patients with increased asthma severity as compared to those whose asthma had improved, suggesting the development of persistent airflow limitation.

We chose to use the GINA score for classifying asthma severity because it is a composite score of asthma control, lung function and treatment.[9] Asthma severity is thus assessed in a broad and realistic perspective, which is important especially when evaluating changes in severity over time. In our opinion, missing data on ACQ-score *or* FEV_1 in a small proportion of our patients was not relevant, because an accurate estimation of the severity score could still be made (see Fig. E1).

Our results could have been biased by the rather large proportion of patients who were lost to follow-up. Patients who were lost to follow-up were more often non-smoking women using lower doses of inhaled corticosteroids than the participants, suggesting that they had milder asthma and did not require regular follow-up. One might argue that the higher number of smokers amongst the participants as compared to those who were lost to follow-up might have influenced our results. However, a small enrichment of the population with (ex-)smokers is not expected to affect the results of a logistic regression analysis.

The most likely explanation for the effect of (ex-)smoking on asthma deterioration is persistent smoking-induced inflammatory pattern, which is glucocorticoid insensitive. Active smoking changes the type of airway inflammation towards more mast cells and neutrophils and less eosinophils.[31;32] Ex-smoking asthmatics are more frequently known to have neutrophilic airway inflammation,[32] which is associated with glucocorticoid insensitivity. Smoking is also known to cause airway remodelling, with an increased epithelial wall thickness and more goblet cells in bronchi, leading to more symptoms of dyspnea and sputum production. A longitudinal study with a follow-up period of 23 years showed that the protective effect of inhaled corticosteroids on lung function decline was absent in patients who had smoked > 5 pack years.[33] However, reduced beneficial effects of inhaled glucocorticoids caused by smoking were found in some, but not in all studies.[34;35] The results of our study support the hypothesis that smoking, even for a short period of time, has persistent effects on corticosteroid sensitivity, leading to rapid increase in asthma severity despite elevation of inhaled steroid doses shortly after asthma diagnosis.

The results from this study have a number of clinical implications. Clinicians should be alert on an increase in asthma severity in patients with new-onset adult asthma who have a history of smoking. These patients are prone to a more rapid increase in asthma severity, and early therapeutic intervention is therefore warranted. Since corticosteroids are less effective in smokers than in non-smokers,[34] novel targeted therapeutic options might be more suitable for these patients.

In summary, we showed that smoking or having smoked in the past is an independent risk factor for increased asthma severity within 2 years after asthma diagnosis. The more pack years smoked, the higher the risk of increased asthma severity. Lung function, sinus disease and type of airway inflammation at the time of asthma diagnosis are not associated with more severe asthma after 2 years. Since (ex)-smokers with new-onset asthma are at increased risk of developing severe asthma, these patients should be coached intensively for stop-smoking, monitored closely, and receive aggressive preventive therapy. Since inhaled corticosteroids are not very effective in these patients, novel targeted therapies need to be developed for this highly prevalent category of patients.

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SUPPLEMENTARY MATERIAL

METHODS

ASTHMA SEVERITY SCORE

Assessment of asthma severity was based on the GINA 2002 guidelines.[E1] Classification takes three steps: the first step is to determine each patient's treatment category, based on the daily dose of inhaled corticosteroids (low, medium or high).[E2] Secondly, symptom score and FEV_1 are assessed: 1) intermittent symptoms or FEV_1 >80% predicted, 2) mild persistent symptoms or FEV_1 >80%, 3) moderate persistent symptoms or FEV_1 60-80%, 4) severe persistent symptoms or FEV_1 <60%. The final step is to combine clinical severity of step 2 with medication dose of step 1 into an overall category (Figure E1).[E1]

Fig E1. Asthma severity score according to the GINA guidelines (2002)[E1].

Classification of Asthma Severity

symptoms & lung function	current treatment step		
	step 1 *intermittent*	*step 2* *mild persistent*	*step 3* *mod. persistent*
step 1: intermittent symptoms - FEV_1 > 80%	intermittent	mild persistent	moderate persistent
step 2: mild persistent symptoms + FEV_1 > 80%	mild persistent	moderate persistent	severe persistent
step 3: mod. persistent symptoms ++ FEV_1 60-80%	moderate persistent	severe persistent	severe persistent
step 4: severe persistent symptoms ++ $FEV_1 \leq 60\%$	severe persistent	severe persistent	severe persistent

Categorization is based on a composite score of treatment with symptoms and lung function. The presence of one feature of severity is sufficient to place a patient in that category.

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Chapter 7.2

REPLY TO: 'OCCUPATIONAL ASTHMA IS A CAUSE OF ADULT-ONSET ASTHMA WITH POOR PROGNOSIS'

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To the Editor

We thank Burge et al.[1] for their relevant commentary on our recently published article.[2] They introduce an interesting hypothesis about the possible influence of occupational exposure on the rapid increase of asthma severity in adults with recent onset asthma in our study. They specifically ask for any data related to possible occupational asthma.

In our cohort we have collected data about current occupation and its influence on asthma symptoms and potential contribution to asthma onset. The specific questions asked to the patients were: "What do you think the possible cause of your asthma could have been (e.g. work)?" and "Did your work influence your asthma symptoms?". If these questions were answered positively, patients were interviewed more in depth about the details of their job and job history and what the possible causative agent could have been.

Ten percent (n=13) of the patients who participated in our follow-up study were considered to have possible occupational asthma. Remarkably, none of these patients showed an increase in asthma severity over the two year follow-up period. On the contrary, 8 out of 13 patients (61.5%) with potential occupational asthma showed an improvement in asthma severity. Four of these patients appeared to have switched jobs because of their asthma symptoms. Unfortunately, we did not have more extensive data about the other half of the patients.

Our observation of improvement of asthma severity fits in with the findings of Burge and colleagues that accelerated FEV_1 decline in patients with occupational asthma returns to normal rates of decline after exposure ceases.[3] Discontinuation or at least decrease of exposure to the causative agent is in line with the currently recommended management strategy for occupational asthma, as this has a possible beneficial influence on the outcome.[4] However, even after stopping exposure a persistent effect can be seen, although the prognosis probably depends on early asthma diagnosis, the duration of exposure and the severity of asthma.[5]

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Chapter 8

PREDICTORS OF FREQUENT EXACERBATIONS IN (EX)SMOKING AND NEVER SMOKING ADULTS WITH SEVERE ASTHMA

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ABSTRACT

BACKGROUND: Persistent eosinophilic airway inflammation is an important driver for asthma exacerbations in non-smokers with asthma. Whether eosinophilic inflammation is also a predictor of asthma exacerbations in (ex)smokers is not known.

OBJECTIVE: The aim was to investigate factors associated with frequent exacerbations in never smokers and (ex)smokers with asthma.

METHODS: (Ex)smoking (n=83) and never smoking (n=70) patients with uncontrolled asthma despite high dose asthma medication (GINA treatment step 4-5) were selected from a cohort of 571 adult-onset asthma patients. Clinical, functional and inflammatory parameters were used in multivariate logistic regression analyses to identify factors associated with frequent exacerbations (≥3 oral corticosteroid (OCS) bursts in the previous year).

RESULTS: Frequent exacerbations in (ex)smokers were independently associated with ICS dose (OR 1.2, 95%CI: 1.1-1.3) and blood neutrophil count (OR 1.5, 95%CI: 1.2-2.1). In never smokers frequent exacerbations were independently associated with blood eosinophil count (OR 18.9, 95%CI: 1.8-202.1).

CONCLUSION AND CLINICAL RELEVANCE: This study shows that never smoking and (ex) smoking patients with severe asthma have different predictors of frequent exacerbations: higher blood neutrophils in (ex)smokers versus higher blood eosinophils in never smokers. This suggests that different types of systemic background inflammation play a role in the aetiology of exacerbations in these patients.

INTRODUCTION

Asthma exacerbations impose a major burden on patients' lives and account for a large part of asthma related health care costs.[1] The importance of persistent eosinophilic airway inflammation in asthma exacerbations has been recognized for many years.[2] Increased levels of sputum eosinophils precede and predict exacerbations.[3,4] Adjusting anti-inflammatory treatment based on sputum eosinophil level has been shown to reduce asthma exacerbations.[5,6] Moreover, suppression of eosinophils with antibodies directed against interleukin (IL)-5 leads to a 50% reduction in exacerbation frequency.[7,8] However, in the majority of asthma studies only non-smoking patients were included.[9] Whether airway eosinophilia is also a predictor of asthma exacerbations in current and exsmoking asthma patients is not known.

In the present study we hypothesized that airway eosinophilia is a predictor of asthma exacerbations in both never smokers and (ex)smokers. Therefore we investigated factors associated with frequent exacerbations in never smokers and (ex)smokers with asthma.

METHODS

STUDY DESIGN AND PARTICIPANTS

This was a cross-sectional study using pooled baseline-data from three observational cohort studies with similar methodologies. These three studies included in total 571 patients with adult-onset asthma between 2009 and 2012 (Netherlands Trial Register numbers: NTR2217, NTR1846 and NTR1838) and aimed at phenotyping patients based on an extensive set of clinical, functional and inflammatory parameters. All three trials were reviewed and approved by medical ethical boards before their initiation.

Adult patients were eligible for the three cohorts if they had a confirmed diagnosis of asthma with onset of the disease after the age of 18 years. Asthma diagnosis was based on a history of variable respiratory symptoms AND documented variable expiratory airflow limitation: reversibility in FEV_1 of >12 % predicted and 200 ml or airway hyperresponsiveness to inhaled methacholine (PC20 <8 mg/ml) or diurnal variation in PEF of ≥20% or history of prompt deterioration in FEV_1 after ≤25% reduction in oral or inhaled corticosteroid dose (within 4 weeks).[1] Patients with other pulmonary diseases, non-related major co-morbidities, and pregnancy were excluded. Smoking was allowed, however, patients with a smoking history of > 10 pack years combined with fixed airflow obstruction and/or reduced diffusion capacity (DLCO/VA <80%) were excluded. Detailed in- and exclusion criteria have been reported previously.[10-12] All patients were informed and gave written consent.

For the present study patients with severe asthma were selected (see Figure 1 for study flowchart); those using high intensity asthma treatment as defined by GINA treatment step 4-5 (use of high dose inhaled corticosteroid and a second controller or systemic corticosteroid use >50% of the previous year)[1] and with uncontrolled asthma defined as either asthma control questionnaire (ACQ)-score >1.5, 2 or more exacerbation per year or presence of airflow limitation with an FEV_1 <80% predicted.[13]

After that, patients were stratified according to smoking status: current smokers and ex-smokers were combined into one group called (ex)smokers,[11] their smoking history was quantified by calculating the number of pack years. Never smokers were patients who had never smoked.

Finally, (ex)smokers and never smokers were divided in two groups: 3 or more exacerbations (frequent exacerbations) or ≤ 1 exacerbations in the previous year (non-frequent exacerbations) as previously reported.[14,15] An asthma exacerbation was defined as an increase in asthma symptoms requiring treatment with a course of oral corticosteroids (OCS) or at least a doubling from a stable maintenance dose for at least three days. Courses of systemic corticosteroid separated by one week or more were recorded as a separate exacerbation.

ASSESSMENTS

Clinical parameters - Systematic medical history was taken with regard to asthma symptoms, including asthma-specific questionnaires (Asthma Control Questionnaire (ACQ)[16] and Asthma Quality of Life Questionnaire (AQLQ)),[17] medication use and asthma related healthcare consumption in the previous year (number of courses oral corticosteroids). Co-morbidities with possible influence on asthma symptoms were recorded; gastro-esophageal reflux disease, chronic rhinosinusitis (based on symptoms and combined with sinus CT-scan or nasal endoscopy if available).

Functional parameters - The following lung function measurements were performed according to international standards: spirometry (prebronchodilator and postbronchodilator FEV_1 and forced vital capacity (FVC)),[18] single breath carbon monoxide diffusing capacity of the lung (TLCOc/VA),[19] static lung volumes by bodyplethysmography (total lung capacity (TLC) and residual volume (RV))[20] and airway hyperresponsiveness to methacholine (provocative concentration causing a 20% drop in FEV_1 (PC20)).[21]

Inflammatory markers - Fraction of exhaled nitric oxide (FeNO) was measured with a portable rapidresponse chemoluminescent analyzer (NIOX system, Aerocrine, Sweden).[22] Venous blood was collected and differential white blood cell counts, total and specific IgE to common allergens (ImmunoCAP, Thermo Scientific, Uppsala, Sweden) measurements were performed. Atopy was defined as one or more specific IgE levels above 0.35 kU/L. Sputum induction and processing was done according to internationally accepted standards as described previously.[23] Results for different sputum cell types are presented as percentage of total non-squamous cell count.

STATISTICAL ANALYSIS

First, characteristics of (ex)smokers and never smokers were compared. Second, (ex)smoking patients with and without frequent exacerbations and never smoking patients with and without frequent exacerbations were compared. Comparisons were made by either student T-test, Mann-Whitney U test or chi square, whenever appropriate.

In order to identify variables potentially associated with frequent exacerbations, all variables with a p-value <0.10 in the comparison between patients with and without frequent exacerbations in the (ex)smoker or never smoker group were used in a univariate logistic regression analysis. After that, variables with a p-value <0.05 in the univariate logistic regression analysis were used in the multivariate logistic regression analysis. The final multivariate models were created by stepwise selection.

RESULTS

Characteristics of (ex)smoking and never smoking patients with severe asthma are given in Table 1.

FREQUENT EXACERBATIONS IN (EX)SMOKERS

(Ex)smokers with frequent exacerbations had a longer duration of asthma, used higher doses of inhaled corticosteroids (ICS), were more likely to be using chronic oral corticosteroids (OCS), had a higher blood neutrophil count and a lower post-bronchodilator FEV_1 (trend) as compared (ex)smokers without frequent exacerbations (Table 2). The univariate logistic regression analysis revealed chronic OCS use, blood neutrophil count, higher ICS dose and asthma duration as factors associated with frequent exacerbations. Blood neutrophils and higher ICS dose were independently associated with frequent exacerbations in the multivariate model (Table 4). Patients with a blood neutrophil count of $\geq 4.3*10^9$ cells/L had an odds ratio of 2.91 (95% CI:1.2-7.2; p=0.021) for frequent exacerbations.

FREQUENT EXACERBATIONS IN NEVER SMOKERS

Never smokers with frequent exacerbations had increased airway hyperresponsiveness to methacholine compared to never smokers without frequent exacerbations (Table 3). Higher blood eosinophil count and higher ICS dose were associated with frequent exacerbations in the univariate logistic regression analysis. Blood eosinophils were the only significant factor when combined in a multivariate model with ICS dose (Table 4). A blood eosinophil cutoff of $\geq 0.25*10^9$ cells/L gave an odds ratio of 2.94 (95% CI:1.1-8.1; p=0.038) for frequent exacerbations.

***Table 1**. Comparison of (ex)smokers vs never smokers.*

	(Ex)smokers (n=83)	Never smokers (n=70)	p-value
Age, years	53 (10)	50 (13)	0.133
Sex, % female	60	77	**0.026**
Age of asthma onset	43 (14)	40 (13)	0.195
Asthma duration, years	5 (1-14)	6 (2-15)	0.531
BMI, kg/m^2	29.0 (5.2)	28.8 (5.4)	0.793
Positive family history of asthma, %	49	32	0.052
Current smoker, %	11	NA	NA
Pack years	9.00 (5-20)	NA	NA
ICS dose *	750 (500-1000)	1000 (500-1000)	0.610
Chronic OCS use, %	28	29	0.906
Atopy, %	30	44	0.070
Chronic rhinosinusitis, %	21	31	0.174
GERD, %	37	38	0.912
ACQ-6 score	2.0 (0.9)	1.9 (0.9)	0.807
AQLQ	4.9 (1.1)	4.8 (1.1)	0.484
Pb FEV_1, % predicted	87 (20)	89 (20)	0.529
Pb FEV_1/FVC, % predicted	87 (14)	91 (14)	0.090
RV/TLC ratio, % predicted	99 (20)	95 (20)	0.321
TLCOc/VA, % predicted	101 (16)	100 (16)	0.553
PC20 (methacholine) mg/ml	3.4 (4.6)	4.7 (5.6)	0.291
FeNO, ppb	22 (13-42)	24 (13-46)	0.595
Blood neutrophils, 10^9 cells/L	4.3 (3.5-5.5)	4.3 (3.4-5.9)	0.959
Blood eosinophils, 10^9 cells/L	0.19 (0.1-0.395)	0.20 (0.1-0.34)	0.926
Total IgE, kU/L	97 (24-247)	64 (30-232)	0.792
Sputum neutrophil %	59.4 (39.3-72.6)	66.5 (38-84.4)	0.322
Sputum eosinophil %	2.2 (0.2-25.6)	2.2 (0.2-20.8)	0.962

Data presented as percentage, mean (SD) or median (interquartile range). * ICS dose in fluticasone equivalent. Pb: postbronchodilator. ppb: parts per billion.

***Table 2**. Comparison of **(ex)smokers** with and without frequent exacerbations.*

	Non-exacerbator (n=45)	Exacerbator (n=38)	p-value
Age, years	53 (10)	54 (11)	0.518
Sex, % female	58	63	0.618
Age of asthma onset	45 (13)	41 (14)	0.225
Asthma duration, years	4 (0-10)	7 (2-21)	**0.040**
BMI, kg/m^2	28.6 (5.0)	29.5 (5.4)	0.404
Positive family history of asthma,%	41	56	0.251
Current smoker, %	11	11	0.932
Pack years	10 (5-24)	8 (4-16)	0.123
ICS dose *	500 (500-1000)	1000 (500-1500)	**0.009**
Chronic OCS use, %	13	45	**0.001**
Atopy, %	38	21	0.098
Chronic rhinosinusitis, %	18	24	0.517
GERD, %	38	36	0.841
ACQ-6 score	1.9 (0.8)	2.1 (1.1)	0.518
AQLQ	5.0 (1.1)	4.8 (1.0)	0.540
Pb FEV_1, % predicted	91 (19)	83 (19)	0.067
Pb FEV_1/FVC, % predicted	88 (14)	85 (15)	0.365
RV/TLC ratio, % predicted	98 (20)	99 (21)	0.976
TLCOc/VA, % predicted	101 (18)	102 (15)	0.871
PC20 (methacholine), mg/ml	3.2 (3.8)	3.9 (6.4)	0.680
FeNO, ppb	19 (13-38)	35 (12-93)	0.131
Blood neutrophils, 10^9 cells/L	4.1 (3.2-5.1)	5.0 (3.8-6.4)	**0.008**
Blood eosinophils, 10^9 cells/L	0.19 (0.13-0.44)	0.18 (0.08-0.34)	0.371
Total IgE, kU/L	63 (22-242)	111 (32-291)	0.370
Sputum neutrophil %	53 (34-71)	63 (39-76)	0.221
Sputum eosinophil %	1.0 (0-37.1)	5.2 (0.4-23.6)	0.469

Data presented as percentage, mean (SD) or median (interquartile range). * ICS dose in fluticasone equivalent. Pb: postbronchodilator. ppb: parts per billion.

***Table 3**. Comparison of **never smokers** with and without frequent exacerbations.*

	Non-exacerbator (n=42)	Exacerbator (n=28)	p-value
Age, years	50 (14)	51 (13)	0.852
Sex, % female	83	68	0.131
Age of asthma onset	41 (14)	39 (11)	0.537
Asthma duration, years	5.0 (1-16)	8.5 (2-15)	0.269
BMI, kg/m^2	28.7 (6.1)	28.9 (4.2)	0.853
Positive family history of asthma, %	40	21	0.132
Pack years	NA	NA	NA
ICS dose *	625 (500-1000)	1000 (500-1250)	0.080
Chronic OCS use, %	21	39	0.105
Atopy, %	38	54	0.202
Chronic rhinosinusitis, %	30	33	0.776
GERD, %	46	27	0.134
ACQ-6 score	1.8 (0.8)	2.2 (1.0)	0.087
AQLQ	5.0 (1.2)	4.5 (1.0)	0.154
Pb FEV_1, % predicted	92 (18)	84 (21)	0.092
Pb FEV_1/FVC, % predicted	92 (15)	89 (14)	0.481
RV/TLC ratio, % predicted	94 (19)	98 (21)	0.414
TLCOc/VA, % predicted	98 (17)	103 (14)	0.245
PC20 (methacholine), mg/ml	5.5 (6.1)	2.0 (1.8)	**0.012**
FeNO, ppb	23 (13-45)	30 (15-61)	0.473
Blood neutrophils, 10^9 cells/L	4.1 (3.1-5.5)	4.5 (3.6-6.3)	0.237
Blood eosinophils, 10^9 cells/L	0.17 (0.10-0.30)	0.28 (0.12-0.65)	0.051
Total IgE, kU/L	54 (27-229)	100 (48-271)	0.195
Sputum neutrophil %	64.1 (3 7.6-86.1)	72.4 (36.8-83.6)	0.872
Sputum eosinophil %	1.9 (0.2-13.7)	6.7 (0.2-33.8)	0.589

Data presented as percentage, mean (SD) or median (interquartile range). * ICS dose in fluticasone equivalent. Pb: postbronchodilator. Ppb: parts per billion.

***Table 4**. Univariate and multivariate logistic regression analysis of potentially associated factors.*

(Ex)smokers

Univariate analysis	OR	95% C.I.		p-value
Chronic OCS use, yes	5.26	1.80	15.36	**0.002**
Blood neutrophils	1.50	1.12	2.02	**0.006**
ICS dose (per 100 µg)	1.16	1.05	1.29	**0.005**
Asthma duration, years	1.04	1.00	1.08	**0.049**
Pb FEV_1 % predicted	0.98	0.96	1.00	0.072
Atopy, yes	0.44	0.16	1.18	0.102

Multivariate analysis	OR	95% C.I.		p-value
Blood neutrophils	1.51	1.10	2.08	**0.010**
ICS dose (per 100 µg)	1.18	1.05	1.32	**0.005**

Multivariate odds ratios are adjusted for OCS use and asthma duration. Blood neutrophils are analysed in absolute numbers.

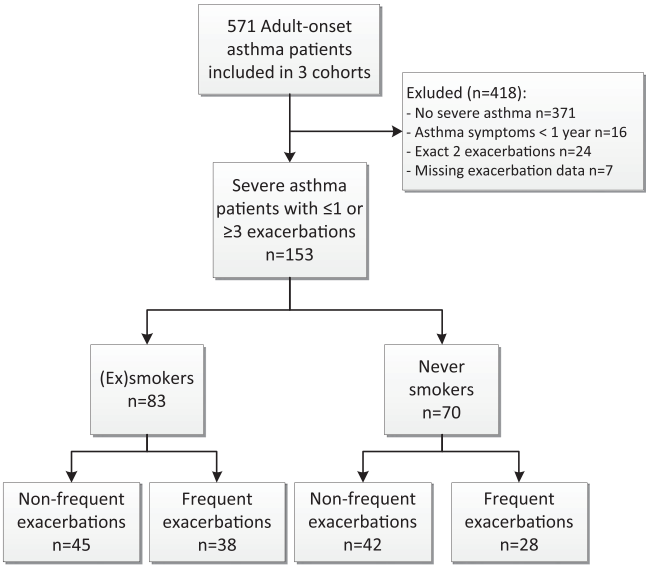
Never smokers

Univariate analysis	OR	95% C.I		p-value
Blood eosinophils	18.9	1.77	202.1	**0.015**
Chronic OCS use, yes	2.37	0.82	6.83	0.109
ACQ-6 score	1.71	0.92	3.17	0.091
ICS dose (per 100 µg)	1.11	1.00	1.23	**0.043**
Pb FEV_1 % predicted	0.98	0.95	1.00	0.095
Methacholine PC20	0.82	0.63	1.07	0.149

Multivariate analysis	OR	95% C.I.		p-value
Blood eosinophils	18.9	1.8	202.1	**0.015**

Multivariate odds ratio is adjusted for ICS dose. Blood eosinophils are analysed in absolute numbers.

Figure 1. *Study flowchart.*



DISCUSSION

This study shows that never smoking and (ex)smoking patients with severe asthma have different predictors for frequent exacerbations. In never smokers higher blood eosinophils are associated with frequent exacerbations, as would be expected. However, in (ex)smokers higher blood neutrophils and higher ICS dose, are associated with frequent exacerbations. These results suggest that different types of systemic background inflammation play a role in the aetiology of frequent exacerbations in (ex)smoking and never smoking adult asthma patients.

In our study we compared predictors of frequent exacerbations between never smokers and (ex)smokers with severe asthma. Previous studies investigated risk factors of frequent exacerbations in asthma in general, without making a distinction between (ex)smokers and never smokers. The association between blood or sputum eosinophilia and exacerbations has been reported in several reports,[4,24] which is in line with our results in never smokers. Other predictors including recent exacerbations,[25] disease activity score,[26] co-morbidities[14] and smoking[15,27] were either not addressed or not confirmed in our study. Thus, in never smokers blood eosinophil counts are clearly associated with frequent exacerbations.

Our results show that (ex)smokers have different risk factors of frequent exacerbations than never smokers. Although all our patients had a confirmed diagnosis of asthma, were measured in a stable phase of their disease, and had a relatively limited smoking history, the results show remarkable resemblance to findings in patients with COPD. One study in COPD patients with frequent exacerbations found an increased white blood cell count and neutrophil count to be associated with exacerbations.[28] Another COPD study found increased levels of blood and sputum neutrophils in patients with bacterial exacerbations.[29] The novel finding of our study is the distinct inflammatory pattern associated with frequent exacerbations in (ex)smoking asthma patients, which suggests a distinct inflammatory phenotype in these patients.

Several mechanisms could explain our findings. Different inflammatory profiles in smokers and exsmokers with severe asthma have been observed previously.[30] Compared to their never smoking counterparts (ex)smoking asthma patients have less eosinophils and more neutrophils in sputum, they have lower levels of FeNO, and are less often sensitized to common allergens.[30] How smoking influences airway inflammation and induces subsequent exacerbations is not fully elucidated and several mechanisms could be involved. One possible pathway could be mobilization and activation of neutrophils via innate immune responses mediated by macrophages and Th17 cells induced by cigarette smoke.[9] Altered immune responses caused by smoking[31] make smokers more susceptible to infections resulting in exacerbations.[32] In addition, changes in airway microbiome and bacterial colonization leading to a dominance of *Haemophilus sp.*, *Streptococcus sp.* and *M. catarrhalis* are also associated with neutrophilic

airway inflammation in severe asthma patients.[33] Furthermore, higher levels of oxidative stress in response to smoking partially cause the corticosteroid resistance often found in smokers with asthma and hence increase their susceptibility to exacerbations.[34] Taken together, an altered airway immune response and increased levels of oxidative stress by smoking, resulting in neutrophilic airway inflammation with more infections and reduced response to asthma therapy, might all contribute to an increased exacerbation risk.

Major strength of this study is the large cohort of extensively characterized adult asthma patients. In contrast to many other asthma-studies, we also included (ex)smokers which enabled us to study this important group of asthma patients as well. We acknowledge there are several limitations in our study. Firstly, the cross-sectional analysis of our data precludes assessing the temporal relationship between exacerbations and patients' characteristics. Therefore, our results have to be interpreted cautiously and ideally should be confirmed in prospective studies with careful documentation of exacerbations. Secondly, the use of oral corticosteroids by a large proportion of our patients may have introduced a bias, since oral corticosteroids are known to increase neutrophil numbers in peripheral blood. However, even after correction for oral corticosteroid use, blood neutrophil count was an independent predictor of frequent exacerbations in the (ex)smoker group, which strengthens our findings.

Our results have clinical implications. In order to improve asthma outcomes, clinicians may have to approach (ex)smokers and never smokers with asthma differently, based on their specific inflammatory cell pattern in peripheral blood. Non-smoking patients with blood eosinophilia should have their steroid treatment adjusted according to eosinophil counts in order to reduce exacerbation frequency, as shown previously.[5] In patients with persistent blood or sputum eosinophilia despite high dose inhaled corticosteroids, novel targeted therapies directed for example against interleukin (IL)-5 might be appropriate.[8] For (ex)smokers with neutrophilic or non-eosinophilic asthma the treatment options are less obvious. Several compounds directed against neutrophils or neutrophil associated pathways have been studied in patients with neutrophilic asthma.[35] For the majority of these compounds the beneficial effects on asthma outcomes or airway inflammation are limited. Some studies have shown that smokers with asthma might benefit from small-size-particle inhaled corticosteroids[36] or low-dose azithromycin as add-on treatment to decrease the number of asthma exacerbations.[37] Yet, the exact pathophysiological role of neutrophils in (ex)smokers with severe asthma and the optimal treatment strategy remains to be elucidated.

In conclusion, blood eosinophil counts in never smokers and blood neutrophil counts in (ex) smokers with asthma are associated with frequent asthma exacerbations. This novel finding suggests that different types of systemic background inflammation are involved in the aetiology of exacerbations in these distinct groups of asthma patients. Contrary to eosinophilic asthma, strategies to reduce exacerbation rates in (ex)smokers with neutrophilic asthma are as yet limited. Our data illustrate once more that the disease mechanisms of (ex)smoking asthma patients are clearly different from those of never smoking patients, which stresses the urgent need for more research in this neglected group of patients.

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Summary

Chapter 9

SUMMARY AND GENERAL DISCUSSION

ADULT-ONSET ASTHMA: PREDICTORS OF CLINICAL COURSE AND SEVERITY

Guus A. Westerhof

SUMMARY AND CONCLUSIONS

Chapter 1. Asthma is a common respiratory disease that affects 334 million people of all ages worldwide.[1] Characteristics of asthma are typical symptoms (wheezing, chest tightness, shortness of breath), reversible airway obstruction, bronchial hyperreactivity and chronic airway inflammation.[2] Asthma has been regarded for a long time as a disease that develops in childhood. Genetic predisposition, atopy and respiratory infections early in life have been established as the major risk factors for developing the disease.[3, 4] The prognosis is generally considered favourable, up to 65% of children has "overgrown" their asthma when they are middle aged.[5] However, since several decades a different asthma type that emerges in adulthood has been recognized, so called adult-onset asthma.[6-9] This type of asthma is considered to have several distinct clinical phenotypes, a more severe course than childhood asthma, with more symptoms and steeper decline in lung function.[10] However, the clinical course of adult-onset asthma over time is still largely unknown. Additionally, eosinophilic airway inflammation plays an important role in the severity of the disease. Eosinophilia is associated with disease activity and can be used to tailor asthma therapy. Direct sampling of the airways is time consuming and costly. Therefore markers of eosinophilic airway inflammation have been described, whether these markers are influenced by clinical asthma phenotype in adult-onset asthma is unknown.

The purpose of this thesis is twofold, first: to examine the diagnostic accuracy of markers of eosinophilic airway inflammation, specifically in different adult asthma phenotypes.

Secondly, identify predictors of asthma remission, increased asthma severity and exacerbations in patients with recent-onset adult asthma in order to elucidate the course of the disease.

Chapter 2 summarizes the known prognostic factors of adult-onset asthma with respect to lung function decline, increased asthma severity and asthma remission. Limited available data suggests that accelerated lung function decline is associated with male gender, atopic status and poor baseline lung function. Increased asthma severity is influenced by smoking and low lung function, whereas current uncontrolled asthma and smoking are associated with uncontrolled asthma in the future. High symptom scores, low lung function and markers of airway eosinophilia can predict asthma exacerbations. The remission rate of adult-onset asthma is considered to be low and mainly seen in patients with mild asthma and short disease duration. Smoking has a profound negative effect on asthma remission.

The number of longitudinal studies investigating the course of adult-onset asthma is limited; many studies in this field have been cross-sectional or conducted in populations not specifically consisting of adult-onset asthma patients. In order to gain more insight in the prognosis of adults with newly diagnosed asthma and identify possible treatable factors, more prospective studies in this specific group of patients are needed.

Part 1 of this thesis examines the diagnostic accuracy of several biomarkers to identify the presence of eosinophilic airway inflammation and serves as a method check for this specific inflammatory asthma phenotype. In **Chapter 3** the influence of different adult-onset asthma phenotypes on biomarkers of sputum eosinophilia is addressed. Levels of eosinophils in blood and sputum, FeNO and total IgE from 336 adult patients, enrolled in 3 prospective observational clinical trials, were analyzed. In the total group the AUC was 0.83 (95%CI 0.78-0.87) for blood eosinophils, 0.82 for FeNO (0.77-0.87) and 0.69 (0.63-0.75) for total IgE. Blood eosinophils and FeNO had comparable diagnostic accuracy (superior to total IgE) to identify sputum eosinophilia in adult asthma patients irrespective of asthma phenotype such as severe, non-atopic, obese and smoking-related asthma. In order to increase the clinical utility of biomarkers, combined markers were tested and high and low cutoff values were used. This method generates more diagnostic certainty for blood eosinophils and FeNO and might be useful to direct therapy adjustment.

Chapter 4 continues on biomarkers of eosinophilic airway inflammation and examines the question: how accurate are surrogate markers in detecting sputum eosinophilia in a general asthma population? A systematic review and meta-analysis to identify and pool all studies about biomarkers of airway eosinophilia in asthma patients have been performed. We included 24 studies in adults with asthma. Three markers had extensively been investigated: Fraction of Exhaled Nitric Oxide (FeNO) (17 studies; 3,216 patients; summary area under the receiver operator curve (AUC) 0.75 (95%CI 0.72-0.78)); blood eosinophils (14 studies; 2,405 patients; AUC 0.78 (0.74-0.82)); total Immunoglobulin E (IgE) (7 studies; 942 patients; AUC 0.65 (0.61-0.69)). Assessment of eosinophilic airway inflammation is possible with these biomarkers; however, one has to bear in mind that their use as a single surrogate marker for airway eosinophilia in asthmatic patients will lead to a substantial number of false positives and/or negatives.

Chapter 5 contains a commentary on a study in which FENO and blood eosinophils were associated with allergic asthma, but only in non-smokers. Although these findings do not necessarily raise questions about the clinical value of these biomarkers in smokers, they do show us that their potential value is likely to be different in this large subgroup of asthma patients. In an era where phenotypoing and subphenotping patients has become increasingly mandatory in the clinical workup of asthma patients; smoking status seems to be an important determinant in this differentiation.

Part 2 of this thesis addresses the clinical course of adult-onset asthma. Results presented in this part of the thesis are mainly based on data collected in the Adonis-study. In this prospective study two hundred adult patients with recently diagnosed (<1 year) asthma were included and followed for 5 years. Clinical, functional and inflammatory parameters were assessed at baseline and at yearly visits.

In **Chapter 6** the first question: 'which factors predict remission and persistence of adult-

onset asthma?' is addressed. Asthma remission (no asthma symptoms for ≥1 year and no asthma medication use for ≥1 year) occurred in 27 patients (15.8%) at 5-year follow-up. In a multivariable logistic regression analysis, bronchial hyperresponsiveness and nasal polyps were independent predictors. Combined in a prediction model, the presence of nasal polyposis or moderate-severe bronchial hyperresponsiveness gives a probability of asthma remission close to nil. Common pathophysiologic mechanisms, related to eosinophilic inflammation, might play a role in the chronicity of both asthma and nasal polyps. With our data, early recognition of patients with persistent asthma is possible and might offer options for adapted management strategies.

In **Chapter 7** factors associated with an increase in asthma severity after 2 years follow-up of the Adonis-study are investigated. 128 patients completed two years of follow-up. 17 patients (13.3%) showed an increase in asthma severity, whereas 53 patients (41.4%) showed a decrease. Multiple regression equations showed that only the number of pack years smoked was independently associated with an increase in asthma severity with an OR 1.4 (95% CI 1.02-1.91) for every 10 pack year smoked. These results imply that adults with new-onset asthma and a positive smoking history are at risk of developing severe disease and might be candidates for early-targeted interventions.

Finally, in **Chapter 8** predictors of asthma exacerbations in smokers and never smoker are addressed. Therefore we selected (ex)smoking (n=83) and never smoking (n=70) patients with severe asthma from a cohort of 571 adult-onset asthma patients. Frequent exacerbations (≥3 oral corticosteroid (OCS) bursts in the previous year) in (ex)smokers were independently associated with ICS dose (OR 1.2, 95%CI: 1.1-1.3) and blood neutrophil count (OR 1.5, 95%CI: 1.2-2.1). In never smokers frequent exacerbations were independently associated with blood eosinophil count (OR 18.9, 95%CI: 1.8-202.1). These different predictors of frequent exacerbations in never smoking and (ex)smoking patients with severe asthma suggest different types of systemic background inflammation might play a role in the aetiology of exacerbations.

GENERAL DISCUSSION

PART 1 MARKERS OF DISEASE ACTIVITY; MEASURING AIRWAY INFLAMMATION

BIOMARKERS OF AIRWAY INFLAMMATION IN ASTHMA PATIENTS; A CRITICAL EVALUATION

Biomarkers are getting increasingly important in medicine, also in the field of asthma.[11] Eosinophilia in sputum has been regarded as a hallmark of asthma since many years.[12] However, the assessment of this important asthma feature is time consuming and laborious. Many studies in patients with mainly childhood onset asthma tried to find more easily accessible biomarkers for the presence of airway eosinophilia. Our meta-analysis in Chapter 3 pooled all these studies[13] and shows that using a single biomarker to detect airway eosinophilia in asthma patients has moderate diagnostic accuracies. The biomarkers analyzed (blood eosinophils, FeNO and total IgE) all have summary estimates of AUC's, sensitivities and specificities that are far from perfect. As a result, when the reported cutoff points are used in clinical practice will lead to many false positive and false negative outcomes. Two other recent reports [14, 15] drew the same conclusion that FeNO and blood eosinophils lack sufficient sensitivity or specificity to be useful as markers of sputum eosinophilia. In order to increase the clinical usefulness of these biomarkers, we conducted a study where we reported biomarker thresholds at either high sensitivity or high specificity and combined markers in a prediction model (Chapter 4).[16] These high or low cutoff levels of the markers are more useful for physicians to respectively confirm or exclude airway eosinophilia with high certainty. However, one should bear in mind that in up to half of the patients with intermediate biomarker levels the diagnostic uncertainty remains; these patients would still need to undergo sputum induction to confirm or exclude sputum eosinophilia.

A general conclusion about part 1 of this thesis will be that biomarkers of airway eosinophilia in adult-onset asthma should be used with caution. Several critical comments can be made when investigating and using biomarkers of airway inflammation in asthma. This starts with choosing the optimal gold standard for research on biomarkers; are sputum eosinophil counts the right measure to use? Several studies have shown that these cells in sputum can be used as a marker to tailor steroid treatment which leads to a lower exacerbation rate.[17] More recent evidence shows that blood eosinophils are better predictors of response to anti-IL5 therapy[18] or periostin as a predictor for response on anti-IL13 therapy than sputum eosionphils are.[19] This raises the question about the relevance of the body compartment where the inflammation is measured: blood, airway lumen, airway wall, small airways, or a combination. If eosinophilic inflammation is present for example in both airway lumen and systemic circulation, there is an association with more severe asthma.[20] Therefore, use of a single biomarker will probably discard the additional information of the extensiveness and location of inflammation.

Secondly, in our study and other studies there is no perfect correlation between sputum and blood eosinophilia or FeNO for example. Probably slightly different underlying mechanisms lead to fluctuations in the levels of these markers. This increases the diagnostic uncertainty of a given biomarker.

Thirdly, finding the optimal cutoff values is always a matter of debate and results differ strongly between several studies.[13] Defining cufoff values for biomarkers should depend on the purpose of the marker: either exclude or confirm a certain condition. This method was used in Chapter 4.

Finally, an important question is whether the biomarker under investigation correlates with the disease outcome and response to therapy. For example, in Chapter 8 we showed that blood eosinophilia in non-smokers correlates with frequent exacerbations, as previously shown.[21] Several studies have addressed response to therapy: for example FeNO or exhaled breath have been shown to predict response to inhaled corticosteroid treatment.[22, 23]

Despite the several issues associated with biomarker use, biomarkers are definitely clinically useful in adult-onset asthma. In an era where we are getting towards precision medicine for more and more diseases, it would be very old fashioned to consider all asthma patients having the same disease (both clinically and biologically). Several studies have described different asthma phenotypes, also based on inflammatory markers like sputum eosinophils. Development of novel targeted therapies stresses the need to use biomarkers for selection of patients who will benefit most. Finally, monitoring disease activity is an important aspect of biomarker use which can be used to adjust treatment dose in an early stage and prevent deterioration of the disease. Biomarker use, with observance of its limitations, will improve care for our patients. Hence we should focus on the possibilities of the available biomarkers rather than the uncertainties.

DIFFERENT BIOMARKERS FOR EOSINOPHILIA IN DIFFERENT CLINICAL ASTHMA PHENOTYPES?

Apart from inflammatory phenotypes, several clinical asthma phenotypes have been described over the past years.[24] For instance asthma associated with obesity, gender, smoking or atopy. It is proposed that there are different mechanisms leading to the asthma and inflammation in these respective phenotypes. However, in all phenotypes, a proportion of one third to half of the patients has eosinophilic airway inflammation. When considering different asthma phenotypes with a possibly different origin of the disease, one can wonder whether the clinical usefulness of biomarkers of eosinophilia would be comparable between the phenotypes. In chapter 4 we have shown that phenotypic characteristics do not influence the accuracy of biomarkers of sputum eosinophilia. This despite the possible different underlying molecular pathways leading to inflammatory mechanisms in distinctive asthma phenotypes.[25] Clinical characteristics like obesity or smoking might also be of influence on the inflammatory pathway and pattern in asthma patients. The classical activation of eosinophilic airway inflammation in

atopic asthma runs via epithelial cells and dendritic cells, where T helper 2 lymphocytes are primed either directly by Il-25 and Il-33 or via naïve T helper cells. Clonal expansion of Th2 cells and activation of mast cells via IgE leads to production of Il-4, Il-5 and Il-13, which recruit and activate eosinophils. In patients with non-atopic asthma eosinophilic airway inflammation also exist, but this is probably initiated via innate lymphoid cell (ILC-2) instead of Th2 cells. Stimuli to the airway epithelial like smoke, pollutants and viruses, directly activate these cells via Il-25 and Il-33. These cells have been shown to be a major source of Il-5 and Il-13, important cytokines for eosinophil activation and survival. The crucial role of Il-5 in asthma has been shown by the extraordinary effect of blocking the cytokine with antibodies: it reduces the exacerbation rate and prednisone maintenance dose in prednisone dependent asthma patients.[26] Whether all cytokines involved in the inflammatory cascade play a crucial role in the clinical disease is questionable, as a recent trial with an anti-Il-13 antibody did not show clear improvement of disease outcomes. [27]

Would there be better biomarkers for inflammation in asthma? Or are the currently used biomarkers able to differentiate properly or is this an oversimplification of reality? The immunology underlying asthma is complex with several interaction and crossing pathways leading to airway inflammation. Perhaps other cells or molecules in these pathways could serve as more accurate biomarkers. For example serum periostin has been shown to be a good predictor of response to anti-IL-13 therapy, although the association with sputum eosinophilia was weak.[28] Another approach could be to look at gene sets associated with activation of a certain inflammatory pathway, such as Th2 High gene set consisting of a set of genes associated with Th2 activation.[29] Furthermore measurement of markers in exhaled breath has potential to discriminate between different inflammatory phenotypes.[30] These alternative biomarkers might prove to be useful for the development of new therapeutic compounds and selection of patients who will benefit most of these therapies.

BIOMARKERS OF AIRWAY INFLAMMATION IN ASTHMA PATIENTS WITHOUT EOSINOPHILIA.

Part 1 of this thesis mainly focuses on eosinophilic inflammation as expression of type-2 inflammation. However, more than half of all adult asthma patients do not have type-2 inflammation (chapter 3). This is a large and important subgroup, as non-type-2 inflammation is associated with poor response to regular asthma therapy[31] and clinical deterioration.[32 33] Activation of Th1 and Th17 cells, via stimuli like cigarette smoke and diesel exhaust, produces Il-1, TNF-α, Il-17 and Il-8. These cytokines and chemokines induce influx of macrophage and neutrophilic inflammation in the airways. Neutrophils might play an important role; especially in smokers as we have shown in Chapter 8 where neutrophils in blood are associated with frequent asthma exacerbations. Measurement of neutrophils as such might therefore serve as biomarker for exacerbations in smoking asthma patients.

In addition, perhaps processes leading to non-eosinophilic inflammation could serve as a

marker of the inflammatory process. For example oxidative stress (markers like 8-isoprostane and 4-hydroxynonenal (HNE)), or signs of systemic inflammation like CRP, IL6 and CD163 have been shown to be associated with poor lungfunction and neutrophilic inflammation.[33-35] Levels of soluble IL6 receptor in serum are associated with more severe asthma and with lower lung function.[36] In obese asthma patients levels of adipokines might be of interest: leptin and adiponectine.

What would be the therapeutic consequences of non-eosinophilic asthma is questionable. Elimination of neutrophils or activating interleukins could theoretically be beneficial. However, these cells do play a crucial role in innate immunity. Trials with anti-Il-8 or anti-Il-17 antibodies have failed to show significant improvement of asthma outcomes. Anti-TNFα also showed no beneficial effect on asthma.[37] Macrolide antibiotics have been shown to reduce the exacerbation rate in non-eosinophilic asthma, the exact working mechanism remains to be elucidated.[38] Unfortunately are the therapeutic possibilities for patients with non-eosinophilic asthma very limited until now and deserve more attention in future research.

PART 2 CLINICAL COURSE OF ADULT-ONSET ASTHMA

CONCLUSION AND COMPARISON WITH LITERATURE

Part two of this thesis focused on predictors of the clinical course of adult-onset asthma. Here we showed in Chapter 6 that a considerable proportion of patients with new-onset asthma experiences clinical asthma remission after 5 years. Persistence of the disease can be predicted by the presence of nasal polyps and moderate to severe bronchial hyperresponsiveness. An increase in asthma severity was already observed after two years of follow-up (Chapter 7). The main risk factor being smoked pack years, in a dose dependent way. Finally, predictors of frequent asthma exacerbations in smokers and never smokers were blood neutrophils and blood eosinophils respectively (Chapter 8).

How do our results compare to literature? And what are the differences compared to childhood onset asthma? The clinical remission rate in our study was higher as compared to previous studies in adult patients with asthma, of which only two study specifically included adult patients with new-onset asthma.[39, 40] When applying a more stringent definition including pathophysiologic remission, the remission rate in our cohort was still higher than previously reported.[39] One explanation for this difference might be the relatively high number of mild asthma patients. However, patients were included after referral to secondary and tertiary clinics which means these patients would be a good representation of what clinicians encounter in daily practice. Compared to childhood onset asthma the rate of asthma persistence is still much higher in adult-onset asthma.[41-43] Based on retrospective studies the probability of asthma remission in adults decreases when asthma exists for a longer time (> 4 years),[42] which suggests that the remission rate in our study is the maximum that can be expected for adult-onset asthma. Epidemiological data also support this hypothesis as an increase of asthma prevalence has been observed with increasing age.[44]

Presence of nasal polyps was a strong predictor of asthma persistence in our study, which is a novel finding. Other factors associated with remission and persistence of asthma are partly comparable for childhood and adult-onset asthma. In children it has been shown that asthma persistence is strongly linked to greater frequency and severity of asthma symptoms, and more severe airway hyperresponsiveness.[45] However, unlike in children, atopic sensitization was not a predictor of persistence in our study of adults with new onset asthma.[46]

Increase in asthma severity was already observed in an early stage of the disease which could be predicted by the number of pack years smoked. This was the first longitudinal study investigating factors associated with an increase in adult-onset asthma severity. Most previous studies were performed in mixed populations with childhood and adult-onset asthma patients.[47-51] One found severe asthma at start of the study as a risk factor for severe disease at follow-up.[52] The deleterious effects of smoking on the prognosis of asthma have been described in several studies. For instance in patients with allergic rhinitis, who developed

asthma, more pack years were associated with more severe asthma.[53] Others found that active smoking predicted an accelerated decline in FEV_1.[49, 51] A recent longitudinal study in patients with adult-onset asthma found smoking as a predictor of uncontrolled asthma[40] and accelerated decline in lungfunction.[54] Altogether, our study fits in with the increasing body of evidence pointing at smoking as important negative determinant for asthma prognosis.

Finally, the association of frequent exacerbations in never smokers with eosinophilia fits in with previous reports as would have been expected.[21, 55] Also in childhood onset asthma eosinophilia has been shown to be predictive of asthma exacerbations. Due to the considerable number of (ex)smokers in our study, we were able to analyze this important subgroup of asthma patients separately. Here we found an association between frequent exacerbations and neutrophilia. For asthma patients this was a novel finding, although it resembles previous findings in COPD patients. Two studies found increased levels of blood neutrophils in COPD patients with exacerbations.[56, 57]

INTERPRETATION OF THE RESULTS

The findings in our study might give clues about the underlying mechanisms determining the clinical course of asthma. Nasal polyps might be a symptom of generalised airways disease, which reflects the severity and extensiveness of the disease. Probably there are common causal mechanisms involved in the development of both nasal polyps and asthma. Stimuli like viruses, bacteria, allergens and toxins lead to activation of type 2 inflammation via T helper 2 (Th2) cells and group 2 innate lymphoid cells (ILC2). Via cytokine release this leads to recruitment and/or activation of mast cells, eosinophils, basophils, goblet cells, M2 macrophages, B cells and tissue responses.[58] Influx and activation of inflammatory cells will eventually lead to nasal polyps and, when lower airways are involved, also to asthma. The relation with asthma persistence and more severe BHR might also be a reflection of this active inflammatory process leading to airway smooth muscle activation and thereby a higher degree of airway hyperreactivity.

From the above mentioned effector cells, eosinophilic inflammation plays a very important role in adult-onset asthma and is not specifically related to allergic inflammation.[59] We have shown that blood eosinophils relate to frequent exacerbations in never smokers with adult-onset asthma, FeNO did not. This might be related to different mechanism leading to an increase in these biomarkers. FeNO increases typically after allergen exposure which leads to production of IL4 and IL13, upregulates iNOS in the airway epithelium followed by production of NO.[60] Whereas IL5 production by ILC2s in response to non-allergic stimuli specifically leads to eosinophilia, and is not necessarily related to allergen exposure.[59] This fits in with the adult-onset eosinophilic non-allergic asthma phenotype.

Smoking is an important determinant for the severity of asthma. The effects might be related to several mechanisms induced by cigarette smoke including altered airway inflammation, airway remodelling and insensitivity to corticosteroids. Smoking is known to cause non

type 2 inflammation in the airways, resulting in an influx of neutrophils. Also inducing basal membrane thickening and mucus hypersecretion by goblet cells.[61] These effects all contribute to corticosteroid insensitivity and increased symptom burden.

POTENTIAL BIAS

One potential pitfall for research on adult-onset asthma is the risk of recall bias; how sure are patients about their asthma history? In order to select patients with real adult-onset asthma for the Adonis-study, extensive intake interviews about pulmonary complaints in childhood were done and strict inclusion and exclusion criteria were used. Furthermore, epidemiological studies have shown that the self-reported year of asthma onset appears to be rather accurate.[62] Therefore we think that the chance of including patients with a relapse of remitted childhood onset asthma is probably very low, but cannot be excluded.

As smokers constitute an important and understudied subgroup of adult-onset asthma patients (up to one third is active smoker[63]), we did include them in our study. However, asthma at older age is often mistaken for COPD, especially when patient are (ex)smokers. To make sure these patients did not have COPD, strict lung function criteria were used and patients were excluded if they had fixed airway obstruction and sings of emphysema.

The definition of asthma remission has been highly variable in different studies. We have chosen to use clinical remission, based on the absence of asthma symptoms and no medication use. This definition might not be as strong as one including pathophysiological remission (normal lung function, no bronchial hyperreactivity, absence of airway inflammation). Due to a limited number of patients with pathophysiological follow-up data, we were not able to do firm analysis on the whole cohort.

CLINICAL IMPLICATIONS

The results of our studies have several clinical implications. We have found a clear predictive clinical profile for asthma persistence, in which bronchial hyperreactivity and nasal polyps are the main determinants. Furthermore, the number of pack years smoked can be used to estimate the chance of an increase in asthma severity. With these clear clinical characteristics, clinicians have tools to recognise patients at risk for persistent asthma and who are prone to deterioration in an early phase. Clinical characteristics combined with inflammatory markers like blood eosinophils and FeNO will give an even more accurate view on the phenotype of the patient in front of us.

The next step would be a therapeutic consequence for the clinical prognostic characteristics. For pathological characteristics like airway eosinophilia this has been shown previously (see Part 1). Treatment of chronic rhinitis has been shown to improve asthma control.[64] Whether aggressive treatment of nasal polyps in an early stage would improve asthma prognosis (no asthma or asthma remission) is unknown. Our observational data suggest a limited effect, as two third of the patients with nasal polyps had already undergone surgical treatment and still

had persistent asthma. Perhaps treatment of a common underlying inflammatory mechanism (associated to eosinophilia) or avoidance of causative agents will lead to a better outcomes of both diseases.

If complete asthma remission, including resolution of pathophysiologic abnormalities, is possible, one could speculate that aggressive treatment of asthma in an early phase of the disease can alter the prognosis. Whether this will be possible in real life will depend on several factors that lead to the clinical expression of asthma: 1) Specific asthma triggers like allergens, infections, pollutants, work related agents and smoke. 2) The extent and type of the present inflammatory patterns caused by the asthma trigger(s) and its consistency. 3) Structural airway changes due to inflammation and triggers (also dependent on the duration and continuation of triggers). 4) Sensitivity to therapy with inhaled corticosteroids and B agonists (genetic predisposition, epigenetic changes). 5) The influence of comorbid conditions like obesity, GERD, atopy and sinonasal diseases. Taken together, the best approach for treatment of an adult asthma patient with the current knowledge, would be a full assessment and treatment of the above mentioned associated factors.

FUTURE DIRECTIONS

Extensive data from the Adonis study is available to address a wide range of research questions:

- Evaluation of lung function over the years, what factors are associated with an accelerated decline in lung function?
- How did asthma severity develop during 5 years? Do we find the same predictors of increased severity as after two years? Or perhaps some patients have fluctuations in severity? Can this be linked to fluctuations in inflammatory markers?
- Investigation of biological data (sputum supernatant, sputum cells, serum, are available at baseline and 5 year follow-up):
- Looking at markers of oxidative stress, one could hypothesize that the level of oxidative stress correlates to clinical decline or decline in lung function.
- Markers of inflammation or inflammatory pathways might be interesting: not just eosinophils but perhaps cytokines or chemokines could teach us more about inflammatory phenotypes and the link to clinical phenotype.

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Chapter 10

NEDERLANDSE SAMENVATTING

Guus A. Westerhof

INLEIDING

Astma is een longziekte waarbij ontsteking en vernauwing van de luchtwegen leiden tot typische klachten. Deze klachten bestaan uit benauwdheid, piepende ademhaling, druk op de borst en hoesten. De meest bekende vorm is astma die op de kinderleeftijd ontstaat en vaak is geassocieerd met allergieën. Bij deze vorm van astma heeft de helft van de kinderen op de volwassen leeftijd geen astma meer heeft. Er is echter ook een andere variant van astma bekend die ontstaat op volwassen leeftijd, zogenaamd adult-onset asthma. Hier richt mijn onderzoek zich op.

Dit laat ontstaan astma kan worden onderverdeeld op basis van het type ontstekingscel in de luchtwegen. Twee belangrijke soorten ontstekingscel zijn eosinofielen en neutrofielen. Behandeling met ontstekingsremmers gericht tegen het eerste type ontstekingscel, de eosinofielen, leidt tot minder astma-aanvallen. Deze ontstekingscellen zijn in meer of mindere mate aanwezig bij een derde van de astma patiënten. Het meten van de hoeveelheid eosinofielen in het slijm uit de luchtwegen is echter zeer bewerkelijk. Daarom is het van belang om stofjes te vinden die wel makkelijk te meten zijn en die samenhangen met het type ontsteking in de luchtwegen. Dit zijn zogenaamde biomarkers. In deel 1 van mijn proefschrift hebben we gekeken naar biomarkers voor eosinofielen.

Deel 2 van mijn proefschrift focust op het verloop van laat ontstaan astma in de tijd. **Hoofdstuk 2** beschrijft wat er bekend was over de prognose van laat ontstaan astma. Vergeleken met kinderastma lijkt het verloop ernstiger, heeft het een snelle achteruitgang van longfunctie en zou het nagenoeg altijd chronisch zijn. Deze aannames zijn echter gebaseerd op onderzoeken die slechts op 1 moment metingen hebben uitgevoerd (cross-sectioneel) of na het verzamelen van gegevens (retrospectief) zijn ingezet. Er was echter nog geen op voorhand opgezet (prospectief) onderzoek dat het verloop van laat ontstaan astma bestudeert. In deel 2 beschrijven we de uitkomsten van ons onderzoek waarbij we een groep van 200 patiënten met recent vastgesteld laat ontstaan astma gedurende 5 jaar hebben gevolgd.

DEEL 1

Hoofdstuk 3 beantwoordt de vraag of er invloed is van allergie, roken, obesitas en ernstig astma (fenotypes) op de betrouwbaarheid van biomarkers voor eosinofielen. We vinden dat er geen invloed is van deze fenotypes op de betrouwbaarheid van de biomarkers FeNO, IgE en bloed eosinofielen. FeNO en bloed eosinofielen zijn het meest nauwkeurig en dus even goed bruikbaar in verschillende astma fenotypes. Daarnaast geven hoge en lage afkapwaarden van de biomarkers meer zekerheid over het respectievelijk aantonen en uitsluiten van eosinofielen in de luchtwegen. Met deze methode kan in de helft van alle patiënten met laat ontstaan astma een zekere uitslag gegeven worden.

In **Hoofdstuk 4** hebben we alle onderzoeken die gedaan zijn naar biomarkers voor eosinofielen op een rij gezet. Met alle data hebben we de gemiddelde betrouwbaarheid van de biomarkers berekend. Hier blijkt een matige gemiddelde betrouwbaarheid te bestaan met wijde variatie aan gerapporteerde afkappunten. Dit betekent dat de beschikbare biomarkers wel een indicatie kunnen geven van eosinofielen in de luchtwegen, maar dat ze met voorzichtigheid gebruikt moeten worden.

Hoofdstuk 5 is een commentaar op een andere studie, waarin bij rokers geen verband blijkt te bestaan tussen de biomarkers en de diagnose astma. Bij niet-rokers is dit wel het geval voor de diagnose allergisch astma. Dit laat zien dat roken een belangrijk klinisch kenmerk van astma patiënten is. Met name vanwege het toenemende belang van individueel gerichte therapie (personalized medicine), is rookstatus zeker een factor die meegenomen moeten worden bij het fenotyperen van astma patiënten.

DEEL 2

In **Hoofdstuk 6** laten we zien dat laat ontstaan astma bij 1 op de 6 patiënten wel degelijk over gaat (remissie) in de eerste 5 jaar. Ook hebben we gekeken welke factoren gemeten bij het vaststellen van de ziekte, de uitkomst na 5 jaar kunnen voorspellen. Zo blijken het hebben van neuspoliepen en matig-ernstige bronchiale hyperreactiviteit de kans op remissie te verlagen tot <1%. Het herkennen van deze hoog-risico patiënten is van belang omdat ze een slechte kwaliteit van leven hebben, lage productiviteit en hoge medische kosten. Eosinofiele ontsteking lijkt een rol te spelen bij zowel aanhoudende astma als neuspoliepen. Mogelijk verbeterd het vroegtijdig behandelen met specifieke moleculen gericht tegen deze ontsteking de prognose op lange termijn.

Hoofdstuk 7 beschrijft de factoren die verslechtering van de astma voorspellen in de eerste twee jaar na de diagnose. Het deel dat een verslechtering van de astma ervaart is 13% van de patiënten, terwijl 41% juist een verbetering van de astma doormaakt. De enige onafhankelijke voorspeller voor astma verslechtering is roken: hoe meer jaren patiënten hebben gerookt, des te groter de kans op verslechtering. Dit betekent dat nieuwe astmapatiënten die roken of gerookt hebben frequenter gecontroleerd zouden moeten worden en wellicht kandidaat zijn voor vroegtijdige intensieve anti-astmatherapie.

In **Hoofdstuk 8** onderzoeken we of er verschillende types ontstekingscellen samenhangen met astma-aanvallen in niet-rokende en rokende ernstig astmapatiënten. Hier blijken de niet-rokende patiënten met frequente aanvallen (3 of meer per jaar) meer eosinofielen in hun bloed te hebben, vergeleken met niet-rokers zonder astma-aanvallen. De rokers met frequente aanvallen gebruiken een hogere dosis astmamedicatie en hebben juist een ander type ontstekingscel, neutrofielen, in hun bloed. Deze bevinding suggereert dat verschillende soorten onderliggende ontsteking een rol spelen bij het ontstaan van aanvallen. Wellicht heeft dit in de toekomst implicaties voor de therapeutische benadering van deze patiënten.

Chapter 11

APPENDIX

- DANKWOORD
- CURRICULUM VITAE
- LIST OF PUBLICATIONS
- PhD PORTFOLIO

DANKWOORD

Geen proefschrift is compleet zonder dankwoord. Ik wil hier graag alle mensen bedanken die hebben geholpen bij het tot stand komen van dit proefschrift en die belangrijk zijn voor alle bijbehorende voorwaarden.

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CURRICULUM VITAE

Guus Alexander Westerhof (geboren 14 november 1986 te Dinxperlo) behaalde in 2005 zijn VWO-diploma aan het christelijk college Schaersvoorde in Aalten. In dat jaar startte hij met de studie geneeskunde aan de Maastricht University. Tijdens de bachelor volgde hij een onderwijsperiode aan de Universiteit van Ferrara, Italië. Gedurende de masterfase liep hij onder andere coschappen in Dharan, Nepal en Pretoria, Zuid-Afrika. In 2011 studeerde hij af als basisarts, waarna hij begon als ANIOS Interne Geneeskunde in het Tergooi in Hilversum/ Blaricum. Vanaf maart 2013 startte hij zijn promotietraject bij de afdeling Longziekten in het AMC, Amsterdam, onder begeleiding van prof. dr. E.H.D. Bel. Per december 2015 is hij in opleiding tot longarts in het AMC, onder supervisie van dr. R.E. Jonkers en dr. E.J.M. Weersink. Hij doet momenteel de vooropleiding Interne geneeskunde in het AMC bij prof. dr. S.E. Geerlings.

LIST OF PUBLICATIONS

1. **GA Westerhof**, EH Bel. Reply to Ayubi: Comments on clinical predictors of remission and persistence of adult-onset asthma.
   J Allergy Clin Immunol. 2017 Sept 26

2. **Westerhof GA**, Coumou H, de Nijs SB, Weersink EJ, Bel EH. Clinical predictors of remission and persistence of adult-onset asthma.
   J Allergy Clin Immunol. 2017 Apr 22.

3. **Westerhof GA**, de Groot JC, Amelink M, de Nijs SB, Ten Brinke A, Weersink EJ, Bel EH. Predictors of frequent exacerbations in (ex)smoking and never smoking adults with severe asthma.
   Respir Med. 2016 Sep;118:122-127.

4. Korevaar DA, **Westerhof GA**, Bel EH. Biomarkers for diagnosing asthma: a smoking gun?
   Clin Exp Allergy. 2016 Apr;46(4):516-8.

5. **Westerhof GA**, Korevaar DA, Amelink M, de Nijs SB, de Groot JC, Wang J, Weersink EJ, ten Brinke A, Bossuyt PM, Bel EH. Biomarkers to identify sputum eosinophilia in different adult asthma phenotypes.
   Eur Respir J. 2015 Sep;46(3):688-96.

6. Korevaar DA, **Westerhof GA**, Wang J, Cohen JF, Spijker R, Sterk PJ, Bel EH, Bossuyt PM: Diagnostic accuracy of minimally invasive markers for detection of airway eosinophilia in asthma: a systematic review and meta-analysis.
   Lancet Respir Med. 2015 Apr;3(4):290-300.

7. **Westerhof GA**, Bel EH. Reply to 'Occupational asthma is a cause of adult-onset asthma with poor prognosis'.
   J Allergy Clin Immunol. 2015 Mar;135(3):837-8.

8. **Westerhof GA**, Vollema EM, Weersink EJ, Reinartz SM, de Nijs SB, Bel EH. Predictors for the development of progressive severity in new-onset adult asthma.
   J Allergy Clin Immunol. 2014 Nov;134(5):1051-6.e2.

PhD PORTFOLIO

PhD TRAINING	YEAR	ECTS
General courses		
Practical Biostatistics	2013	1.1
Basic course regulations and organization for clinical investigators (BROK)	2013	0.9
Oral presentation in English	2014	0.8
AMC World of Science	2014	0.7
Basic laboratory safety	2014	0.4
Seminars, workshops, symposia		
NRS young investigators symposium	2013, 2014, 2015	0.6
Lung Amsterdam, evening symposium	2013, 2014, 2015	0.3
Lung Amsterdam, mini-symposium	2014, 2015, 2016	0.3
GSK Zeister Longsymposium	2015	0.2
COPD en astma huisartsen adviesgroep (CAHAG)	2017	0.3
(Inter)national conferences		
American Thoracic Society (ATS) conference	2014, 2016	2.5
European Respiratory Society (ERS) conference	2013, 2014, 2015	3.8
Nederlandse Longdagen	2013, 2014, 2015	1.5
Presentations and posters		
Longdagen, thematic poster	2013, 2014, 2015	1.5
ERS, Thematic poster	2013	0.5
ERS, Poster discussion	2013, 2014, 2015	1.5
ATS, Poster discussion	2014	0.5
ATS, Thematic poster	2016	0.5
CAHAG, 2 Oral presentations	2017	1
Other activities		
Journalclub Respiratory medicine	2013-2016	6
Research meeting Respiratory Medicine	2013-2016	6
Peer reviewer (Lancet, Cochrane Database of Systematic Reviews, European Respiratory Journal, Clinical Experimental Allergy, American Journal Respiratory Critical Care Medicine, Respiration)	2014-2015	1.8

TEACHING	YEAR	ECTS
Supervising		
Bachelor thesis lung function technician in training	2015	1
Bachelor thesis Medical student	2015	1

PARAMETERS OF ESTEEM	YEAR	ECTS
Abstract award, ATS	2014	

Total ECTS	**34.7**