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DIAGNOSTIC AND PROGNOSTIC MARKERS IN ADULT-ONSET ASTHMA

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Table of contents

Chapter 1

General introduction and aims of the thesis

Asthma

Asthma is a common disease worldwide and affects people across all age groups[1]. Typically, this disease develops in childhood and is characterized by variable symptoms of wheeze, dyspnea and chest tightness caused by (fully reversible) airflow obstruction[1-3]. A large part of these children will "outgrow" their asthma and do not experience any symptoms during adulthood[3]. On the other hand, asthma can develop in adulthood and is then much more likely to persist, as the remission rates for this subtype are much lower[4, 5]. Nevertheless, the majority of all adults with asthma can be successfully treated with inhaled corticosteroids and have well controlled disease. However, a small group of patients continues to have severe symptoms despite standard treatment[6, 7]. These patient with severe asthma often develop persistent airflow limitation, have frequent exacerbations and use (maintenance) oral corticosteroids[6, 8-10]. Not surprisingly, severe asthma is accountable for a large part of all asthma costs[11].
In order to get more insight into the complexity of (severe) asthma, recent studies focusing on asthma heterogeneity have identified asthma phenotypes[12-14]. Phenotypes are based on observable patient characteristics, and for asthma these are mostly clinical, functional and inflammatory parameters. The landmark study by Haldar and colleagues identified 4 clear phenotypes; namely, "early-onset atopic", "obese noneosinophilic", "early-onset symptom-predominant" and "inflammation predominant" asthma[15]. The phenotypes "obese noneosinophilic" and "inflammation predominant" asthma were both characterized by disease onset in adulthood, but differed in symptom score, gender, and most importantly, presence of eosinophilic inflammation[15]. Other studies have identified similar phenotypes and thereby underlined the importance of the type of (airway) inflammation present in a patient and the age of asthma onset[16-20].

Eosinophilic inflammation in asthma

It has been long recognized that eosinophilic inflammation is a frequent phenomenon in asthma[21, 22] and persistent airway eosinophilia is associated with greater severity of the disease[23], poorer symptom control[24, 25] and an increased risk of exacerbations [26, 27]. Eosinophilic airway inflammation in asthma is presumed to develop along several pathways, of which two have

been studied best; one is thought to be triggered through allergens, the other through non-allergic triggers[28-30] (Figure 1). The most important difference between these two pathways are the key-cells regulating the inflammatory response. In general it is thought that in allergic eosinophilic asthma an allergen leads to activation of T-helper cells (Th-2 cells) via dendritic stimulation[31]. In nonallergic eosinophilic asthma it is thought that pollutants, microbes and glycolipids activate innate lymphoid cells type 2 (ILC-2) via IL-25, IL-33 and thymic stromal lymphopoetin (TSLP)[29]. Activation of Th-2 cells or ILC-2 leads to release of so called Type-2 cytokines. Th2 cells produce interleukins (IL) 5, 4, 9 and 13. These cytokines act on eosinophils, B-cells, mast cells, smooth muscle cells and goblet cells, which results in eosinophilic inflammation, IgE release, mastcell degranulation and mucus hypersecretion[31, 32], respectively. On the other hand, ILC-2 produce high amounts of IL-5 and IL-13[33], which leads to eosinophilic inflammation (IL-5) and affects smooth muscle cells (IL-13)[29], but has less effect on mast cells or IgE production (IL-9, IL-4).
Inflammation that is characterized by the presence of Type-2 cytokines is called Type 2 inflammmation. However, in a large proportion of patients with asthma no Type 2 inflammation can be observed [34-36]. These patients show either sputum neutrophilia (i.e. neutrophilic asthma) or no signs of airway inflammation (i.e. pauci-granulocytic asthma)[12]. Yet, the inflammation patterns of these non Type-2 asthma phenotypes are poorly understood[36-38].

The increased knowledge on the pathophysiology has altered the way we think about asthma and has led to the suggestion that a "one-size fits all" approach is no longer appropriate for asthma management[39]. Instead, disease management based on specific patient characteristics has been proposed[40]. One of these specific characteristics is the type of airway inflammation that can be identified and treated, particularly Type 2 inflammation[28]. Identification of the type of airway inflammation is assessed by the use of sputum induction, but this technique is not widely available and not always successful. Therefore, several surrogate markers have been proposed, such as blood eosinophils and exhaled nitric oxide (FeNO) to assess Type 2 (airway) inflammation[41-44]. Blood eosinophil levels are also important to determine whether patients are eligible for various novel anti-eosinophilic treatments targeting IL-5 or its receptor, and IL-4/IL-13 by blocking the alpha subunit of the interleukin-4 receptor [45]. These novel treatments targeting Type 2 cytokines have been a break-through for

patients with severe eosinophilic asthma[28]; they have shown to be very effective in patients with severe asthma, they reduce exacerbation rate, improve quality of life and enable patients to taper the dose of their maintenance oral corticosteroid treatment[46-52]. These improvements are only attained when the right patients are selected for the right treatment[53, 54]. However, the accuracy of currently available biomarkers such as blood eosinophil and FeNO to diagnose Type-2 inflammation is not optimal[55, 56] and the diagnosis of this asthma phenotype is often based on one single biomarker measurement. Therefore, one part of this thesis will focus on improving the diagnosis of Type-2 asthma with the currently available biomarkers by using repeated blood eosinophil measurements to identify patients with persistent eosinophilic inflammation.

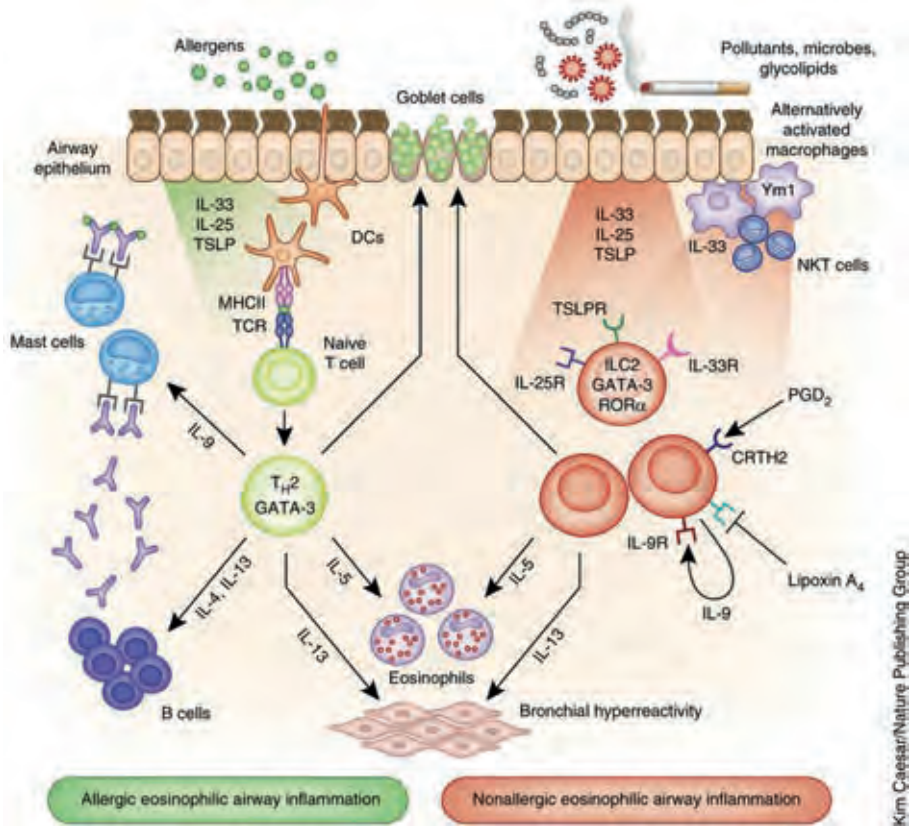

Figure 1.Simplified schedule of two pathways leading to eosinophilic airway inflammation in asthma. CRTH2; chemoattractant receptor homologous molecule expressed on Th2 cells, DC; dendritic cell, GATA-3; GATA-binding protein 3, IL; interleukin, ILC-2; innate lymphoid cell type 2, MHCII; major histocompatibility complex II, NKT cells; natural killer T cells, PGD2; prostaglandin D2, RORα; retinoic acid receptor-related orphan receptor α, TCR; T-cell receptor, Th-2; T-helper cell 2, TSLP; thymic stromal lymphopoietin. Adapted from Lambrecht and Hammad[57].

Adult-onset asthma

In almost 50% of adults with asthma the disease has started in adulthood. which makes adult-onset asthma an important subtype[58]. Adult-onset asthma differs from childhood-onset asthma in several aspects. For example, is has been associated with the development of persistent airflow limitation[8], which is observed even despite a shorter duration of the disease as compared to childhood-onset asthma, which suggests a faster decline in lung function in adult-onset asthma[59]. Another important difference with childhood-onset asthma is the etiology of the disease. Genetic predisposition is a key factor for childhood-onset asthma but not for adult-onset asthma[60, 61]. Adult-onset asthma often develops after a specific trigger such as an infection[62], occupational exposure[63, 64] or a stressful life event[65].

During the last decade it has become clear that adult-onset asthma is heterogeneous and may be divided into separate phenotypes with distinct characteristics[15, 17, 66]. For example, Type 2 adult-onset asthma has been associated with less atopy, air trapping and chronic rhinosinusitis with nasal polyposis[67-71], [72]. Another study found an association between adult-onset asthma, male gender and persistent airflow limitation[73]. Yet another adult-onset asthma phenotype is observed in obese females, and characterized by high symptom scores, frequent exacerbations and less Type 2 inflammation[16, 17]. However, most studies in adult-onset asthma have a cross-sectional design, and information on long-term outcomes is lacking[74]. Identifying prognostic biomarkers is important as this might provide insight in pathogenesis of adult-onset asthma. Moreover, these markers can help to identify patients at risk of severe disease who might benefit from early treatment with novel targeted treatments[46-50, 75, 76]. Therefore, part of this thesis will focus on the prognosis of new-onset asthma in adults and on markers to predict the course of the disease.

Thesis objectives:

- To review biomarkers that are currently used to diagnose eosinophilic asthma.
- To investigate whether one single measurement of blood eosinophils or exhaled nitric oxide is adequate to diagnose persistent blood eosinophilia.

- To summarize the current knowledge on the prognosis of adult-onset asthma.
- To investigate which markers can identify patients at risk of accelerated decline in lung function.
- To identify which markers can predict asthma remission/persistence
- To assess whether the patient-reported trigger of asthma onset is associated with specific phenotypic characteristics, or with a specific asthma outcome

For these objectives we used data from the ADONIS (ADult-ONset asthma and Inflammatory Subphenotypes)-cohort. In this prospective cohort study 200 adult patients with recently (<12 months) diagnosed asthma were included. Patients were extensively assessed at study-inclusion; clinical, functional and inflammatory parameters were collected (Figure 2). Then patients were invited to participate in follow-up visits at yearly intervals, with an additional follow-up after the first 6 months. At the follow-up visits only a selection of the parameters was collected. After 5 years all study patients were invited for extensive reassessment, repeating all measurements that were performed at study-inclusion.

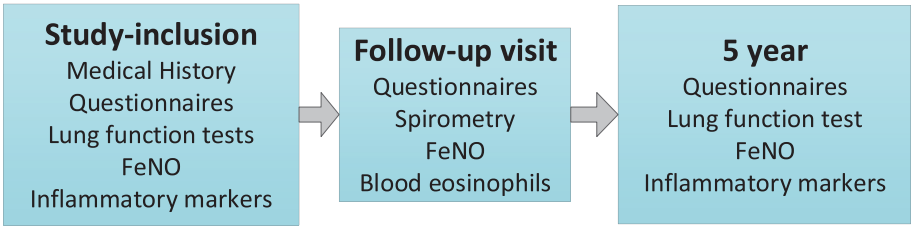

Figure 2. Study design of the ADONIS cohort study.

Outline of this thesis

This thesis discusses diagnostic and prognostic markers of adult-onset asthma. The first part focuses on diagnostic markers of eosinophilic asthma. Chapter 2 contains a literature overview of current methods to diagnose eosinophilic asthma and discusses options to improve this process. Chapter 3 describes a study investigating whether persistent blood eosinophilia can be diagnosed with one single measurement of blood eosinophils or exhaled nitric oxide. Chapter

4 contains a correspondence letter to address the importance of age of asthma-onset with regard to the interpretation of blood eosinophil levels in obese asthma patients.
Chapters 5-8 are focusing on prognostic markers of adult-onset asthma, investigating which markers are able to predict the course of adult-onset asthma. Chapter 5 contains a literature overview of the current knowledge on adult-onset asthma prognosis. In chapter 6 we investigate potential clinical, functional and inflammatory predictors for accelerated decline in lung function in the first 5 years after asthma diagnosis. In chapter 7 prognostic markers for asthma remission and persistence are investigated. In chapter 8 we compare clinical, functional and inflammatory characteristics between patients with different triggers of asthma onset, and relate these triggers to asthma outcome.

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Chapter 2

Improving the diagnosis of eosinophilic asthma

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1. Introduction

Traditionally, asthma was considered a single disease, but it has now been recognized that it is a heterogeneous mix of subtypes with distinct clinical, physiological, and inflammatory characteristics [1]. In each asthma subtype, unique pathophysiologic mechanisms drive symptoms, airway smooth muscle contraction and mucosal inflammation, that influence the response to conventional therapies [2]. One subtype that has gained increasing attention in recent times is the 'eosinophilic asthma' subtype [3]. Patients with this asthma subtype have evidence of uncontrolled eosinophilic inflammation in the airways, which puts them at risk of asthma exacerbations. Patients in whom eosinophilic airway inflammation persists despite high doses of inhaled corticosteroids (ICSs) typically have severe asthma, with poor symptom control, frequent exacerbations, fixed airflow limitation, and oral corticosteroid dependency [3]. For these patients with severe eosinophilic asthma, new biological agents that block eosinophil specific interleukins (IL) such as IL-5 have recently become available [4]. Treatment with these new biologicals results in a marked reduction in exacerbations, a significant improvement in quality of life [5,6] and the ability to taper or even discontinue chronic prednisolone treatment [6]. Diagnosing eosinophilic asthma at an early stage is important, but not without challenges. Here, we will review the current and upcoming approaches for diagnosing eosinophilic asthma and give our personal view on how it can be improved. We will first address the definition of eosinophilic asthma, and why it is important to diagnose this asthma subphenotype. We will then consider the current diagnostic methods and the use of surrogate biomarkers. Next, we will review the value of combined biomarkers and adding clinical patient characteristics. Finally will give our expert opinion including a diagnostic algorithm and conclude with a 5-year view on how diagnosing eosinophilic asthma will evolve.

2. How is eosinophilic asthma defined?

There exists no exact definition of 'eosinophilic asthma.' In the literature, term 'eosinophilic asthma' is used in different contexts. First, it is used to describe an asthma phenotype that is distinct from non-eosinophilic asthma. Non-eosinophilic asthma was initially described by Douwes and colleagues in 2002 [7]. They defined

non-eosinophilic asthma as symptomatic asthma in the absence of eosinophilic airway inflammation. By reviewing the literature, they found that up to 50% of all asthmatics had no evidence of eosinophilic inflammation, and a proportion had evidence of neutrophilic inflammation. This was confirmed by McGrath and colleagues in a longitudinal study of 995 patients with asthma, showing that about 50% of patients had persistently noneosinophilic asthma, that did not respond well to ICSs [8]. Eosinophilic asthma, defined as symptomatic asthma in the presence of airway eosinophilia with or without neutrophils, is generally associated with a good response to glucocorticosteroids [9], and asthma management guided by eosinophils in sputum rather than according to asthma control measures results in better outcomes [10,11]. Cowan and colleagues found that the proportion of patients with eosinophilic asthma was influenced by the use of ICSs. After withdrawal from ICSs, 67% of patients had eosinophilic asthma, whereas after treatment with ICSs, only 39% had eosinophilic asthma [12]. This and other factors may explain why the eosinophilic phenotype is not consistent over time in some studies [13]. Second, the term eosinophilic asthma is used to describe a subphenotype of severe asthma, that is, asthma that is characterized by active eosinophilic inflammation, which remains uncontrolled and is associated with active eosinophilic inflammation despite the adequate use of high-dose corticosteroid treatment [14]. Wenzel and colleagues first described this 'eosinophil high' severe asthma phenotype when performing bronchial biopsies and showed that it was associated with airway remodeling and risk of near-fatal asthma attacks [15]. Others showed that severe eosinophilic asthma is characterized by frequent exacerbations and often requires systemic corticosteroid treatment for control of the disease [14,16,17]. Persistent airway eosinophilia (with or without neutrophils) is the key characteristic of this asthma subphenotype, which appears to be reasonably stable over time [18]. In the present review, we will use the term 'eosinophilic asthma' in both senses. It is important to acknowledge that airway eosinophilia and/or blood eosinophilia are not exclusively related to asthma. Tissue eosinophilia and peripheral blood eosinophilia are also characteristic for other hypereosinophilic conditions including eosinophilic granulomatosis with polyangiitis allergic bronchopulmonary aspergillosis, eosinophilic pneumonia, hypereosinophilic syndrome (HES), and other rare conditions [19,20]. Typical for eosinophilic asthma is the presence of elevated numbers of eosinophils in airway tissue, in the absence of vasculitis, fungus infection, pulmonary infiltrates, bone marrow abnormalities, and organ involvement other than airway mucosa [21]. These other hypereosinophilic conditions will not be addressed in this review.

3. Why should we diagnose eosinophilic asthma?

Diagnosing eosinophilic asthma is important, both in primary practice and secondary or tertiary care. For the general practitioner, it is important to know whether in a patient with symptoms suggestive of asthma treatment with ICSs should be initiated or not. Patients with symptoms and evidence of eosinophilic inflammation are likely to respond to ICSs [9], but in the absence of airway eosinophilia, patients should not be treated with ever increasing doses of corticosteroids. Also for secondary and tertiary care physicians who deal with patients with difficultto- treat asthma, it is important to know whether the patient has airway eosinophilia. Those with persistent eosinophilia despite being treated with inhaled or oral corticosteroids are at risk of severe exacerbations and airway remodeling and should therefore be monitored more intensively [11]. In all settings, it is important to use all available resources and information to better predict whether a patient has eosinophilic asthma.

4. Current methods for diagnosing eosinophilic asthma

The diagnosis of eosinophilic asthma relies on the demonstration of eosinophilic inflammation in the airways of patients with asthma, but there is still no standard diagnostic test. Several methods are being used to identify airway eosinophilia in the airways including bronchial biopsies, induced sputum, blood, and exhaled breath. Traditionally, airway biopsies or bronchoalveolar lavage is considered as the gold standard for assessing airway inflammation [15] but this method is too invasive for routine clinical use. Therefore, there has been great interest in methods to assess airway inflammation noninvasively and in a convenient and inexpensive way. The most common and best validated method to diagnose eosinophilic asthma is detection of eosinophils in induced sputum [22]. A recently published meta-analysis on the diagnostic accuracy of minimally invasive markers for detection of airway eosinophilia in asthma found that most (88%) of the research on this subject was done with sputum eosinophils as reference standard [23]. Also in the present review, we will use sputum eosinophilia as reference for eosinophilic airway inflammation. Sputum induction is not without risk in patients with severe asthma nor is it always successful [24]. Processing requires time and expertise and results are not readily available. This has led to surrogates to detect airway

eosinophilia including blood eosinophils, the fraction of exhaled nitric oxide (FeNO), serum periostin, and serum immunoglobulin E (IgE) [25]. Most of these markers have proven their use in clinical setting. However, the accuracy of these markers is far from ideal [23].

5. Use of surrogate markers for diagnosing eosinophilic asthma

5.1. Blood eosinophils

Complete blood cell counts with differential can be performed at a lower cost and greater accessibility than induced sputum. Therefore, eosinophil counts in peripheral blood have become popular in identifying asthma patients with airway eosinophilia, in particular in studies with anti-IL-5 therapies [5]. However, although there exists a significant association between eosinophils in blood and in sputum, the use of blood eosinophils to identify sputum eosinophilia is still debated because of the relatively high false-negative and false-positive rates [26]. This was illustrated in the 328 patients of the severe asthma research programme (SARP) showing that the accuracy of blood eosinophils to predict sputum eosinophilia was low, leading to considerable misclassification of patients with eosinophilic or non-eosinophilic asthma. This low accuracy might be due to the high variability of blood eosinophil counts in patients with asthma, both in the short (24 h) and in the long (1 year) run [13,27]. So, merely for the diagnosis of 'eosinophilic asthma,' one single measurement of blood eosinophils does not seem to be of great value. The most important role for blood eosinophils is probably its role in identifying patients who are likely to respond to treatment with the new biological agents against Type 2 inflammation [25]. However, they seem to be less suitable to monitor treatment response, given the fact that biologicals such as anti-IL-5 strongly reduce blood eosinophils but affect tissue eosinophils to a much lesser extent [28]. This may also explain why anti-IL-5 treatment can effectively reduce exacerbations and systemic corticosteroid requirement but has only little effect on the forced expiratory volume in 1 s (FEV_1) [16].

5.2. FeNO

FeNO is being regarded as one of the most practical noninvasive tests to identify eosinophils in sputum from patients with asthma, because it is easy, quick, cheap, and generally available. Still, there are many unresolved questions. After the first

observation that FeNO was increased in patients with asthma [29], many studies have suggested that this biomarker could be used as a test to diagnose or monitoring asthma in clinical practice [30]. In particular, for identifying eosinophilic airway inflammation, FeNO was put forward as an ideal noninvasive biomarker [31]. Many studies have shown a positive relationship between FeNO and the number of percentage sputum eosinophils [23,32,33], and one study showed that FeNO measurement could identify the subgroup of patients with severe eosinophilic asthma [34]. However, in the SARP study, a high rate of misdiagnosis was found when using FeNO for diagnosing eosinophilic asthma based on sputum eosinophils ≥2% [26]. One possible explanation for this discrepancy might be that FeNO values in severe asthma can be confounded by many factors [30]. But another, more plausible explanation is that FeNO and blood eosinophils reflect different pathways of Type-2-mediated inflammation. This is illustrated by two trials in which a discrepancy was observed between the treatment response as measured by eosinophils or FeNO. In one trial, mepolizumab, a monoclonal antibody against IL-5 given to patients with eosinophilic asthma, significantly reduced blood and sputum eosinophils but had no effect on FeNO levels [14]. In another trial, lebrikizumab, an anti-IL-13 monoclonal antibody, had no effect on eosinophils but was associated with a significant reduction in FeNO levels and improvement in lung function, in particular in patients with high periostin levels [35]. Apparently, the molecular pathways leading to increased FeNO levels differ from those regulating eosinophil recruitment and activation. Increased FeNO seems to be primarily related to pathways involved in Th2-mediated asthma, whereas blood eosinophils may relate more to type 2 innate lymphoid cell (ILC2)-mediated (severe) asthma [36,37]. These different pathways may also explain why sputum eosinophils can be used to titrate treatment with ICSs in asthma patients with frequent exacerbations [10,11], whereas FeNO values are not adequate for this purpose [38]. Inhaled corticosteroids are known to directly affect inducible nitric oxide synthase, irrespective of their effect on eosinophils, which is very important when interpreting FeNO values [39]. FeNO values are high in patients with allergies, irrespective of blood eosinophils counts, whilst FeNO levels are reduced in asthma patients who smoke or have consumed alcohol [30]. Even age and gender are known to affect FeNO levels, which reduces its value as a biomarker for predicting eosinophilic airway inflammation. Up till now, there is no consensus how and where to use FeNO in clinical practice. In primary care, FeNO might be used as a screening tool to exclude Type 2 inflammation

in patients with symptoms of cough and wheeze [40]. Alternatively, for patients with severe asthma who are candidates for the new expensive biologicals directed against Type 2 inflammation, FeNO could be suitable to exclude nonadherence with ICS treatment as the cause of eosinophilia in blood or sputum [41].

5.3. Periostin

Periostin is a relatively new kid on the block for detecting eosinophilic airway inflammation. Several reports have shown significant correlations between periostin in serum and eosinophils in sputum [42,43]. Woodruff et al. were the first to show upregulated periostin gene expression in bronchoalveolar lavage from patients with asthma as compared to normal control subjects, which was associated with increased concentrations of IL-4, IL-5, and IL-13 [44]. These patients also had higher eosinophil counts in lavage fluid, suggesting that increased periostin levels might reflect a Th2-high profile. This fits well with the observed correlation between periostin and specific IgE in sputum [45]. After this initial report, several investigators confirmed the association between periostin and sputum eosinophils, even proposed serum periostin as a biomarker for airway eosinophilia [42]. However, subsequent studies showed that the strength of the correlation between periostin and sputum eosinophilia, although significant, was relatively weak, and that neither serum nor sputum periostin was suitable for use as a surrogate marker of airway eosinophilia [43]. Wagener et al. showed that serum periostin, when compared to blood eosinophils and FeNO, showed the lowest accuracy for distinguishing eosinophilic (sputum eosinophils ≥3%) from non-eosinophilic inflammation in two independent cohorts of patients with asthma [46]. Although the diagnostic accuracy to predict eosinophilic airway inflammation is relatively modest, periostin may have clinical relevance in predicting the response to some of the new biologicals against Type 2 inflammation. This was suggested by the observation that increased serum periostin levels were found in patients with a greater response to therapy with lebrikizumab, a monoclonal antibody against IL-13. In patients with low periostin levels, the response was minimal, suggesting that periostin might identify patients with an IL-13-dependent type 2 inflammation who are likely to benefit from anti-IL-13 therapy [35] although this has yet to be confirmed in prospective studies.

5.4. Total IgE

IgE has an essential role in allergic asthma [47]. Allergic patients produce IgE antibodies specific for antigens such as house dust mite and pollens, which bind to IgE-specific receptors on mast cells and basophils. Cross-linking of IgE molecules causes the release of mediators (histamine, arachidonic acid metabolites) and cytokines (tumor necrosis factor alpha, IL-4, and IL-5) which are responsible for the immediate and latephase allergic response, and the associated influx of eosinophils in the airway [48]. Several studies have found an association between serum IgE levels and airway eosinophilia in asthma, and treatment with anti-IgE has been shown to be associated with a significant reduction in tissue eosinophils [49]. However, despite these findings, the use of IgE as a biomarker for eosinophilic inflammation is not recommendable. A recent meta-analysis by Korevaar and colleagues showed low accuracy for this biomarker to detect sputum eosinophilia, and inferiority as compared to FeNO [23]. When comparing blood eosinophils with IgE, the results were not conclusive. Remarkably, Westerhof and colleagues, when comparing atopic and non-atopic patients with asthma, found poorer accuracy of total IgE for detecting sputum eosinophilia in atopic patients, suggesting that in these patients IgE has no value in distinguishing between those with or without sputum eosinophilia [50]. Thus, of all currently available biomarkers to detect eosinophilic asthma, IgE seems to be the least useful. This does however not exclude that IgE can be used to identify patients who will likely benefit form anti-IgE therapy, nor that anti-IgE can have a beneficial effect in subgroups of patients with eosinophilic asthma [51].

6. Combinations of blood eosinophils, FeNO, periostin, and IgE

By recognizing that eosinophilic asthma has a complex underlying pathogenesis involving different molecular pathways, it seems illogical to expect to find one perfect biomarker for this phenotype. However, since different biomarkers may represent different pathways of type 2 airway inflammation [23,52], combinations of two or more biomarkers could increase sensitivity and specificity for detection of eosinophilic airway inflammation. Several clinical trials investigating the effects of monoclonal antibodies against key cytokines of Type 2 inflammation have already taken such approach. For example, the DREAM study evaluating the effect of anti-

IL-5 therapy with mepolizumab used FeNO ≥50 parts per billions (ppb) or blood eosinophils ≥0.3 × 10^9/L to select patients with eosinophilic asthma [16]. A more complex method was used in another trial with and IL- 5R-alpha [53]. Here, the ELEN-index was used for stratifying the eosinophilic status of the patient. This index used the absolute ratio between eosinophils and lymphocytes (EL) and the absolute ratio between eosinophils and neutrophils (EN) as a predictor for the presence of sputum eosinophilia [54]. A study with anti-IL-13 used a combination of total serum IgE level and peripheral-blood eosinophil count [35]. Both biomarkers had to be high enough in order to classify the subject as 'type 2 high.' Yet, another study investigating whether periostin correlated with type 2 immunity [55] combined blood and sputum eosinophils, FeNO and IgE and created the 'type 2 immunity composite score.' Apparently, the authors of all above-mentioned studies assumed that a combination of several biomarkers provided more information than one single biomarker. Only few studies have specifically looked at the accuracy of a combination of two or more biomarkers to detect eosinophilic airway inflammation [26,46,50]. Unfortunately, the results of these studies are not fully conclusive. One study did not find an improvement in accuracy when combining blood eosinophils, FeNO, and periostin as compared to one of the single biomarkers [46], whereas another study did find a small enhancement in accuracy when combining FeNO and blood eosinophils [50]. Adding IgE to these models did not lead to any improvement of the results. Thus, theoretically the use of a combination of biomarkers could improve the diagnosis of eosinophilic asthma, but there are currently not enough data available to confirm this. Hopefully, the results of the Medical Research Council United Kingdom Refractory Asthma Stratification Programme (RASP-UK) will shed more light on this issue. The RASP-UK Consortium is a large scale research project that aims to explore novel biomarker stratification strategies in severe asthma to improve clinical management and target treatments effectively in patients with severe asthma. This study will examine a novel composite biomarker strategy including FeNO, blood eosinophils, and serum periostin as biomarkers for diagnosing Type 2 inflammation [56]. Up till now, there is a general feeling and some evidence that combining different biomarkers, in particular FeNO and blood eosinophils, might improve the accuracy to diagnose eosinophilic asthma. The role for periostin is unclear and with some certainty it can be concluded that IgE does not provide added value to detect active eosinophilic airway inflammation. However, this does not exclude that using single or combined biomarkers might be helpful to select the right asthma patients for specific targeted treatments.

7. Cutoff points for blood eosinophils, FeNO, periostin, and IgE

One of the problems in defining eosinophilic asthma is lack of consensus regarding biomarker cutoff values. This lack of consensus is reflected in research papers where several different values are being used, even for sputum eosinophilia as the reference standard. In some studies, sputum eosinophilia is defined as ≥2% eosinophils, which is based on the upper limit of the normal range in the healthy population [57], but other studies use higher levels, up to ≥3%. Also values for the surrogate biomarkers vary widely. The cutoff values for blood eosinophils differ between 0.22 and 0.32 × 10^9/L, for FeNO levels between 10 and 41 ppb, and for IgE 72 and 900 IU/mL [23]. For periostin, a value of 25–50 ng/mL or the median of the study population is mostly used [35,42,58]. These cutoff values are based on the optimal combination of sensitivity and specificity. However, for clinical use of these biomarkers, it might be better to use more than one threshold, so that one can either choose for high specificity OR high sensitivity instead of a suboptimal combination of both. These thresholds can thus be used to rule out (high sensitivity) or rule in (high specificity) eosinophilic inflammation in an asthmatic patient [23]. This principle of using different thresholds was applied in an elegant study in patients with severe asthma [59]. Sputum eosinophils and blood eosinophils were collected and different cutoff points for blood eosinophils (0.15/0.3/0.45 × 10^9/L) were used to predict ≥2% sputum eosinophilia. The results showed that changing the threshold from 0.3 to 0.45 × 10^9/L improved the specificity from 84.4% to 97%. From these results, the authors concluded that using a cutoff of 0.45 × 10^9/L or higher for blood eosinophilia could safely predict airway eosinophilia. Also other studies reported that a blood eosinophil threshold ≥0.4 × 10^9/L could predict sputum eosinophilia with high probability [8,50]. Westerhof et al. not only found a specificity of 95% for blood eosinophils ≥0.41 × 10^9/L to rule in, but also a sensitivity of 96% for ≤0.09 × 10^9/L eosinophils to rule out sputum eosinophilia [50]. This value is in accordance with values used in trials with the anti-IL-5 monoclonal antibody reslizumab, in which the authors have chosen a blood eosinophil threshold of ≥0.4 × 10^9/L to select patients with a high likelihood of having active eosinophilic airway inflammation [60,61]. This choice was based on a subgroup analysis of a phase 3 trial with this agent, showing that patients with baseline eosinophils <0.4 × 10^9/L, exhibited no significant improvement in FEV_1 after reslizumab treatment compared with placebo [62].

In the subgroup with eosinophils ≥0.4 × 10^9/L eosinophils, however, treatment with reslizumab was associated with much larger improvements in FEV_1, asthma symptoms, rescue medication use and forced vital capacity (FVC) compared with placebo. These findings contrast with those for mepolizumab, another anti-IL-5 monoclonal antibody. In three independent large trials, it was found that a threshold of blood eosinophils ≥0.15 × 10^9/L predicted treatment response to mepolizumab, in that it reduced the rate of exacerbations and the dose of chronic oral glucocorticoids [5,6,16]. Blood eosinophils 0.15 × 10^9/L correctly predicted the response sputum eosinophilia ≥3% with a sensitivity of 85% and specificity of 75%, whilst increasing the threshold to 0.3 × 10^9/L had minimal influence on sensitivity, but decreased specificity substantially [63]. Remarkably, sputum eosinophils did not predict treatment response to mepolizumab, but blood eosinophils counts did [64]. This latter finding suggests that blood eosinophilia, rather than sputum eosinophilia is the 'gold standard' for patients to be eligible for mepolizumab treatment. For treatment with reslizumab, another anti-IL-5 monoclonal antibody, the threshold for blood eosinophils was higher, ≥0.4 × 10^9/L. This difference in thresholds might be explained by the relatively large proportion of patients who were on systemic corticosteroid treatment in the mepolizumab studies. Yet another value for blood eosinophils (0.3 × 10^9/L was found to be optimal for the selection of patients likely to respond to lebrikizumab, the monoclonal antibody against IL-4R-alpha (dupilumab),and IL-5R-alpha (benralizumab) [53,58,65]. Taken together, the threshold for blood eosinophils to be used for treatment with Type 2 biologicals is variable and depends on medication used and the biological of choice. Threshold values with high sensitivity or high specificity to detect elevated sputum eosinophils have also been reported for FeNO [8,34,42,50,66–68]. However, these thresholds vary widely depending on the technique that is being used, and the population under study. In steroid naïve asthma patients the presence of sputum eosinophils ≥3% was ruled in at a FeNO level of >41 ppb [68], and ruled out at a levels of ≤21 ppb (sensitivity 97%) [67]. Among ICS users, the sensitivity was considerably lower for the rule-out threshold (sensitivity 81%). Another study in corticosteroid-treated asthma patients showed that sputum eosinophils ≥3% could be ruled out if FeNO levels were ≤12.2 ppb and ruled in if FeNO levels were ≥64.5 ppb [50]. A similar high threshold (>73 ppb) for FeNO to rule in eosinophilic inflammation in patients on corticosteroid treatment was seen when bronchial biopsies were used as the reference standard for eosinophilic airway inflammation [34]. Taken together, in patients with mild asthma FeNO levels of around 40 ppb or higher are associated

with airway eosinophilia, whereas in steroid-treated asthma patients higher levels of FeNO (>65–70 ppb) are indicative of persistent airway eosinophilia. Only one study specifically looked at thresholds for IgE to detect sputum eosinophilia [50]. This study showed that the threshold for total IgE to rule out sputum eosinophilia ≥3% was <13.5 IU/L and to rule in eosinophilia was ≥764 IU/L (sensitivity ≥ 95%). Clearly, these widely spaced thresholds of total IgE do not seem to have much value in clinical practice. To our knowledge, there are no studies that have looked at thresholds for serum periostin to rule out or rule in airway eosinophilia.

8. Repeated measurements of biomarkers

Although studies have shown that the eosinophilic asthma phenotype is reasonably stable over time [18,69], there is no doubt that sputum inflammatory profiles and their associated biomarkers vary under the influence of many factors, including change in medications, adherence to treatment, exacerbations, pollution, and allergens' exposure. It is therefore rational to assume that one-off clinic readings may not be the best method of utilizing biomarkers, and that repeated measures are required to obtain reliable phenotyping data on subjects with asthma. With respect to blood eosinophil measurements, Katz and colleagues showed that using an average of multiple measurements of blood eosinophils spread over a prolonged period of time only marginally increased the sensitivity to detect sputum eosinophils ≥3% [64,70]. Repeated measurements of FeNO, however, do seem to increase the diagnostic accuracy for detecting sputum eosinophilia. Nanda and colleagues showed that an average FeNO measurement over a moving period of 5–7 days gave a better insight into the level of sputum and blood eosinophils than a single measurement [71]. Thus, repeated measurements of blood eosinophils for diagnosis of eosinophilic asthma do not seem to provide additive value, whereas serial measurement of FeNO may do.

9. Added value of clinical features to improve the diagnosis of eosinophilic asthma

In clinical practice, high or low levels of blood eosinophils and FeNO may be used for ruling in or ruling out eosinophilic asthma, but often the levels of these

biomarkers are inconclusive. In these patients, the clinical profile may have added value. A recent study looking at the typical clinical profile of adult patients with eosinophilic asthma found that patients with this phenotype differed from patients with non-eosinophilic asthma with respect to a number of distinctive clinical features [72]. As compared to non-eosinophilic asthma patients, those with the eosinophilic phenotype had more often adult-onset asthma, were more often male, and had more often chronic rhinosinusitis with nasal polyposis. In addition, they showed more often persistent airflow limitation and airtrapping, which is typically associated with dyspnea on exertion. Other studies looking at patients with eosinophilic asthma found similar clinical characteristics [18]. So, this typical set of clinical parameters seems point toward an asthma phenotype that is likely to have active eosinophilic airway inflammation. This implies that clinical pattern recognition by the treating physician could improve the diagnosis eosinophilic asthma if combined with biomarkers in blood or serum. Currently, there are no published studies that have examined the diagnostic accuracy of combined clinical characteristics and biomarkers for diagnosing eosinophilic asthma, but it would be worth considering.

10. Future options to improve the diagnosis of eosinophilic asthma

10.1. Volatile organic compounds and other 'omics' techniques

A promising new technique for assessing airway inflammation involves the use of volatile organic compound (VOC) patterns in exhaled breath [73]. Exhaled breath contains a complex gas mixture of several thousands of individual VOCs, which are derived from systemic and local metabolic, inflammatory, and oxidative processes. Individual molecular compounds in a VOC mixture can be measured by gas chromatography–mass spectrometry (GC–MS), whereas patterns of different VOCs can be captured by an 'electronic noses' (breathomics) [74]. Until now, no single compound has been identified as disease specific, presumably due to overlap of compounds between diseases. However, various research groups demonstrated that VOCs profiles could accurately distinguish patients with a pulmonary disease from healthy controls. The current hypothesis is that pulmonary diseases are characterized by a diseasespecific breath-print, as distinct profiles were found in patients with different lung diseases [75]. Exhaled breath has been used in the

detection of lung diseases such as lung cancer, tuberculosis, and cystic fibrosis [74], but its most promising application lies in stratification of inflammatory airways disease into different subphenotypes [76]. In asthma, specific exhaled VOCs profiles defined by GC–MS appear to be associated with the eosinophilic phenotype and are associated with disease activity [76]. The distinctiveness of breath prints for inflammatory subtypes was also confirmed in a study with an electronic nose [77]. In accordance, steroid responsiveness in patients could be predicted by the electronic nose with even greater accuracy than sputum eosinophils or FeNO [78]. There are still some hurdles which need to be taken in order to make prints breath applicable in the clinic [74]. But large multicenter trials such as U-BIOPRED study [79] validating this diagnostic 'biomarker' are currently underway. U-BIOPRED focusses on several new 'omics' techniques such as gene expression (transcriptomics), proteins (proteomics), lipids (lipidomics), and metabolites (metabolomics) in the lungs, blood, and urine [79]. Results of this large study are expected to reveal more accurate biomarkers for identification of all existing asthma phenotypes and endotypes.

10.2. Multiple molecular pathways leading to eosinophilic airway inflammation

Until a few years ago, airway eosinophilia was considered the key characteristic feature of Th2-mediated inflammation. This concept has been challenged by the observation that blocking eosinophil recruitment and activation by a monoclonal antibody against IL-5 had no effect on the Th2-related symptoms, in particular, on the early and late asthmatic response to inhaled allergens [80]. The most likely explanation that has been put forward recently is that multiple mechanisms involving multiple molecular pathways can lead to an eosinophilic inflammatory response [81]. In allergic eosinophilic asthma, Th2 cells are driving the pathobiology of the disease, but there is accumulating evidence that in adult-onset nonallergic eosinophilic asthma, ILC2s may play a more prominent role [36]. Air pollutants, microbes, and glycolipids induce the release of epitheliumderived cytokines that activate ILC2s, which are then able to produce high amounts of IL-5 and IL-13 leading to eosinophilia [82,83]. ILC2s are present in greater numbers in sputum and blood of patients with severe eosinophilic asthma compared to mild allergic asthmatics and produce high levels of IL-5 and IL-13 in sputum [84]. Thus, ILC2s and its cytokines can promote the persistence of airway eosinophilia independent of allergen exposure, which explains the lack of response of anti- IL-5

in the allergen challenge model, and the presence of eosinophilic inflammation in nonallergic asthma [36]. Because of the close association between ILC2s in peripheral blood and sputum eosinophils, ILC2s have even been proposed as novel biomarkers for eosinophilic asthma [85]. It appeared that the diagnostic accuracy of ILC2s, expressed in percentage of lymphocytes (%ILC), was better than that of blood eosinophils and FeNO. Using 0.076% ILC2 as a cutoff value, eosinophilic airway inflammation was detected with a sensitivity of 67.7% and a specificity of 95.3%. With these numbers, %ILC2s can be a promising biomarker to be used in the clinic to rule in eosinophilic asthma and identify patients for anti-IL-5 therapy. The finding of at least two different pathways with different drivers of eosinophilic inflammation may explain why markers such as blood eosinophilia, FeNO, periostin, and IgE have different predictive value for sputum eosinophilia in several studies. Therefore, it seems sensible to look for a biomarker that is representative of multiple Type 2 pathways. Airway epithelial cells constitute the first physical, chemical, and immunological barrier for all type 2 immune cell stimuli including allergens, pollutants, viruses, and fungi [86]. There is now increasing evidence that in asthma, a dysregulated epithelial responses or barrier function could be the driver of the disease process. Potential markers of such epithelial cell dysregulation are IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), known as epithelial alarmins. These cytokines initiate pathways resulting in the type 2 inflammatory cascade and eosinophilia [86]. TSLP, in particular, has been proposed to play a key role in eosinophilic asthma. This upstream cytokine has convincingly been shown to be driving classical allergeninduced asthma and eosinophil influx into the airways [87] but is also an important component of the innate type 2 immune system. TSLP plays an important role in the inflammation seen in patients with nasal polyposis [88] and is supposed to contribute to the pathogenesis of aspirin induced asthma [89]. It would be interesting to see if serum TSLP or another upstream molecule could be used as a biomarker of the multiple pathways that lead to eosinophilic airway inflammation in allergic, nonallergic, and aspirin-induced asthma. However, the question remains whether a biomarker for diagnosing eosinophilic airway inflammation 'per se' would be the best predictor of response to targeted therapies, when compared to specific pathway-related biomarkers like periostin for anti-IL-13 or IgE or for anti-IgE therapy.

10.3. Genetic markers, cell-surface markers, and microbiome

Genetic markers, in particular single-nucleotide polymorphisms (SNPs), have been found to control epithelial barrier function (e.g. filaggrin) and control the production or responsiveness to epithelial cytokines, such as TSLP and IL 1 receptorlike 1 (which encodes the receptor for IL-33). These SNPs are involved in biological pathways leading to eosinophilic airway inflammation and might thus be considered as indirect biomarkers [90]. Surface markers of eosinophils have also been proposed as biomarkers to distinguish between different eosinophilic conditions. For example, Siglec-8 correlates with blood eosinophils and has been identified as a marker for HESs [91]. This protein might even become a target for new therapies as this protein plays a role in apoptosis of eosinophils [92]. Whether this surface marker is useful as biomarker or therapeutic target in eosinophilic asthma needs to be further explored. Another interesting approach to diagnose eosinophilic asthma may be the microbiome. Recently, it has been shown that the dysbiosis of the lower airway microbiome correlates with clinical characteristics of chronic persistent asthma, including airflow obstruction, use of corticosteroid medications, and presence of airway eosinophilia [93,94]. This suggests that the composition of the airway microbiome may be important in severe eosinophilic asthma, and as such might become a unique biomarker in the future.

11. Expert commentary

The diagnosis of eosinophilic asthma is currently based on sputum eosinophils as the 'gold standard.' However, the measurement of sputum eosinophils is time consuming and requires specific technical expertise. Therefore, biomarkers such as blood eosinophils, FeNO, serum IgE, and periostin are being used as surrogates. Blood eosinophils and FeNO have the highest diagnostic accuracy, but to reduce the likelihood of misclassification as much as possible, these biomarkers should be used in the most effective manner. First, repeated measures of biomarkers, in particular FeNO, are likely to reduce misclassification, and combined measures of blood eosinophils and FeNO have been demonstrated to improve diagnostic accuracy. Second, these biomarkers should primarily be used to rule in or rule out eosinophilic asthma by using high and low cutoff values, respectively (Figure 1). For patients who cannot be classified by these biomarkers alone, the clinical profile may be of help. If the patient exhibits typical features such as adult-onset asthma,

chronic rhinosinusitis with nasal polyposis, persistent airflow limitation, and air trapping, the probability will be high that the patient has indeed eosinophilic asthma. If both biomarkers and clinical profile are inconclusive, sputum induction will be necessary to confirm the diagnosis. However, a diagnosis of eosinophilic asthma is no guarantee for response to treatment with biologicals targeting Type 2 inflammation, because several molecular pathways may lead to eosinophilic inflammation. For this purpose, it should be recommended to evaluate the biomarker appropriate for the specific treatment of choice (Figure 1). Blood eosinophils, periostin or IgE measurements could be used to select patients for treatment with anti-IL-5, anti-IL-5Ralpha, anti-IL-4R-alpha, anti-IL-13, and anti-IgE, respectively. When the biomarker in question is labeled as 'high,' the diagnosis of eosinophilic asthma may not be certain but there will be a chance of success for the chosen treatment, which may be more relevant for the patient.

12. Five-year view

Currently, we are at the tipping point concerning asthma diagnosis. In the next few years, results of large multicenter studies as the IMI-EU sponsored U-BIOPRED study will certainly lead to the discovery and development of new biomarkers for eosinophilic asthma. In the most ideal situation, even a new gold standard will be set. This could be one particular biomarker representing the entire complex Type 2 pathway or a welldefined combination of various biomarkers representing multiple pathways conjointly. However, the biggest change for asthma will not lie in the area of biomarkers. We believe that the diagnosis of eosinophilic asthma will become less important, but the focus will be on the diagnosis of eosinophilic airway inflammation as a treatable option. The current debate in the asthma and chronic obstructive airways disease (COPD) field emphasizes on changing the current strategy of traditional diagnoses to acknowledging the complexity of chronic airway diseases. Instead of clear-cut disease definitions, the focus is shifting toward features of chronic airway diseases that are amenable to specific therapies. This concept is not entirely new but was the hall mark of the Dutch Hypothesis in the sixties and has also been put forward some years ago by the Leicester asthma research group as the 'A to E of airway disease' [95,96]. With the introduction of 'treatable traits,' important steps are being made as we speak. Agusti and colleagues [40] have reinforced this fundamental concept and strongly

Figure 1. Algorithm to diagnose and treat eosinophilic asthma.

*reference values can be found in the text. ICS: inhaled corticosteroids; FeNO: fraction of exhaled nitric oxide; ppb: parts per billion; GINA: global initiative for asthma; OCS: oral corticosteroids; IgE: immunoglobuline E; IL: interleukin; Rα: receptor alpha.

encourage the use of 'label-free patients.' This in order to work toward personalized medicine and thereby improving patient's outcome [97]. These traits can be pulmonary (e.g. airflow limitation, eosinophilic inflammation), extrapulmonary

(e.g. obesity, rhino-sinusitis), or behavior/lifestyle risk factors (e.g. smoking, adherence). By defining which treatable traits are present in a patient with airways disease, whether it is asthma or COPD, a tailor-made treatment plan can be used to manage the patient. This approach is still in early stages, and the currently suggested treatable traits will certainly be adapted in the coming years. Nonetheless, there is a high probability that eosinophilic inflammation will be one of the key traits to be targeted. Diagnosing eosinophilic inflammation might be done with the suggested use of biomarkers in this review, and hopefully new biomarkers will be identified and available for daily use in the near future. But most of all, it will be interesting to see if within the next 5 years physicians and researchers are 'brave enough' to take the next steps in order to let go of the past and embrace the new era that is in front of us [98].

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Chapter 3

Diagnosing persistent blood eosinophilia in asthma with single blood eosinophil or exhaled nitric oxide level.

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1. Introduction

Asthma is a heterogenic disorder with several distinct phenotypes, which are generally based on clinical, functional or inflammatory characteristics [1–6]. One important phenotype is severe eosinophilic asthma, defined by elevated levels of eosinophils in the airways or in blood despite treatment with medium to high dose inhaled corticosteroids and long-acting beta-agonists [7,8]. This is an important phenotype because it is characterized by poor symptom control, fixed airflow limitation and frequent exacerbations [9]. New biological agents targeting interleukin (IL)-5, a key interleukin in the pathway of eosinophilic inflammation, are able to reduce the exacerbation rate in these patients and also increase their quality of life [10–12]. This provides new treatment options for this severe asthma phenotype. It is therefore important to identify patients with this specific phenotype in an early stage of the disease, before severe exacerbations and loss lung function occur. In clinical trials, single blood eosinophil or fraction of exhaled nitric oxide (FeNO) levels are commonly used to identify patients with the eosinophilic phenotype [7,11]. However, it remains highly questionable whether one single measurement of these biomarkers is adequate to diagnose the eosinophilic asthma phenotype. In this study we selected adults with recent onset asthma to investigate whether a single measurement of blood eosinophils or FeNO could predict the eosinophilic asthma phenotype, thereby assuming that persistent blood eosinophilia precedes the development of this severe phenotype. For clinical practice we also defined cut-off values.

2. Methods

2.1. Subjects

The present study was part of the ADONIS-project (Adult-onset asthma and inflammatory subphenotypes) in which adults with recently diagnosed asthma were included. For this study 358 patients were approached, of which 200 agreed to participate. There was no difference in patient characteristics between participants and non-participants except for gender (56% vs 70% females, respectively, $p < 0.01$). Asthma was diagnosed<12 months before inclusion and based on typical asthma symptoms, combined with documented reversibility in FEV_1 of 12% predicted and 200 ml in absolute values, or presence of airway hyperresponsiveness [13]. Patients

with a history of chronic airway disease in childhood were excluded. (Ex)smokers with>10 pack years of smoking had to have $FEV_1/FVC>0.7$ and normal diffusing capacity of the lungs for carbon monoxide (> 80%). The study was approved by the AMC Medical Ethics Board and registered in the Dutch trial register (NTR1846). All participants gave written informed consent.

2.2. Design and study procedures

In the ADONIS-project patients underwent an extensive assessment at baseline, and were invited to for reassessment at yearly intervals up to 5 years, and at one additional visit at 6 months. Peripheral blood cell counts and FeNO (NIOX System, Aerocrine, Sweden) [14] were collected at every visit, exacerbations, defined as a burst of oral corticosteroids, were reported and asthma control was assessed by the asthma control questionnaire (ACQ-6). Extensive assessments at baseline and at 4–5 years have been described previously [15] and included demographics, medical history, lung function measurement, atopic status, and nasal endoscopy. Differential cell counts in induced sputum were assessed according to international guidelines [16].

2.3. Defining persistent blood eosinophilia

Blood eosinophilia was defined by threshold levels of either $\geq 0.30\times10^9$/L [8,9,12] or $\geq 0.40\times10^9$/L [17–20], as both values are frequently used in clinical and pharmaceutical asthma research. Persistent blood eosinophilia (hereafter: "eosinophilic asthma") was defined by levels consistently above the threshold during follow-up. Absence of blood eosinophilia (hereafter: "non-eosinophilic asthma") was defined by levels consistently below this threshold. Patients who could not be classified in one of these two groups were considered as "fluctuating". Blood eosinophil levels measured<2 weeks after an exacerbation were not included.

2.4. Statistical analysis

Patients with at least one reassessment over 5 years were included in the analysis. Patients were excluded if they did not use inhaled corticosteroids at baseline or were on maintenance oral corticosteroid therapy. Receiver operating characteristic curve (ROC) analysis was used to determine the diagnostic value of one single blood eosinophil or FeNO level at baseline to diagnose "eosinophilic" and "non-eosinophilic" asthma during follow-up. For both biomarkers the area under the curve (AUC) was calculated. Cut-off values were defined at a positive predictive

value (PPV) of 95%, or in case this value couldn't be reached, the highest achievable PPV below 95%. Corresponding sensitivity, specificity and negative predictive value (NPV) and accuracy were calculated. In addition, cut-off points for predicting persistent sputum eosinophilia (≥3%) were calculated, but since we expected the number of patients capable of producing adequate sputum samples to be low, we did not choose sputum eosinophilia to be the primary outcome. Analyses were performed in SPSS version 24.0 (IBM SPSS, Chicago, Ill).

3. Results

Data from 114 patients could be used in the analysis (Fig. 1). When comparing these patients with those that were excluded, no differences in baseline characteristics were observed, except for a slightly higher body mass index and lower dose of inhaled corticosteroids in the latter group (*data not shown*). The median (interquartile) number of visits was 5 (4–6), the number of visits per patient can be found in Fig. 1. Characteristics of the included patients are shown in Table 1.

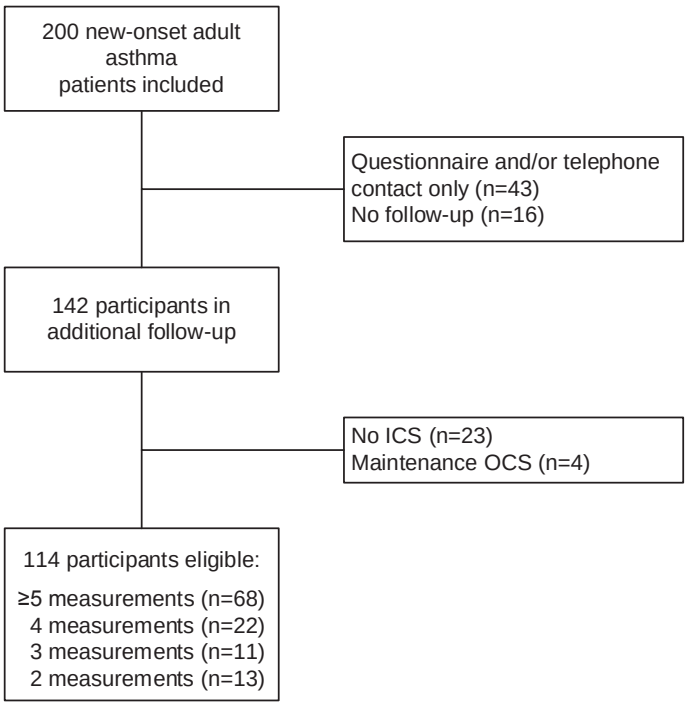

Figure 1. Consort diagram. ICS; inhaled corticosteroids, OCS; oral corticosteroids.

Table I. Baseline characteristics.

Subjects (n)	114	
Gender, % female	52	
Age (years)	49	±14
BMI (kg/m^2)	28	±5
Never/ex/current-smoker, %	40/54/5	
Pack Years	4	(0-14)
ACQ6-score	1.35	±0.94
ICS dose, fluticasone equivalent(μg)[§]	500	(250-500)
Exacerbation (n in pervious year)	0	(0-1)
IgE	68	(28-201)
Atopy, %	43	
Nasal polyps, %	19	
postFEV$_1$ (% pred)	100	±17
FeNO (ppb)	20	(13-40)
Blood eosinophils (x10^9/L)	0.17	(0.09-0.26)
Neutrophils (x10^9/L)	3.88	±1.32
Sputum eosinophils (%)[¥]	0.70	(0.10-4.30)

Data are mean ± SD, median (interquartile range), or percentage. BMI, Body mass index; ACQ, asthma control questionnaire; ICS, inhaled corticosteroids; post, post-bronchodilator; FeNO, fraction of exhaled nitric oxide; ppb, parts per billion. [§]Reported daily dose, [¥]n=86

3.1. "Eosinophilic asthma" defined by blood eosinophil threshold ≥0.30×10^9/L

Nine percent of the patients had persistent blood eosinophil levels ≥0.30×10^9/L at all visits, 72% had persistent blood eosinophils levels< 0.30×10^9/L. Characteristics of these two groups as well as the group patients with fluctuating blood eosinophil levels are shown in Table 2.

ROC analysis showed an AUC (95% Confidence Interval (CI)) of 0.89 (0.73–1.00) (Fig. 2a) for one single measurement of blood eosinophils to predict "eosinophilic asthma". For FeNO the AUC was 0.73 (0.54–0.93) (Fig. 2a). The cut-off value of blood eosinophils for diagnosing "eosinophilic asthma" was ≥0.47×10^9/L with a PPV of 83%, and for FeNO ≥83 ppb with a PPV of only 40%. Cut off values for diagnosing "non-eosinophilic asthma" were≤0.17×10^9/L eosinophils and FeNO of ≤15 ppb, respectively. Corresponding ROC-curves, sensitivity, specificity, PPV and NPV for all cut offs can be found in Fig. 2 and Table 3.

Sixty-three percent of patients showed blood eosinophils ≥0.47×10^9/L or ≤0.17×10^9/L, and of these patients, 93% was correctly classified as having "eosinophilic" or "non-eosinophilic" asthma. Forty-four percent of patients had FeNO values of ≥83 ppb or ≤15 ppb of whom only 84% were correctly classified.

3.2. "Eosinophilic asthma" defined by blood eosinophil threshold ≥0.40×10⁹/L

Seven percent of the patients had persistent blood eosinophil levels ≥0.40×10⁹/L, 80% had persistent blood eosinophil levels<0.40 ×10⁹/L. Characteristics of these two groups as well as the group patients with fluctuating blood eosinophil levels are shown in Table 4.

ROC analysis showed an AUC (95% CI) of 0.79 (0.39–1.00) (Fig. 2c) for one single measurement of blood eosinophils to predict "eosinophilic asthma". For FeNO the AUC was 0.85 (0.72–0.98) (Fig. 2c). The cut-off value of blood eosinophils for diagnosing "eosinophilic asthma" was ≥0.49×10⁹/L with a PPV of 80%, and for FeNO ≥83 ppb with a PPV of only 40%. Cut off values for diagnosing non-"eosinophilic asthma" were ≤0.21×10⁹/L eosinophils and FeNO of ≤15 ppb, respectively. Corresponding ROC-curves, sensitivity, specificity, PPV and NPV for all cut offs can be found in Fig. 2 and Table 3.

Seventy-five percent of patients had blood eosinophils ≥0.49×10⁹/L or ≤0.21×10⁹/L, and of these patients, 93% was correctly classified as having "eosinophilic" or "non-eosinophilic" asthma. Forty-four percent of patients had FeNO values of ≥83 ppb or ≤15 ppb of whom only 84% were correctly classified.

3.3. "Eosinophilic asthma" defined by sputum eosinophil threshold ≥3%

Adequate sputum samples at baseline ànd at 4–5 years follow-up were available in 46 patients. Seven patients had ≥3% sputum eosinophils at both occasions, 34 patients had less than 3% eosinophils at both occasions. Patients with blood eosinophils ≥0.46×10⁹/L had a PPV of 80% for persistent sputum eosinophilia ≥3%, and those with ≤0.19×10⁹/L had a PPV of 96% for persistently low sputum eosinophils (< 3%). Patients with FeNO levels ≥78 ppb had a PPV of 60% for persistent sputum eosinophilia ≥3%, and those with≤12 ppb had a PPV of 91% for persistently low sputum eosinophils (< 3%).

Table II: Characteristics of patients with persistent blood eosinophilia (≥0.30x10^9/L), fluctuating levels and those who never showed blood eosinophilia.

	Persistent blood eosinophil levels <0.30x10^9/L		"Fluctuating"		Persistent blood eosinophil levels ≥0.30x10^9/L	
Subjects (n)	82		22		10	
Gender, % female	59		32		40	
Age (years)	47	±14	51	±14	57	±12
BMI (kg/m2)	28.1	±4.8	30.6	±5.3	26.5	±7.2
Never/ex/current-smoker, %	44/52/4		32/55/14		30/70/0	
Pack Years	4	(0-13)	7	(0-14)	9	(0-24)
ACQ6-score	1.39	±0.95	1.26	±0.92	1.22	±0.95
ICS dose, fluticasone equivalent(μg)[§]	500	(250-500)	500	(250-500)	500	(250-500)
Exacerbation (n in pervious year)	0	(0-1)	0	(0-1)	1	(0-2)
IgE	50	(23-122)	185	(75-401)	241	(52-366)
Atopy, %	40		50		50	
Nasal polyps, %	16		27		30	
postFEV$_1$ (% pred)	100.4	±16.8	99.3	±17.0	98.2	±14.3
FeNO (ppb)	16	(12-30)	48	(24-89)	56	(18-130)
Blood eosinophils (×10^9/L)	0.13	(0.07-0.18)	0.28	(0.20-0.38)	0.55	(0.50-0.80)
Neutrophils (×10^9/L)	3.91	±1.32	3.47	±1.42	4.56	±0.77
Sputum eosinophils (%)[¥]	0.3	(0.1-1.2)	2	(0.6-19.8)	10.1	(1.90-47.9)
Sputum neutrophils (%)[¥]	74.6	(58.6-87-2)	59.6	(49.4-72.6)	48.4	(39.9-78.0)

Data are mean (±SD) or median (interquartile range) or percentage. BMI, Body mass index; ACQ, asthma control questionnaire; ICS, inhaled corticosteroids; post, post-bronchodilator; FeNO, fraction of exhaled nitric oxide; ppb, parts per billion. [§]Reported daily dose, [¥]n=52/15/8

Table III: Diagnostic accuracy of blood eosinophils and FeNO for persistent eosinophilia.

	AUC (95% CI)	Cut point	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy (95% CI)
To predict blood eosinophil levels persistent ≥0.30 x10^9/L							
Blood eosinophils	0.99 (0.97-1.00)	≥0.47x10^9/L	1.00 (0.69-1.00)	0.98 (0.93-1.00)	0.83 (0.52-0.98)	1.00 (0.96-1.00)	0.98 (0.94-1.00)
FeNO	0.73 (0.54-0.91)	≥83 ppb	0.44 (0.14-0.79)	0.94 (0.87-0.98)	0.40 (0.12-0.74)	0.95 (0.89-0.98)	0.90 (0.83-0.95)
To predict blood eosinophil levels persistent <0.30x10^9/L							
Blood eosinophils	0.90 (0.83-0.97)	≤0.17x10^9/L	0.70 (0.58-0.79)	0.91 (0.75-0.98)	0.95 (0.86-0.99)	0.54 (0.40-0.67)	0.75 (0.66-0.83)
FeNO	0.80 (0.71-0.90)	≤15 ppb	0.48 (0.37-0.60)	0.93 (0.78-0.99)	0.95 (0.83-0.99)	0.41 (0.29-0.53)	0.61 (0.51-0.70)
To predict blood eosinophil levels persistent ≥0.40x10^9/L							
Blood eosinophils	0.99 (0.97-1.00)	≥0.49x10^9/L	1.00 (0.63-1.00)	0.98 (0.93-1.00)	0.80 (0.44-0.97)	1.00 (0.96-1.00)	0.98 (0.94-1.00)
FeNO	0.85 (0.72-0.98)	≥83 ppb	0.57 (0.18-0.90)	0.94 (0.88-0.98)	0.40 (0.12-0.74)	0.97 (0.91-0.99)	0.92 (0.85-0.96)
To predict blood eosinophil levels persistent <0.40x10^9/L							
Blood eosinophils	0.89 (0.79-0.98)	≤0.21x10^9/L	0.78 (0.68-0.86)	0.83 (0.61-0.95)	0.95 (0.87-0.99)	0.49 (0.32-0.65)	0.79 (0.70-0.86)
FeNO	0.77 (0.64-0.89)	≤15 ppb	0.43 (0.33-0.54)	0.90 (0.70-0.99)	0.95 (0.83-0.99)	0.28 (0.17-0.40)	0.52 (0.43-0.62)
To predict sputum eosinophil levels persistent ≥3%							
Blood eosinophils	0.89 (0.76-1.00)	≥0.46x10^9/L	0.57 (0.18-0.90)	0.97 (0.87-1.00)	0.80 (0.28-0.99)	0.93 (0.80-0.98)	0.91 (0.79-0.98)
FeNO	0.68 (0.42-0.94)	≥78 ppb	0.43 (0.10-0.82)	0.94 0.81-0.99)	0.60 (0.15-0.95)	0.89 (0.75-0.97)	0.86 (0.71-0.95)
To predict sputum eosinophil levels persistent <3%							
Blood eosinophils	0.88 (0.76-1.00)	≤0.19x10^9/L	0.68 (0.49-0.83)	0.92 (0.62-1.00)	0.96 (0.79-1.00)	0.50 (0.28-0.72)	0.74 (0.59-0.86)
FeNO	0.71 (0.52-0.91)	≤12 ppb	0.32 (0.17-0.51)	0.91 (0.59-1.00)	0.91 (0.59-1.00)	0.32 (0.17-0.51)	0.48 (0.32-0.64)

AUC: area under the curve; CI, confidence interval; PPV: positive predictive value; NPV: negative predictive value; FeNO: fraction of exhaled nitric oxide; ppb, parts per billion.

Table IV: Characteristics of patients with persistent blood eosinophilia (≥0.40x10^9/L),fluctuating levels and those who never showed blood eosinophilia

	Persistent blood eosinophil levels <0.40x10^9/L		"Fluctuating"		Persistent blood eosinophil levels ≥0.40x10^9/L	
Subjects (n)	91		15		8	
Gender, % female	55		40		38	
Age (years)	47	±14	53	±14	55	±14
BMI (kg/m2)	28.3	±4.9	31.2	±5.1	25.3	±7.4
Never/ex/current-smoker, %	42/53/5		33/60/7		38/63/0	
Pack Years	4	(0-12)	7	(0-30)	5	(0-14)
ACQ6-score	1.36	±1.34	1.46	±0.98	1	±0.81
ICS dose, fluticasone equivalent(μg)[§]	500	(250-500)	500	(250-500)	500	(250-500)
Exacerbation (n in pervious year)	0	(0-1)	0	(0-1)	1	(0-2)
IgE	51	(26-140)	243	(87-472)	243	(49-517)
Atopy, %	42		47		50	
Nasal polyps, %	19		13		38	
postFEV$_1$ (% pred)	100.7	±17.5	94.7	±10.8	101.8	±13.6
FeNO (ppb)	18	(13-33)	48	(18-71)	86	(35-159)
Blood eosinophils (×10^9/L)	0.14	(0.08-0.20)	0.31	(0.20-0.47)	0.62	(0.52-1.05)
Neutrophils (×10^9/L)	3.80	±1.34	4.07	±1.45	4.44	±0.80
Sputum eosinophils (%)[¥]	0.4	(0.1-1.9)	1.5	(0.5-10.4)	25.4	(3.6-55.5)
Sputum neutrophils (%)[¥]	73.8	(54.2-86.6)	58.5	(49.5-70.4)	44.5	(33.8-83.3)

Data are mean (±SD) or median (interquartile range) or percentage. BMI, Body mass index; ACQ, asthma control questionnaire; ICS, inhaled corticosteroids; post, post-bronchodilator; FeNO, fraction of exhaled nitric oxide; ppb, parts per billion. [§]Reported daily dose, [¥]n=52/15/8

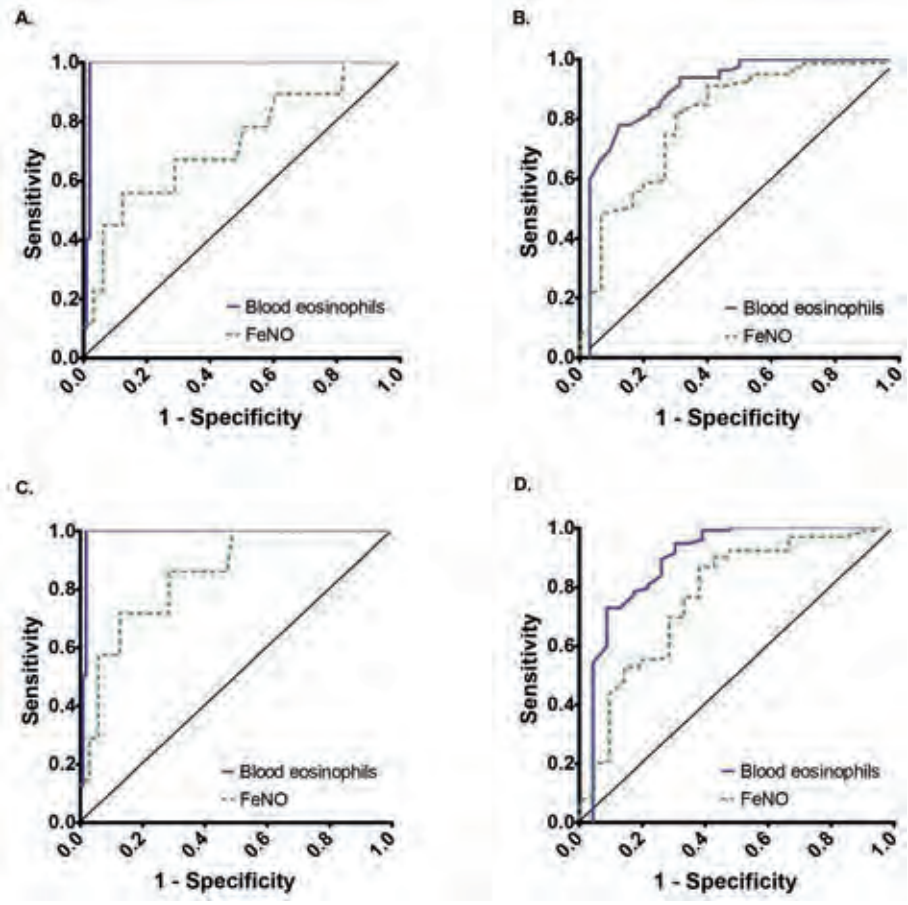

Figure 2. Receiver operating characteristic curves for single blood eosinophils and FeNO level. A. blood eosinophil levels persistent ≥0.30 x10^9/L; B. blood eosinophil levels persistent <0.30 x10^9/L; C. blood eosinophil levels persistent ≥0.40 x10^9/L; D. blood eosinophil levels persistent <0.40 x10^9/L.

4. Discussion

This study shows that one single measurement of blood eosinophils can be used to accurately diagnose the "eosinophilic phenotype" in new-onset asthma. To diagnose "eosinophilic asthma" defined as asthma with persistently high (≥0.30×10^9/L) blood eosinophils over a period of 5 years, we found a cut-off value of ≥0.47×10^9/L for blood eosinophils at baseline. When using levels of ≥0.40×10^9/L blood eosinophils or sputum percentages of ≥3% to define "eosinophilic asthma",

the cut-off values were ≥0.49×10^9/L and ≥0.46×10^9/L, respectively. This was in contrast to FeNO, showing poor predictive value and low accuracy.

Our study shows that one single measurement of blood eosinophils in the initial phase of adult-onset asthma can be sufficient to predict the "eosinophilic" or "non-eosinophilic" phenotype. Earlier studies to predict "eosinophilic asthma" with blood eosinophils were mostly crosssectional, and used sputum eosinophilia instead of blood eosinophilia to define "eosinophilic asthma". Fowler and colleagues found a PPV of 80% to predict sputum eosinophilia (≥2%) with a cut-off for blood eosinophils of ≥0.45×10^9/L [21]. Also, a study from our group in a large adult-onset asthma population defined a cut-off for 3% sputum eosinophils at 0.41×10^9/L with a PPV of 79% [20]. A post-hoc analysis of recent trials with mepolizumab in severe asthma looked into the added value of repeated blood eosinophil measurements for treatment response [22]. Remarkably, in these trials patients with only one single measurement of blood eosinophils<0.15×10^9/L did not respond to mepolizumab. This fits with our results as we observed that patients with eosinophil levels ≤0.17×10^9/L never showed blood eosinophilia over a period of 5 years. However, in the mepolizumab studies only a minority of the severe asthma patients had blood eosinophils<0.15 ×10^9/L and blood eosinophilia was less consistent over time. This suggests that persistent eosinophilia in recent onset asthma and response to anti-IL-treatment in severe asthma cannot be used interchangeably.

In our study blood and sputum samples were collected prospectively per protocol, which means that there was no specific clinical indication. Consistently elevated levels of eosinophils are therefore likely to reflect ongoing airway inflammation, despite inhaled corticosteroid treatment. Only 40% of our study population was able to provide a repeated sputum sample, and this number was not sufficiently high to use sputum eosinophils as the gold standard. Remarkably, blood eosinophils and sputum eosinophils showed similar diagnostic cut-off points and all patients with consistent blood eosinophilia who were able to produce sputum also showed consistent sputum eosinophilia.

Our study has some limitations. First, the relatively small number of patients with persistent eosinophilia, which could have influenced the diagnostic accuracy of the single eosinophil measurement at asthma onset, and could therefore be seen as a pilot study. However, this low number underestimates rather than overestimates the diagnostic accuracy. Secondly, our patients were assessed at variable times of the day. This might have influenced our results, since it has been shown that blood eosinophils show diurnal variations [23]. Also the season in which patients

were assessed could have influenced the stability of blood eosinophils, but this appeared not to be the case (data not shown). A third potential limitation is that we excluded patients using maintenance oral corticosteroids or were not using inhaled corticosteroids, which limits the generalizability of our results. Also the doses of inhaled corticosteroids might have confounded our results. However, since only a small portion (12%) of our patients was using high dose of inhaled corticosteroids, and reanalysis of the data after exclusion of these patients did not change our findings (data not shown). Finally, one might not agree with our assumption that persistently elevated blood eosinophils is pathognomonic for "eosinophilic asthma", and is a precursor of the severe eosinophilic asthma phenotype. However, the patients with persistent eosinophilia in our study, although their disease was still relatively mild, already showed typical features of the "inflammatory predominant" asthma phenotype described in the landmark study by Haldar and colleagues [1], including adult-onset asthma, frequent exacerbations and male predominance.

In contrast to blood eosinophils, FeNO was less accurate to diagnose "eosinophilic asthma". This is remarkable, because FeNO is often associated with eosinophilic airway inflammation both in mild and severe asthma [24–26]. The most plausible explanation is that FeNO and blood eosinophils represent different pathways associated with airway eosinophilia [27.] This is also illustrated by the observation that treatment directed against IL-4/IL-13, which play a central role in asthmatic airway inflammation, shows significant reduction in FeNO levels and an increase in blood eosinophils [28], whereas treatment with monoclonal antibody against IL-5 does not affect FeNO, while profoundly reducing blood eosinophils [7].

Our study has clinical implications because it shows that in a large part of the adult-onset asthma patients "eosinophilic asthma" can be diagnosed by using 2 cut-off values (high and low) of blood eosinophils (Figure E1). Identifying patients with "eosinophilic asthma" at an early stage of the disease and treating them with novel anti-eosinophil drugs might be important to prevent exacerbations and improve asthma outcomes. Alternatively, for symptomatic asthma patients with low blood eosinophil counts no such treatment should be considered as these patients are unlikely to have ongoing eosinophilic airway inflammation. From our data it appears that FeNO is less feasible to diagnose "eosinophilic asthma" in new-onset asthma in adults, because of its low accuracy. For patients with intermediate ($> 0.17\times10^9$/L and $<0.47\times10^9$/L) blood eosinophil levels new biomarkers are required for further phenotyping.

In conclusion, we showed that one single measurement of blood eosinophils, but not FeNO, in the initial phase of new-onset asthma in adults can be used to predict "eosinophilic" and "non-eosinophilic" asthma. By using two cut-off points we could classify 74% of the patients into the "eosinophilic" or "non-eosinophilic" phenotype, with an accuracy of 93%. These cut-off values may become very useful for clinical application of personalized treatments and implementation of the "treatable traits" approach [29].

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Supplementary Material

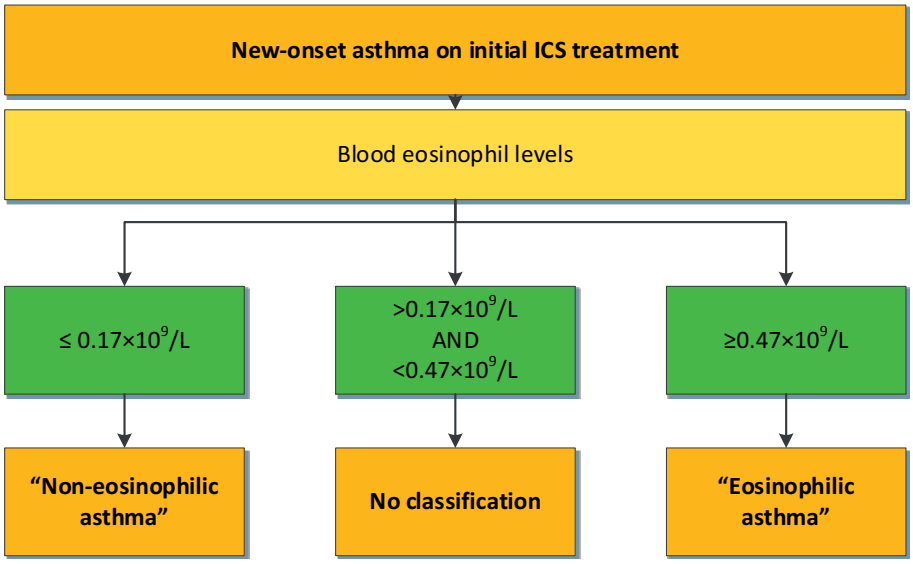

Figure E1. Flow diagram to define the persistent eosinophilic phenotype, defined by a threshold of blood eosinophils always/never ≥0.30x10⁹/L. *For persistent eosinophilic asthma defined by blood eosinophils always/never ≥0.40x10⁹/L cut-offs are ≤0.21x10⁹/L and ≥0.49x10⁹/L, respectively.*

Chapter 4

Biomarkers in obese asthma, age of asthma onset matters!

Coumou H*, Westerhof GA*, Bel EH.

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To the Editor:

With great interest we read the recently published article in the Journal by Lugogo *et al.*[1] They report poor predictive values of conventional type 2 biomarkers in obese asthma patients. Receiver operating characteristics (ROC)-curve analysis is used to calculate biomarkers cut points for predicting ≥2% sputum eosinophils. For obese asthma patients these cut-off values are lower than for lean and overweight patients. The authors conclude that in obese asthma conventional biomarkers are poorly predictive of eosinophilic airway inflammation.

This conclusion contrasts with the findings of a previous study form our group.[2] Our study showed a fairly good predictive value of blood eosinophils as well as FeNO to diagnose >3% sputum eosinophilia. Furthermore, we found no difference in ROC area under the curves (AUC) for obese and non-obese patients (eg AUC for blood eosinophils: 0.82 *vs*. 0.83, p=0.82).[2] Only for IgE the predictive value was similarly poor as in the study by Lugogo and colleagues.

We believe this discrepancy can be explained by several factors. First, the two studies differ with respect to the characteristics of included patients. The study by Lugogo and colleagues mainly included patients with childhood-onset atopic asthma (range 4-25 yr), whereas our study exclusively included adult-onset (>18yrs) asthma patients (53±13 yr) of whom 68% was non-atopic. From the literature it is well-known that asthmatic subjects are differentially affected by obesity based on whether they had asthma early (<12 years of age) or later in life[3], suggesting that these two groups of subjects represent two different obese asthma phenotypes. It is well conceivable that in the adult-onset obese asthma patients, the relationship between FeNO or blood eosinophils and sputum eosinophils is more clear-cut than in a mixed group of obese patients with different ages of disease onset. Second, the patients in the study by Lugogo and colleagues showed relative low levels of biomarkers and sputum eosinophils, whereas our study showed a wide range of both biomarkers and sputum eosinophils. Obviously, wide ranges enable more accurate analyses than values with little spread. Third, the cut point used for sputum eosinophilia by Lugogo is 2% *vs.* 3% in our study, probably because of the relatively low number of patients with ≥3% eosinophils in their study. This higher cut-point could also have improved the diagnostic accuracy of the biomarkers in our study.

In conclusion, we agree with Lugogo and colleagues that in obese patients with childhood onset atopic asthma biomarkers should be used with caution to predict

eosinophilic inflammation. However, in adult-onset obese asthma patients, FeNO and blood eosinophils seem to be well-suited suited to predict eosinophilic or non-eosinophilic asthma.

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Chapter 5

The prognosis of adult-onset asthma, a literature overview

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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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Chapter 6

Predictors of accelerated decline in lung function in adult-onset asthma

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Introduction

Asthma is a heterogeneous disease comprising several sub-phenotypes with different clinical, inflammatory and functional characteristics[1, 2]. Age of disease onset appears to be an important distinctive feature of these sub-phenotypes, since studies have shown that patients with adult-onset asthma differ in many respects from those in whom asthma started in childhood[3]. As adult-onset asthma covers more than 50% of the novel diagnoses of asthma[4, 5] this is a clinically important phenotype. Adult-onset asthma is associated with more (persistent) eosinophilic airway inflammation and more chronic sinus disease[6, 7]. It has also has been suggested that these patients have a more rapid decline in FEV_1 both in cross sectional and longitudinal studies[8, 9].

Potential factors that have been shown to contribute to accelerated lung function decline in the general asthma population are smoking[10],recurrent exacerbations[11], and low baseline FEV_1[12]. However, severity of inflammation could also be an important contributor by inducing airway remodelling[13].

Early identification of patients at risk of accelerated decline in lung function is important because irreversible airflow obstruction is known to be associated with increased morbidity and mortality[14, 15]. In addition, determining the nature of risk factors could help identifying the right preventive treatment for the right patient.

In this study we hypothesized that adult-onset asthma patients at risk of an accelerated decline in lung function can be identified at diagnosis. The aim of this study was to identify clinical, functional or inflammatory risk factors of accelerated FEV_1 decline in a prospective 5-year study in adults with newly diagnosed asthma.

Methods

Subjects

Two hundred adults who were included in the ADONIS study (Adult-onset asthma and inflammatory subphenotypes) were prospectively followed for 5 years. Patients aged 18-75 years old were eligible if they were recently(<12 months) diagnosed with asthma. Asthma diagnosis was based on typical symptoms with at least 200ml and 12% improvement in FEV_1 % predicted values or presence of airway hyperresponsiveness[16]. Current smoking or a smoking history >10

pack years was allowed if there was no airflow obstruction (FEV_1/FVC <0.7) and normal diffusion capacity (diffusing capacity of the lungs for carbon monoxide, corrected for haemoglobin and alveolar volume >80%). Patients with a history of asthma or chronic airways disease in childhood were excluded. The study was approved by the AMC Medical Ethics Board and registered in the Dutch trial register(NTR1846). All participants gave written informed consent.

Study Design

The study design included 7 visits over 5 years. At baseline, patients underwent a comprehensive assessment of clinical, functional and inflammatory parameters. Follow-up occurred at yearly intervals, with one additional follow-up visit after 6 months. At the final visit all baseline measurements were reassessed.

Study measurements

At baseline, the following data were collected: demographics, medical history, medication use, asthma control(Asthma Control Questionnaire ACQ-6) [17], standardized pre- and postbronchodilator spirometry (FEV_1 and forced vital capacity)[18], airway hyperresponsiveness defined as the provocative concentration of methacholine to establishing a 20% fall in FEV_1 (if no fall was reached, a concentration of 32 mg/ml was used), atopy defined as IgE >0.35 Ku/L for at least one out of a set of common aeroallergens (ImmunoCAP), peripheral blood cell counts, fraction of exhaled nitric oxide(FeNO) (NIOX System, Aerocrine, Sweden)[19], sputum cell differentials according to international standards[20], and nasal polyposis based on nasal endoscopy and/or nasal polypectomy in the history of the patient . At subsequent visits medication use, ACQ, AQLQ, exacerbations (defined as courses of oral corticosteroids in the previous follow-up period), postbronchodilator(pb) FEV_1, peripheral blood cell counts and FeNO were assessed.

At the final visit all baseline assessments were repeated with the exception of atopic status and presence of nasal polyposis.

Definition of accelerated decline

Annual $pbFEV_1$ decline per patient was calculated as the slope of the regression line of the individual $pbFEV_1$ measurements over time. Annual $pbFEV_1$ decline per patient was dichotomized at the lower quartile ($\leq 25^{th}$ percentile). Values below this lower quartile were defined as accelerated decline.

Statistical analysis

Data from patients who only attended the baseline visit without follow-up data were excluded. Linear mixed effect model of the longitudinal $pbFEV_1$ values over time (ml/year) was used to determine the relationship between the annual $pbFEV_1$ decline and covariates. Change of $pbFEV_1$ over time (years) was modelled as a linear function. Patients individual intercept and slope over time were included as random effect in the linear mixed effects model. We assessed whether the slopes of these individual regression lines were different between variable levels by inclusions of interaction terms of the predictor with follow-up time in years in the mixed effect model.

Univariable mixed models were used to select variables for the multivariable mixed models ($p<0.10$),containing either continuous or dichotomized predictor variables. Backward selection was used in the multivariable continuous and dichotomized models, and all models we adjusted for baseline $pbFEV_1$ (expressed as $pbFEV_1$% of predicted) by including this variable as main effect. Thereafter, exacerbations during follow-up were added to the multivariate model.

Subsequently, receiver operating characteristic analysis based on the results of the mixed effect model was performed with accelerated $pbFEV_1$ decline as outcome. We defined cut-off values of the predictor variables as the threshold with 90% specificity for each predictor variable.

Statistical significance was set at a p-value of less than 0.05. SPSS software, version 23(IBM SPSS, Chicago, Ill) and Rstudio V1.0.136(Free Software Foundation, Inc.) were used for statistical analyses.

Results

Of 200 patients included in the study, repeated lung function tests of 141 patients (71%) were available over a period of 5 years. The 59 patients with incomplete datasets were more often female (71% vs 50%; $p<0.01$) and used lower doses of inhaled corticosteroids (250 µg/day vs 313 µg/day; $p=0.02$) than the patients with complete datasets (Table E1). Baseline characteristics of the 141 adults with new-onset asthma are shown in Table 1. The median time between asthma diagnosis and baseline visit was 2 [range 1-5] months. The average follow-up time was 4.6 (SD±1.4) years, with a median of 5 [range 5-6] visits. The median exacerbations rate was 0 [0-0.25]/yr. 68% of the patients did not report any exacerbation during

follow-up and 19% experienced only 1 exacerbation. The median change in $pbFEV_1$ was -17.5 ml/yr. The limit of the lower quartile was at -54.2 ml/yr and the limit for the upper quartile was +22.4 ml/yr.

Table I: Baseline characteristics of participating patients

Subjects (n)	141	
Follow-up (Years)	4.6	±1.4
Gender, % female	50	
Age (years)	49	±15
BMI (kg/m^2)	28	±5
Never/ex/current-smoker, %	41/51/8	
Pack Years	4	[0-13]
ACQ6-score	1.27	±0.93
GINA severity score[16]	3	[2-4]
LABA/LAMA use, %	67	
LTRA use, %	1	
ICS use, %	84	
ICS dose, fluticasone equivalent(μg)[§]	313	[250-500]
Exacerbation (n in pervious year)	0	[0-1]
IgE	68	[28-203]
Atopy, %	47	
Nasal polyps, %	20	
$pbFEV_1$ (% pred)	101	±17
$pbFEV_1$/FVC (% pred)	0.76	0.10
FeNO (ppb)	22	[13-47]
Blood eosinophils ($\times 10^9$/L)	0.16	[0.09-0.26]
Neutrophils ($\times 10^9$/L)	3.79	±1.32
Sputum eosinophils (%)[¥]	0.6	[0.1-3.9]
Annual FEV_1 decline (ml/yr)	-17.48	[-54.22 - +22.44]

Data are presented as mean±SD or median (interquartile range), unless otherwise stated. BMI, Body mass index; ACQ, asthma control questionnaire; GINA, Global Initiative for Asthma; LABA, Long-acting beta-agonists; LAMA, Long-acting muscarinic antagonists; LTRA, leukotriene receptor antagonist; ICS, inhaled corticosteroids; pb, post-bronchodilator; FeNO, fraction of exhaled nitric oxide. Data are mean (±SD) indicated by *, median [interquartile range] indicated by [#], or percentage. [§]Reported daily dose, [¥]n=86

Predictors for accelerated lung function decline

Univariable linear mixed effect analysis showed that the presence of nasal polyps, lower body mass index (BMI), higher number of blood eosinophils, higher % of sputum eosinophils and higher levels of FeNO, were significantly associated with a steeper decline in $pbFEV_1$ (Table II, Figure I). Also male gender tended to be associated with a steeper decline in $pbFEV_1$, (p=0.08). Other variables were not associated with lung function decline.

Table II. Univariable mixed effect model

	change in pbFEV$_1$ (ml/yr)*	p-value
FeNO (ppb)	-0.60	**<0.001**
BMI (kg/m^2)	2.96	**0.011**
Nasal polyps (yes)	-33.24	**0.023**
Sputum eosinophils (%)	-1.35	**0.022**
Blood eosinophils (×10^9/L)	-32.98	**0.044**
Gender (female)	20.89	0.084
(ex)-smoker (yes)	-18.98	0.126
Age (years)	-0.65	0.128
ACQ6-score	10.83	0.129
GINA severity score[16]	8.31	0.194
Pack Years (n)	-0.60	0.210
ICS use (no)	-20.63	0.214
Exacerbations (n in pervious year)	-4.82	0.300
Neutrophils (×10^9/L)	4.34	0.340
Atopy (yes)	-9.46	0.440
IgE	-0.004	0.549
Airway hyperresponsiveness (mg/mL)	0.164	0.772
ICS dose, fluticasone equivalent(μg)[§]	0.004	0.811

Pb, postbronchodilator; FeNO, fraction of exhaled nitric oxide; BMI, Body mass index; ACQ, asthma control questionnaire; ICS, inhaled corticosteroids. * change in pbFEV$_1$ in ml per year, per increase of variable. [§]Reported daily dose.

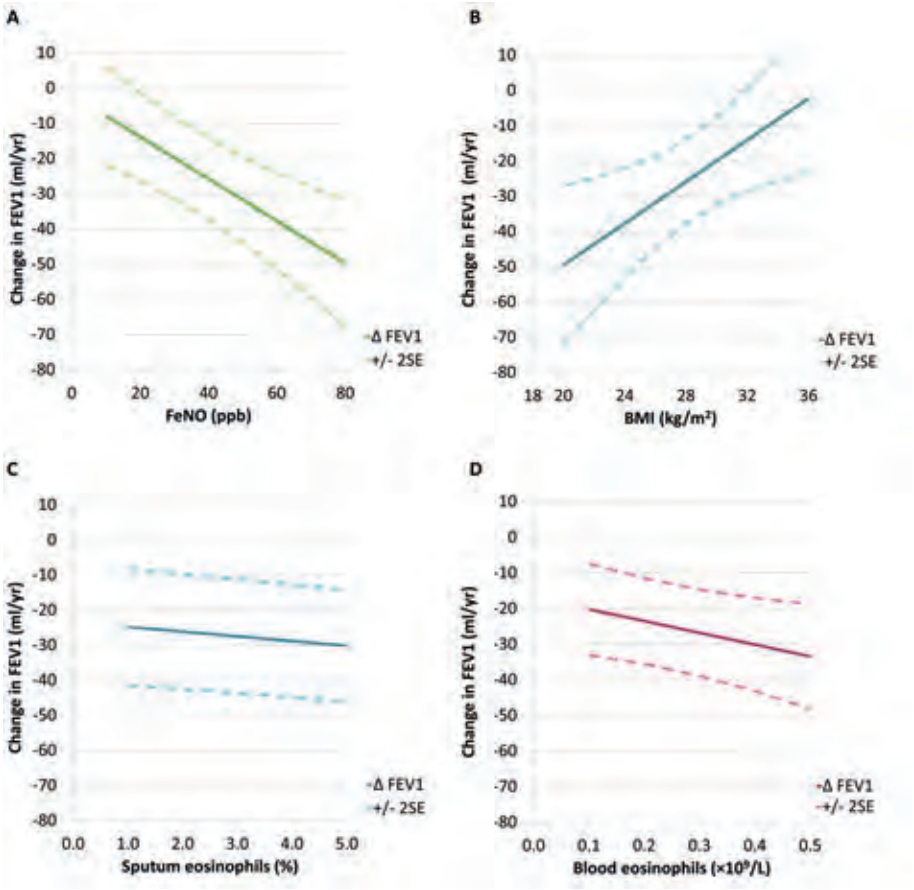

Figure I. Univariable models for FEV_1 decline with different biomarkers. **A**. FeNO *(p<0.001)* **B**. BMI *(p=0.011)* **C**. Sputum eosinophils *(p=0.022)* **D**. Blood eosinophils *(p=0.044)*. Solide line, change in $pbFEV_1$ in ml per year, per increase of biomarker; dotted line, +/- 2 standard error. FeNO, fraction of exhaled nitric oxide; ppb, parts per billion; BMI, body mass index; SE, standard error.

When putting all significant (p<0.10) continuous variables from the univariate analysis into a backward selection multivariable model, only higher FeNO and lower BMI were significantly associated with decline in $pbFEV_1$ with values of -0.55ml/ppb/yr (p=0.001) and 2.20ml/kg/m^2/yr (p=0.049), respectively.

When the calculated cut-off values of the variables were put in the backward selection multivariable model, FeNO (≥57ppb) and BMI (≤23.05kg/m^2) were significantly associated with a change in $pbFEV_1$ of-37.9 ml/yr (p=0.015) and -49.9 ml/yr (p=0.003), respectively (Table III).

Table III. Multivariable mixed effect model with use of cut-off values

	change in pbFEV$_1$ (ml/yr)*	p-value
BMI ≤23.05kg/m^2	-56.80	**0.002**
FeNO ≥57ppb	-37.78	**0.015**
Blood eosinophils ≥0.39×10^9/L	-24.59	0.234
Gender (female)	12.69	0.272
Sputum eosinophils ≥6.6%	19.17	0.564
Nasal Polyps (yes)	-10.13	0.609

Pb, postbronchodilator; BMI, Body mass index; FeNO, fraction of exhaled nitric oxide.
* change in pbFEV$_1$ in ml per year, per increase of variable.

The negative predictive values of FeNO ≥57ppb or BMI ≤23.05kg/m^2 were fairly good, 77% and 79%, respectively (table IV). The positive predictive values for FeNO ≥57ppb or BMI ≤23.05kg/m^2 were 45% and 41%, respectively. However when combining FeNO ≥57ppb and BMI ≤23.05kg/m^2 the positive predictive value for accelerated lung function decline was 100%.

When adding exacerbations to the continuous model, BMI and FeNO remained associated with lung function decline, but there appeared to be no association between exacerbations and lung function decline (p=0.253). Also no association was seen in the model using the cut-off variables.

Table IV. Test characteristics

	Sensitivity	Specificity	PPV	NPV
FeNO ≥57ppb	30	88	45	80
BMI ≤23.05kg/m^2	20	91	41	77
FeNO ≥57ppb **AND** BMI ≤23.05kg/m^2	15	100	100	78

PPV, positive predictive value; NPV, negative predictive value; FeNO, fraction of exhaled nitric oxide; BMI, body mass index

Discussion

This study shows that in adult patients with new onset asthma higher levels of FeNO and lower BMI are independently associated with faster decline in FEV$_1$. When using cut-off values to identify patients at highest risk of accelerated lung function decline, (defined as the lowest quartile of decline in FEV$_1$), it appeared that all patients with combined FeNO ≥57ppb and BMI ≤ 23.05kg/m^2 showed accelerated decline accelerated decline. No influence of current or ex-smoking,

or airway hyperresponsiveness on lung function decline was observed. These results suggest that amongst adults with new onset asthma non-obese patients with relatively severe airway inflammation are at highest risk of lung function impairment in the future.

This prospective longitudinal study focused specifically on the prognosis of new-onset asthma in adults, with a special emphasis on the predictive value of clinical, functional and inflammatory markers. One other study in a Finnish adult-onset asthma population reported a smoking history >10 pack years to be associated with accelerated decline in FEV_1[21], which was not confirmed by our data, and might be explained by the low numbers of pack-years, few current smokers and exclusion of patients especially prone to the effects of smoking (i.e. those having FEV_1/FVC <0.7 or lowered DLCO) in the present study. Neither could we confirm increased airway hyperresponsiveness or asthma exacerbation rate to be risk factors of accelerated decline in FEV_1 as was reported in several other studies, but not all[11, 21-24]. These discrepancies might be due to differences in study population, being the adult-onset asthma population in our study versus the general asthma population in the other studies. This underscores the importance of taking age of disease onset into consideration in asthma studies[2]. With respect to BMI, similar results to ours were found in one study showing a faster FEV_1 decline in non-obese adult asthmatics compared with obese[25]. Remarkably, in the general population the contrary has been described, showing an association between high BMI and lung function decline [26]. High FeNO levels have been associated with excess decline in lung function in previous studies in long-standing adult asthma [27, 28], and our study provides evidence that already at the start of the disease high FeNO levels predict a substantial loss of lung function over the first five years.

The strengths of our study are its focus on a relatively underexposed and poorly studied asthma phenotype, the prospective design of the study, and the extensive clinical, functional and inflammatory characterization of the patients. Further, a robust statistical analyses was possible given the relatively high visit rate per patient (median attendance: 5 out of 7 visits), and the high percentage of participants in the follow-up study. Indeed, the linear mixed effect model, in which all available annual lung function measurements were taken into account, guaranteed a realistic and accurate representation of the course of FEV_1 decline over 5 years.

Unfortunately, we were not able to obtain adequate sputum cell samples in all patients, which might have reduced the potential of this biomarker as a predictor. This fits in with the observation that the % eosinophils in sputum was associated with decline in lung function in the univariable analysis, but was overruled in the multivariable analysis. This does not apply to blood eosinophils, meaning that FeNO was more strongly associated with decline in lung function than blood eosinophilia. Our study did not include a control group, which could be regarded as a limitation. However, solid longitudinal data about lung function decline in healthy volunteers (22 ml/yr) and asthma patients (38 ml/yr) are available[10]. In the present study we choose the lower quartile for lung function decline to define accelerated decline in pbFEV$_1$ (54.2 ml/yr). Thus, this definition seems to be clinically relevant.

How to explain the association between high FeNO and accelerated decline in lung function? An obvious explanation would be that patients with high levels of FeNO were not using adequate amounts of anti-inflammatory medications due to poor adherence or undertreatment. Inhaled corticosteroids have been shown to decrease FeNO values[29], and prevent deterioration in lung function over time in newly diagnosed asthma[30]. However, when comparing patients with and without accelerated decline in lung function, there was no difference in inhaled corticosteroids dosing, asthma symptoms or airway hyperresponsiveness. This implies that high FeNO levels were not associated with poorer asthma control or airways dysfunction so that there was no clinical reason to assume that patients were nonadherent or needed a step-up in asthma treatment. Another explanation could be that the FeNO levels are caused by nasal polyposis[31]. Although nasal polyposis was not independently associated with loss of lung function, this might be due to the fact that half of the patients diagnosed with this condition were treated with topical corticosteroids. Therefore, we cannot fully exclude that nasal polyps are associated with decline in lung function, which would fit with the observation that nasal polyposis is a characteristic of a specific severe adult-onset asthma phenotype[32]. In fact, the high FeNO levels in these patients might reflect a "silent" mechanism leading to loss of lung function. Many studies have shown that high FeNO levels are associated with eosinophilic airway inflammation[33-35], and it is generally accepted that persistent airway inflammation leading to airway remodeling is a cause of accelerated lung function decline[13]. Remarkably, asthma characterized by predominant eosinophilic airway inflammation is typically

associated with adult-onset asthma and low symptoms scores[1], which fits in with our concept that FeNO is a marker of asymptomatic airway inflammation in this population.

Lower body weight at baseline was independently associated with an accelerated decline in FEV_1. This was an unexpected finding, and we can only speculate on possible mechanisms. In previous studies associations between weight gain and decline in lung function have been observed both in patients with asthma[25] and in the general population[26, 36]. However, this association was most marked in patients who became obese during the follow-up period[36]. In our study patients with a low BMI did not become obese; the mean BMI for this group at the 5 year visit was only slightly higher as compared to baseline. Another possibility might be that patients with a very low BMI have low muscle mass due to lack of exercise, or a mild wasting syndrome related to ongoing inflammation. This fits with our observation that the patients with combined low BMI and high FeNO were at greatest risk of accelerated decline in lung function. Finally, obese and non-obese asthmatics may represent different asthma phenotypes, not only with respect to clinical and inflammatory mechanisms but also with respect to airway remodeling and long-term prognosis[37, 38]. A recent cluster analysis also points in this direction, showing less lung function decline in obese patients with asthma, as compared to lean patients[39].

Our study has clinical implications because it provides a new and important role for FeNO in daily practice. Our results suggest that FeNO is an easy to measure, noninasive biomarker of ongoing eosinophilic airway inflammation, even in patients who have controlled disease without exacerbations. It also suggests that a high FeNO level at asthma diagnosis should be taken seriously, especially in patients who already received initial treatment, since it may reflect ongoing or refractory inflammation and loss of lung function on the long run. FeNO-guided treatment is currently advocated only for adults and children with frequent asthma exacerbations[40, 41], but this recommendation is based on studies that have not taken the rate of lung function decline into account. Therefore, studies investigating whether FeNO-guided treatment can prevent accelerated decline in lung function should be high on the research agenda.

In conclusion, our study shows that FeNO is a prognostic biomarker for accelerated decline in lung function in adults with recently diagnosed asthma. In particular non-obese patients with high FeNO levels seem to be most at risk. High FeNO levels might represent refractory eosinophilic airway inflammation leading to airway remodelling, even in patients with controlled asthma. FeNO might become an important biomarker for clinicians for guiding asthma treatment in daily practice.

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Supplementary Material

Results

Table E1. Baseline characteristics of patients with and without lung function data at 5 year visit.

	Patients with complete datasets (n=141)		Patients with incomplete datasets (n=59)		p-value
Gender, % female	50		71		**0.011**
Age (years)	49	±15	47	±15	0.458
BMI (kg/m^2)	28	±5	27	±6	0.155
(Ex)-smoker, %	59		50		0.316
Pack Years	4	[0-13]	1	[0-21]	0.890
ACQ6-score	1.27	±0.93	1.46	±0.92	0.221
Inhaled corticosteroid dose (µg)	313	[250-500]	250	[0-500]	**0.020**
Exacerbation (n)	0	[0-1]	0	[0-1]	0.213
IgE (kU/L)	68	[28-203]	45	[21-240]	0.181
GINA severity score[16]	3	[2-4]	3	[2-4]	0.400
Atopy, %	47		40		0.444
Nasal polyps, %	20		14		0.436
pbFEV$_1$ (% pred)	100	±17	100	±18	0.761
FeNO (ppb)	22	[13-47]	17	[13-35]	0.184
Blood eosinophils (×10^9/L)	0.17	[0.09-0.27]	0.15	[0.10-0.28]	0.950
Neutrophils (×10^9/L)	3.79	±1.32	3.75	±1.61	0.870
Sputum eosinophils (%)	0.6	[0.1-3.9]	0.7	[0.1-3.5]	0.736

Data are presented as mean±SD or median (interquartile range), unless otherwise stated. BMI, Body mass index; ACQ, asthma control questionnaire; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroids; pb, post-bronchodilator; FeNO, fraction of exhaled nitric oxide; ppb, parts per billion.

Table E2. Baseline characteristics of patients with normal and accelerated decline.

	Normal decline		Fast decline	
Subjects (n)	106		35	
Gender, % female	53		43	
Age (years)	48	±15	51	±13
BMI (kg/m^2)	29	±5	27	±5
Never/ex/current-smoker, %	46/48/6		29/60/11	
Pack Years	2	[0-10]	5	[0-20]
ACQ6-score	1.34	0.94	1.07	±0.90
GINA severity score[16]	3	[2-4]	3	[2-3]
ICS dose, fluticasone equivalent(µg)[§]	375	[250-500]	250	[125-500]
Exacerbation (n in pervious year)	0	[0-1]	1	[0-2]
Exacerbation (n/yr during follow-up)	0	[0-0.25]	0	[0-0.50]
IgE (kU/L)	68	[27-201]	68	[33-280]
Atopy, %	48		43	
Nasal polyps, %	18		26	
pbFEV$_1$ (% pred)	99	±16	105	±19
pbFEV$_1$/FVC (% pred)	97	±10	93	±12
FeNO (ppb)	20	[13-40]	37	[13-66]
Blood eosinophils (×10^9/L)	0.16	[0.09-0.24]	0.17	[0.09-0.35]
Neutrophils (×10^9/L)	3.89	±1.30	3.49	±1.37
Sputum eosinophils (%)[¥]	0.40	[0.10-1.73]	2.15	[0.38-8.65]

Data are presented as mean±SD or median (interquartile range), unless otherwise stated. BMI, Body mass index; ACQ, asthma control questionnaire; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroids; pb, post-bronchodilator; FeNO, fraction of exhaled nitric oxide; ppb, parts per billion. [§]Reported daily dose, [¥]n=42/11

Table E3. Area under the receiver operator characteristics curve per biomarker

	AUC
FeNO (ppb)	0.70
BMI (kg/m^2)	0.60
Sputum Eosinophils (%)	0.58
Blood eosinophils (×10^9/L)	0.57

AUC, Area under the curve; FeNO, fraction of exhaled nitric oxide; ppb, parts per billion; BMI, body mass index

Table E4. Cut-off points for predicting accelerated lung function decline.

	High specificity		
	Cut-off	Sensitivity	Specificity
FeNO (ppb)	≥57	0.36	0.90
BMI (kg/m^2)	≤23.05	0.17	0.90
Sputum Eosinophils (%)	≥6.6	0.18	0.90
Blood eosinophils (×10^9/L)	≥0.39	0.18	0.90

FeNO, fraction of exhaled nitric oxide; ppb, parts per billion; BMI, body mass index

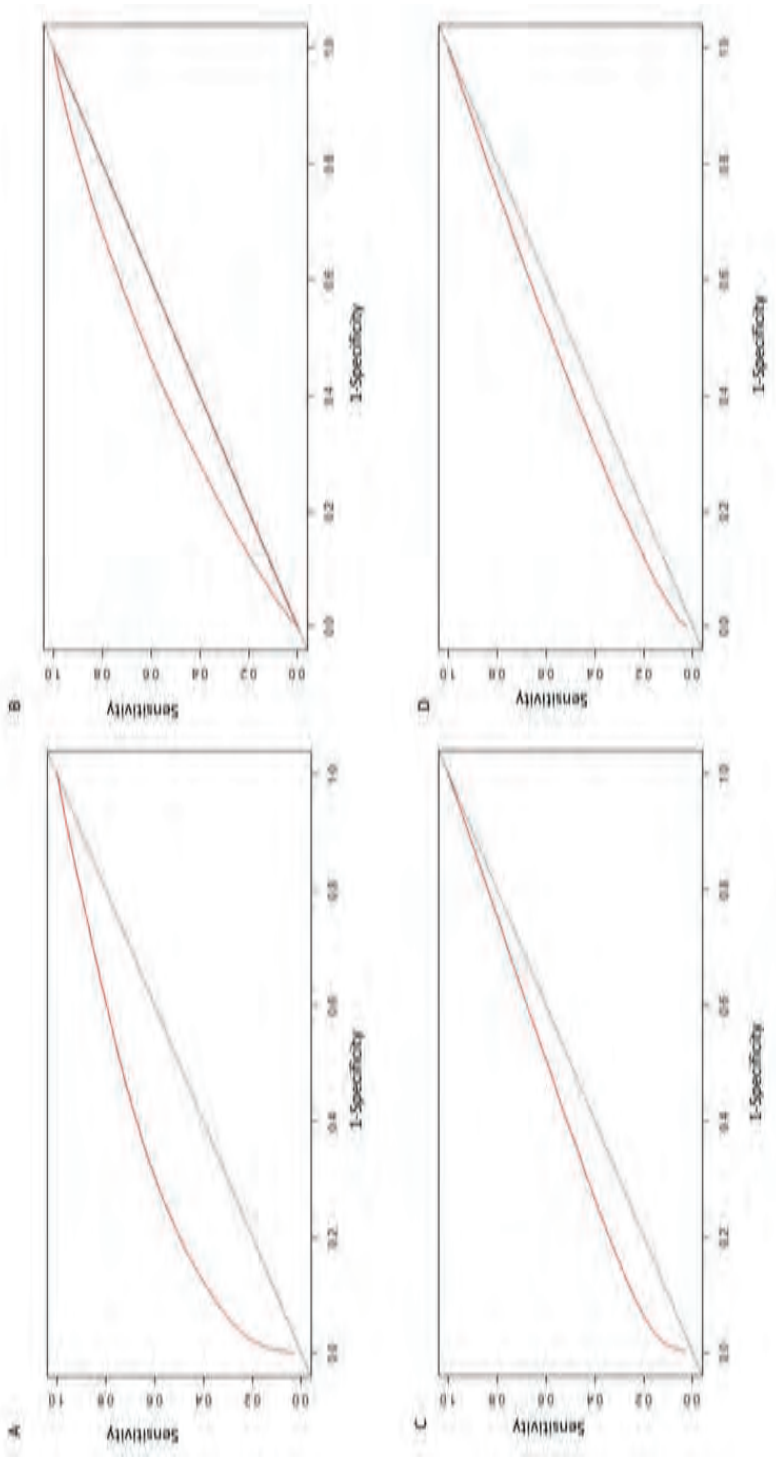

Fig E1. Receiver operating characteristic curves for accelerated lung function decline. A. Exhaled Nitric Oxide (n=135), B. Body Mass Index. (n=141), C. Sputum Eosinophils (n=86), D. Blood Eosinophils (n=141).

Chapter 7

Clinical predictors of remission and persistence of adult-onset asthma

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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/m² *	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 mean (±SD) indicated by * , median (interquartile range) indicated by # 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/m² *	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 mean (±SD) indicated by * or median (interquartile range) indicated by #.

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

	Absence of nasal polyps (n=107)			Nasal polyps (n=35)			p-value
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 mean (±SD) indicated by * , median (interquartile range) indicated by # or percentage.

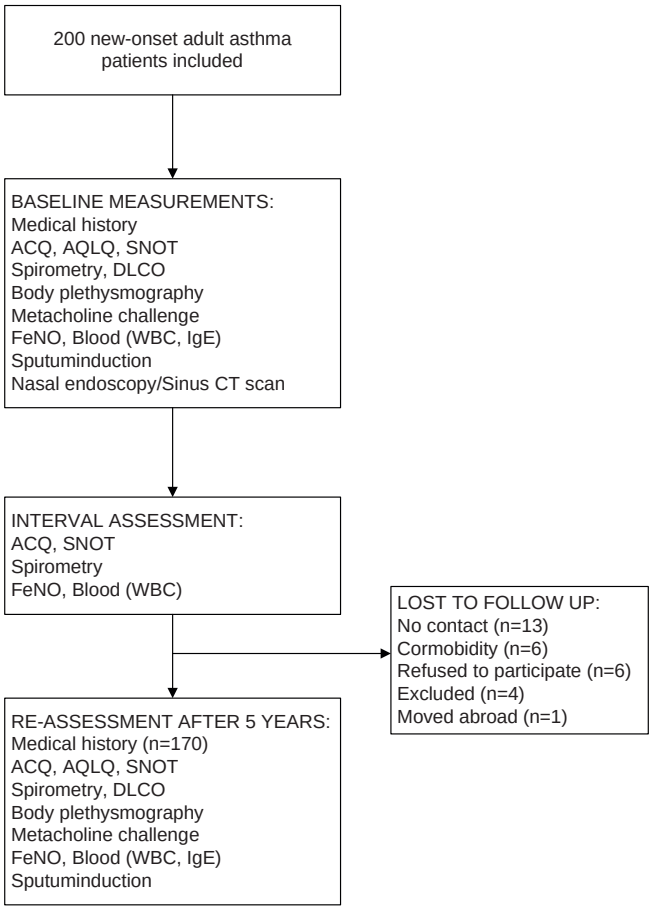

Figure 1. Consort diagram.

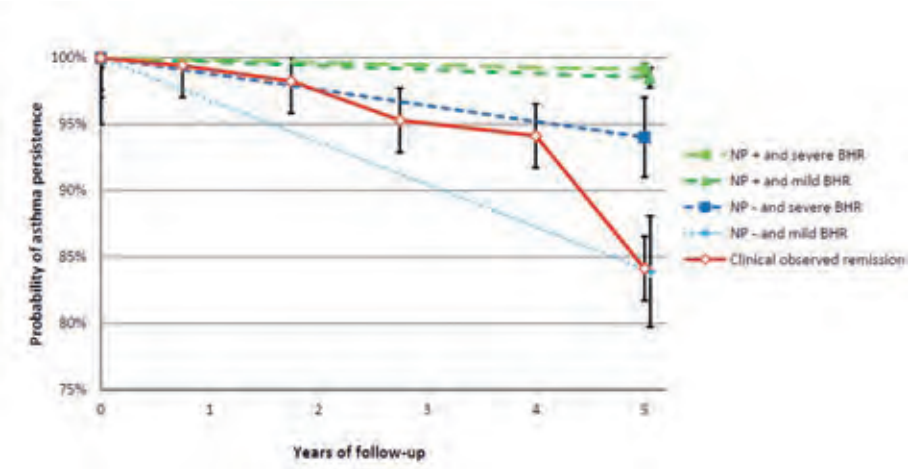

Figure 2. Probability of Asthma Persistence

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.

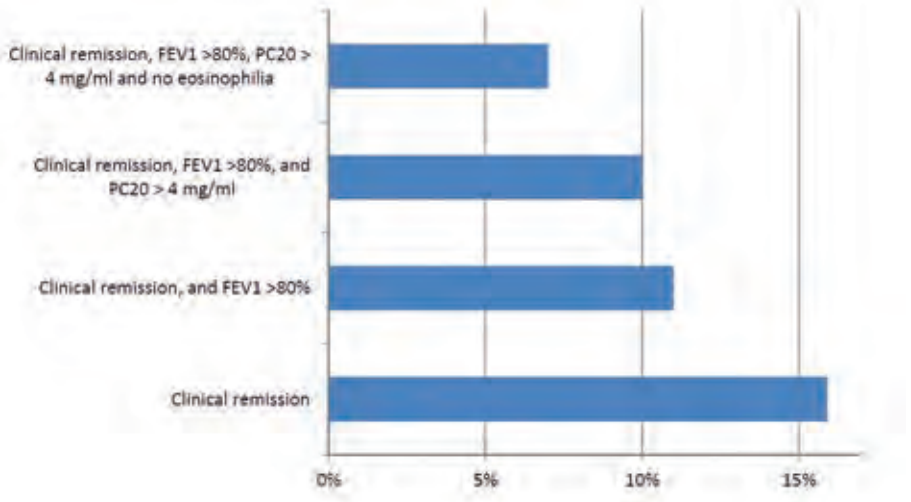

Figure 3. Asthma Remission Incidence Based on Different Definitions of Remission.

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 on-going 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 8

New-onset asthma in asthma; what does the trigger history tell us?

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Introduction

Age of disease onset is an import differentiator in asthma phenotyping(1). Asthma starting in childhood has been extensively studied over the last decades(2), whereas asthma starting in adulthood is relatively understudied(3). Over the years it has become clear that adult-onset asthma is not associated with a clear genetic predisposition(4, 5), is often severe (6) and may be incited by a specific trigger(7, 8).

Several triggers are known be associated with asthma onset in adulthood(9-11). For example, aspirin(12), smoking(13, 14) and occupational agents like isocyanates(15, 16) are well-known, but also other, less known inciters for asthma have been reported including a stressful life events (17) and hormonal factors (10). Also respiratory infections are commonly reported just before the onset of asthma (18, 19), and atopy, although generally associated with childhood asthma, has also been associated with adult-onset asthma, in particular if a new allergen is encountered (20). All these triggers have been associated with asthma onset in large population studies. However, it is still unknown whether these triggers are associated with specific phenotypic characteristics, or with a specific asthma outcome.

We hypothesized that the triggers which seemingly elicited asthma onset affect different type of patients and that they are associated with different asthma outcomes. The aim of the present study was to compare clinical, functional and inflammatory characteristics between patients with different triggers of asthma onset, and relate these triggers to asthma outcome (remission or persistence).

Methods

Patients

Two-hundred adults with recently (≤12 months) diagnosed asthma were included in this study, which was part of the ADONIS-project (Adult-onset asthma and inflammatory sub phenotypes)(21, 22). Patients were recruited from 1 academic and 2 secondary care hospitals. Asthma diagnosis was based on typical symptoms and an improvement in forced expiratory volume in 1 s (FEV_1) of 12% and 200ml, and/or a positive methacholine-challenge with a drop in FEV_1 of 20% (PC_{20} methacholine < 8 mg/ml) (23). Patients with a history of childhood asthma were excluded, as were patients with a smoking history of >10 pack years smoked with fixed airflow obstruction (post bronchodilator FEV_1 < 80% and FEV_1/FVC < 0.70) and a diffusion capacity of < 80%. All patients gave written informed consent for the study. The study was approved by the Amsterdam Medical Centre's Medical Ethics Board and registered in the Dutch trial register (NTR1846).

Study design

In this sub-study of the ADONIS project patients were comprehensively assessed at baseline, including clinical, functional and inflammatory characteristics(21, 22). Four to five years after asthma diagnosis patients were invited for reassessment to evaluate remission or persistence of the disease. If patients weren't able to visit our laboratory for reassessment, information was collected by phone, email or postal mail. Otherwise, basic information about asthma symptoms and medication was collected via their general practitioner(22).

Outcome parameters

The trigger of asthma onset was patient reported and defined by the question "What, in your opinion, elicited your asthma?". All answers were evaluated and divided into 10 different categories. Clinical, functional and inflammatory characteristics included 23 variables (Table I). Asthma remission was defined as: No asthma symptoms AND no medication for > 1 year at the reassessment after 5 years(22).

Statistical analysis

Statistical analysis was performed on trigger categories with at least 10 cases. Comparison of characteristics was done by using Kruskal-Wallis (normally

and nonnormally distributed data) and Fisher's exact test (nominal variables). Comparison of remission rates was analysed by using Fischer Exact test in patients in whom remision data were available. Statistical significance was set on P-value 0.05. Analysis was performed with SPSS version 24.0 (IBM SPSS, Chicago, Ill).

Table I. Baseline characteristics of included patients

n	194	
Gender, % female	56	
Race (caucasic)	84	
Age (years)	48	±15
BMI (kg/m^2)	27.8	±5.3
Never/ex/current-smoker, %	44/47/9	
Pack Years	3	(0-14)
ACQ6-score	1.32	±0.94
AQLQ-score	5.58	±0.94
ICS dose, fluticasone equivalent(µg)	250	(250-500)
Exacerbation (n in pervious year)	0	(0-1)
Total IgE	61	(27-207)
Atopy, %	46	
Nasal polyps, %	17	
Post-FEV$_1$ (% pred)	99.9	±17.2
Post-FEV$_1$/VC (% pred)	95.4	±10.8
RVTLC ratio (% pred)	87.4	±19.1
PC_{20} methacholine (mg/mL)*	2.18	±2.92
FeNO (ppb)	21	(13-42)
Blood eosinophils (×10^9/L)	0.16	(0.09-0.27)
Blood neutrophils (×10^9/L)	3.76	±1.41
Sputum eosinophils (%)¥	0.6	(0.1-4.0)
Sputum neutrophils (%)¥	71	(50.6-84.5)

Data are mean ± SD, median (interquartile range), or percentage. BMI, Body mass index; ACQ, asthma control questionnaire; AQLQ, asthma quality of life questionnaire; ICS, inhaled corticosteroids; post, post-bronchodilator; % pred, % predicted; FeNO, fraction of exhaled nitric oxide; ppb, parts/billion. *Geometric mean; ¥n=105.

Results

Two hundred patients were included at baseline of whom 6 did not provide information on the onset-trigger. In the remaining 194 patients, data on asthma remission was available in 165 patients.

The triggers of asthma onset were classified in 10 categories: , "upper respiratory tract symptoms" (rhinitis, common cold, coughing), "pneumonia" (physician

diagnosed), "occupational exposure", "smoking cessation", "aspirin use", "post-menopausal", "stressful life event", "new allergic sensitization", "no trigger identified" and "other". Percentage reported for all 10 categories are shown in Figure I.

The five most reported categories were "no trigger identified" (38%), "upper respiratory tract symptoms" (22%), "new allergic sensitization" (11%), "pneumonia" (8%) and "stressful life event" (7%). Characteristics of patients reporting the five most common triggers can be found in Table II. Characteristics of patients reporting the remaining reported triggers can be found in Table E I.

Group characteristics

Characteristics of patients in each trigger category are shown in Table II and Figure II. The "new allergic sensitization" category was associated with a relatively young age at onset, a high percentage of non-Caucasians and a high percentage of never-smokers. Over 90% of these patients were atopic, with elevated levels of total IgE and were treated with low dose of inhaled corticosteroids. Patients with "pneumonia" as inciting trigger were often ex-smokers with low IgE. Those with "upper respiratory symptoms" at start of their disease had relatively high exhaled nitric oxide (FeNO) and blood or sputum eosinophils, and low percentages of sputum neutrophils. The category in which "no trigger was identified", showed relatively low blood eosinophils and high sputum neutrophils. Patients reporting a "stressful life event" as their trigger of asthma onset had high symptom scores and high medication usage, but relatively low FeNO and blood eosinophils.

Outcome after 5 years

Baseline and follow-up characteristics of patients per trigger category can be found in Table III. A difference in asthma remission between the groups was seen (Fischer's exact p=0.046). In the "pneumonia" and "no trigger identified" groups, relatively high remission rates of 33% and 26% were observed, respectively. Low remission rates were observed in the other 3 groups, with 0% asthma remission in patients who reported a stressful life event as the disease trigger. See also figure II and II.

Table II. Baseline characteristics of patients reporting the five most common triggers

	No trigger identified		Upper respiratory tract symptoms		New sensitization		Pneumonia		Stressful life event		p-value
n	74		42		22		16		13		
Gender, % female	55		60		59		55		54		0.988
Race (caucasic)	88		83		50§		100		92		**<0.001**
Age (years)	50	±15	50	±15	38§	±13	48	±14	41†‡	±14	**0.003**
BMI (kg/m^2)	27.6	±5.4	27.3	±4.6	28.1	±6.8	27.7	±4.6	29.3	±6.3	0.976
Never/ex/current-smoker, %	44/47/9		38/57/5		77/14/9†‡‖		19/75/6		39/39/23		**0.007**
Pack Years	4	(0-18)	3	(0-10)	0§	(0-2)	8	(1-23)	5	(0-16)	**0.037**
ACQ6-score	1.23	±0.90	1.15	±0.92	1.28	±1.11	1.20	±1.00	1.74	±0.90	0.374
AQLQ-score	5.68	±0.83	5.79	±0.89	5.56	±1.14	5.65	±0.98	5.12	±1.15	0.412
ICS dose, fluticasone equivalent(μg)	250	(125-500)	500†	(250-500)	250‡‖	(0-500)	250	(31-500)	500	(250-875)	**0.032**
Exacerbation (n in pervious year)	0	(0-0)	0	(0-1)	0	(0-1)	0	(0-1)	1	(0-1)	0.157
Total IgE	48.5	(22-126)	96†〒	(49-283)	180†〒‖	(66-575)	23	(9-125)	38	(23-240)	**<0.001**
Atopy, %	42		49		91§		38		39		**0.001**
Nasal polyps, %	16		20		18		13		23		0.936
Post-FEV$_1$ (% pred)	101.4	±15.6	98.5	±16.7	96.5	±20.1	99.7	±17.7	106.3	±17.3	0.488
Post-FEV$_1$/VC (% pred)	96.9	±9.4	94.3	±9.9	98.3	±11.9	94.7	±11.6	94.9	±11.7	0.375
RVTLC ratio (% pred)	86,0	±15.7	87.6	±18.6	87.5	±18.0	93.1	±26.9	87.6	±23.2	0.373
PC$_{20}$ methacholine (mg/mL)*	3.61	±2.38	2.20	±3.12	1.64	±2.58	2.25	±1.66	1.24	±3.53	0.227
FeNO (ppb)	19	(13-33)	39†〒	(16-75)	21	(13-51)	17	(12-37)	13‡	(11-19)	**0.011**
Blood eosinophils (x10^9/L)	0.14	(0.07-0.24)	0.19†	(0.13-0.43)	0.19†	(0.14-0.30)	0.18	(0.13-0.30)	0.15	(0.07-0.24)	**0.022**
Blood neutrophils (x10^9/L)	3.75	±1.37	3.49	±1.37	3.49	±0.99	4.28	±1.27	4.56	±1.15	0.200
Sputum eosinophils (%)¥	0.6	(0.1-2.3)	1.5	(0.3-6.5)	0.5	(0.1-1.1)	1.2	(0.2-4.9)	1.1	(0.2-7.6)	0.425
Sputum neutrophils (%)¥	74.2	(59.8-85.9)	57.1	(43.4-82.5)	72.6	(50.7-97.1)	66.3	(43.4-79.9)	66.2	(36.9-85.3)	0.659
Remission (%)#	26		8†〒		11		33		0		**0.046**

Only groups with > 10 patients are shown. Data are mean ± SD, median (interquartile range), or percentage. Values in boldface are statistically significant. BMI, Body mass index; ACQ, asthma control questionnaire; AQLQ, asthma quality of life questionnaire; ICS, inhaled corticosteroids; post, post-bronchodilator; % pred, % predicted; FeNO, fraction of exhaled nitric oxide; ppb, parts/billion. *Geometric mean; ¥n=40/25/7/10/8, respectively. #available remission data
§p<0.05 to all other triggers, †p<0.05 vs no trigger identified, ‡p<0.05 vs upper respiratory tract symptoms, #p<0.05 vs new sensitization, 〒p<0.05 vs pneumonia, ‖p<0.05 vs stressful life event.

Table III. Baseline and follow-up characteristics of patients per trigger category∓.

	No trigger identified				Upper respiratory tract symptoms				New sensitization			
	Baseline		5 y		Baseline		5 y		Baseline		5 y	
BMI (kg/m^2)	27.5	±5.3	27.1	±4.5	27.2	±4.6	27.8	±4.9	29.1	±6.7	29.6	±7.1
ACQ-6 score	1.22	±0.9	1.03	±0.99	1.09	±0.94	0.79	±0.71	1.27	±1.16	0.69	±0.68
AQLQ score	5.72	±0.77	5.87	±0.89	5.92	±0.89	6.07	±0.81	5.55	±1.28	6.27†	±0.62
ICS dose, fluticasone equivalent(µg)	250	(125-500)	125	(0-500)	500	(250-500)	250†	(0-500)	250	(125-500)	250	(0-500)
Post-FEV_1 (% pred)	101.6	±14	102.5	±19.2	97.2	±17.3	96.7	±14.3	93.8	±22.1	95.8	±21.1
Post-FEV_1/VC (% pred)	96.6	±9.9	94.0†	±11.6	93.2	±10.7	89.1†	±11.7	94.3	±13.7	93.3	±10.7
RVTLC ratio (% pred)	88.2	±14.8	84.1	±14.7	86	±19.4	85.4	±13.7	91.9	±15.5	83.8†	±8.0
PC_{20} methacholine (mg/mL)*	4.04	±2.45	3.12	±3.4	2.49	±2.75	2.89	±3.58	2.61	±2.52	1.99	±2.41
FeNO (ppb)	22	(14-32)	18	(10-33)	51	(16-90)	29	(17-65)	20	(13-47)	25	(20-40)
Total IgE (kU/L)	42	(16-155)	38	(13-117)	138	(38-291)	134	(45-419)	198	(76-565)	172	(57-578)
Blood eosinophils (x10^9/L)	0.16	(0.07-0.25)	0.15	(0.10-0.25)	0.19	(0.14-0.39)	0.22	(0.13-0.36)	0.18	(0.14-0.29)	0.16	(0.11-0.27)
Blood neutrophils (x10^9/L)	3.66	±1.08	3.45	±1.16	3.67	±1.34	3.54	±0.96	3.55	±1.13	3.28	±0.9

Data are mean ± SD, median (interquartile range). BMI, Body mass index; ACQ, asthma control questionnaire; AQLQ, asthma quality of life questionnaire; ICS, inhaled corticosteroids; post, post-bronchodilator; % pred, % predicted; FeNO, fraction of exhaled nitric oxide; ppb, parts/billion. *Geometric mean. †Significant different from baseline (Wilcoxon signed rank test $p<0.05$)

∓Only patients with 5-year follow-up data are shown. Percentages of missing follow-up data ranged from 19% for ICS dose, 28% for ACQ and AQLQ, 43% for lung function, blood measurements and BMI, to 55% for PC_{20} methacholine.

Table III continued.

	Pneumonia				Stressful life event			
	Baseline		5 y		Baseline		5 y	
BMI (kg/m^2)	30.4	±5.1	30.5	±5.1	30.5	±6.5	30.7	±7.6
ACQ-6 score	1.00	±1.02	0.74	±0.67	1.56	±0.77	1.17	±0.77
AQLQ score	6.01	±0.80	6.24	±0.73	5.07	±0.87	5.64	±0.54
ICS dose, fluticasone equivalent(µg)	188	(0-313)	0	(0-313)	500	(250-875)	500	(63-891)
Post-FEV_1 (% pred)	97.4	±17.7	109.1†	±14.1	102.1	±16.7	101.9	±24.7
Post-FEV_1/VC (% pred)	96.3	±9.6	97	±8.7	91.9	±13.8	91.4	±14.3
RVTLC ratio (% pred)	85.7	±18.3	82.9	±10.0	76.6	±20.1	85	±23.5
PC_{20} methacholine (mg/mL)*	2.74	±2.18	8.74†	±2.06	0.58	±4.05	8.5	±2.15
FeNO (ppb)	29	(13-55)	23	(9-28)	13	(11-16)	12	(9-17)
Total IgE (kU/L)	20	(12-74)	27	(11-54)	59	(24-407)	94	(27-438)
Blood eosinophils (x10^9/L)	0.21	(0.13-0.31)	0.13	(0.07-0.27)	0.14	(0.08-0.25)	0.22	(0.17-0.28)
Blood neutrophils (x10^9/L)	4.06	±1.51	3.7	±1.71	4.65	±2.56	3.77	±1.2

Data are mean ± SD, median (interquartile range). BMI, Body mass index; ACQ, asthma control questionnaire; AQLQ, asthma quality of life questionnaire; ICS, inhaled corticosteroids; post, post-bronchodilator; % pred, % predicted; FeNO, fraction of exhaled nitric oxide; ppb, parts/billion. *Geometric mean.
†Only patients with 5-year follow-up data are shown. Percentages of missing follow-up data ranged from 19% for ICS dose, 28% for ACQ and AQLQ, 43% for lung function, blood measurements and BMI, to 55% for PC_{20} methacholine.

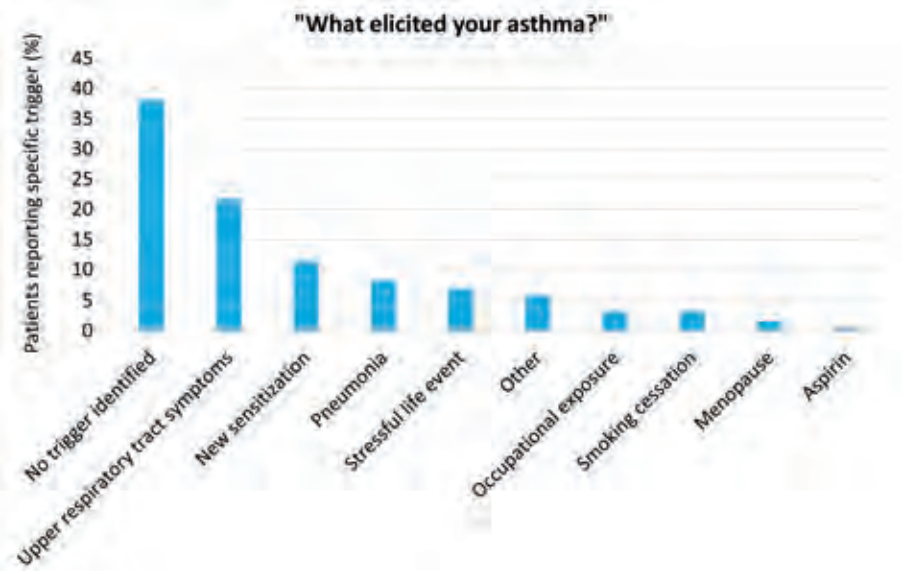

Figure I. Patients reporting triggers.

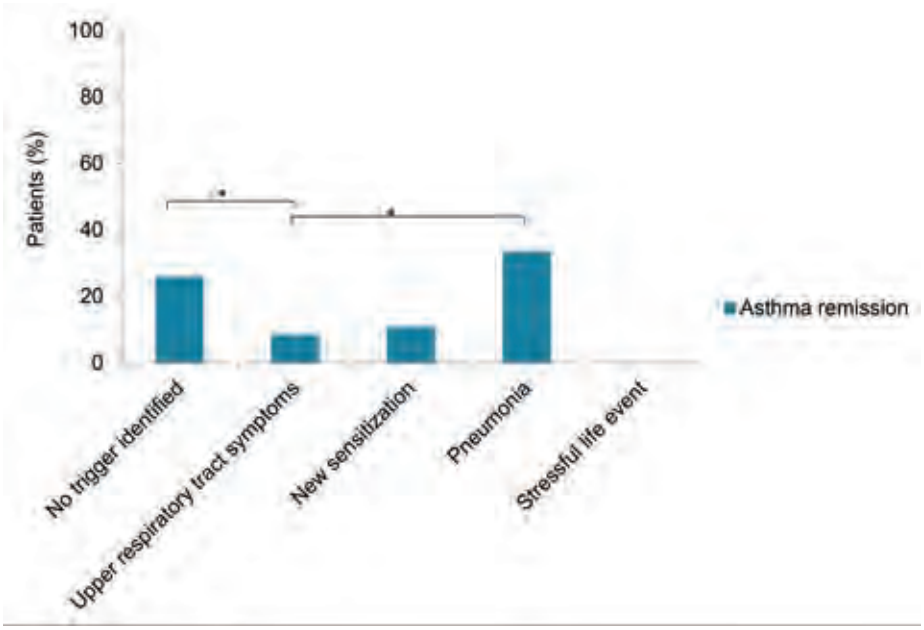

Figure II. Asthma remission rate among the reported triggers.

Overall significance p=0.046. Significant pairwise comparison ($p<0.05$) is indicated by *. Missing data varied between 31% and 11%, and were not different between the groups (p=0.259).

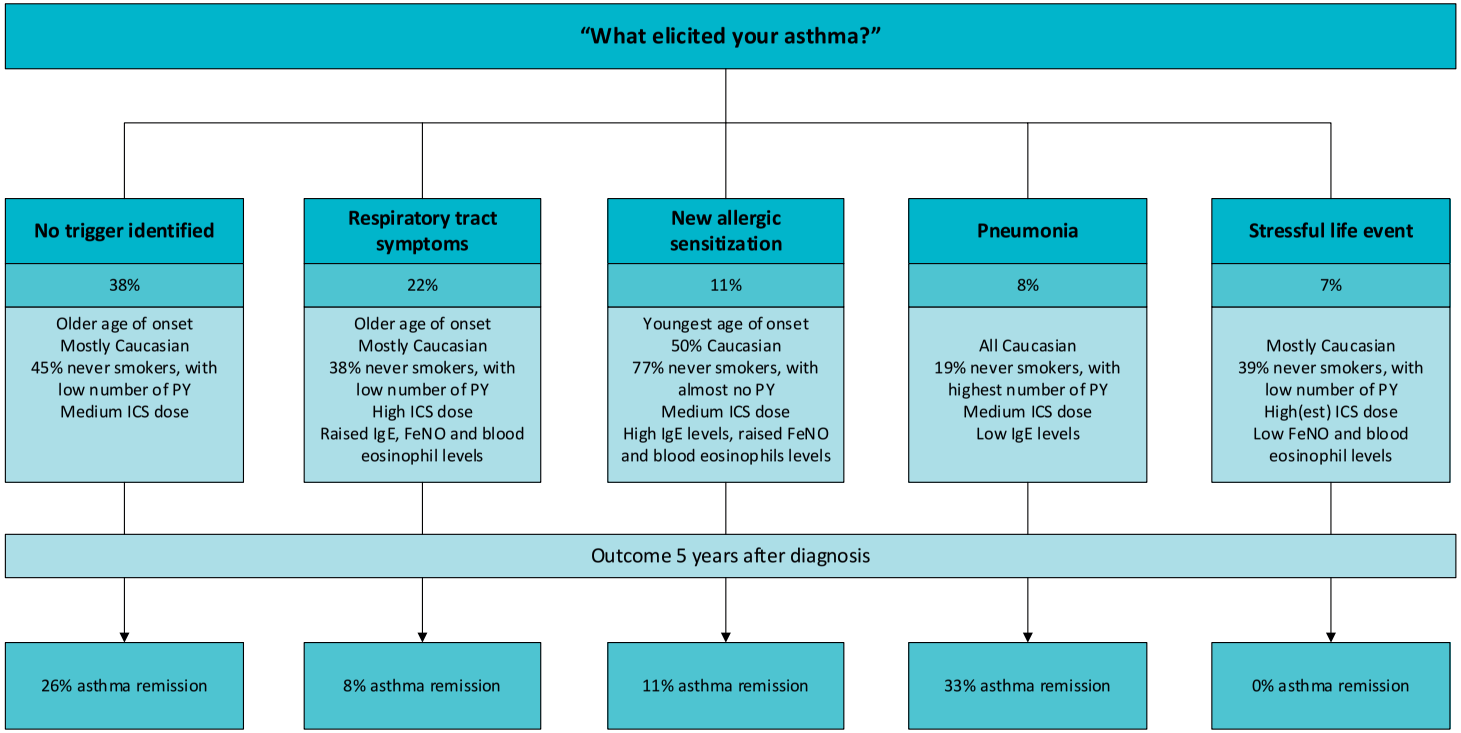

Figure III. Patients' profiles of the reported triggers. Only groups with > 10 patients are shown. PY; pack years smoked, ICS; inhaled corticosteroids, IgE; immunoglobulin E, FeNO; fraction of exhaled nitric oxid

Discussion

This study shows that clinical and inflammatory characteristics of adults with new-onset asthma differ according to the trigger that seemingly elicited the disease. Also, the triggers of onset were associated with different asthma remission rates, ranging from 33% in patients with onset after a pneumonia, to 0% in the patients with a stress-related event as asthma onset trigger. These results suggest that new-onset asthma in adults is heterogeneous from the start of the disease and that new-onset asthma in adults has a different outcome based on the onset trigger as reported by the patient.

The patients in our study reported triggers that have been previously described in the literature (7-9, 17, 18, 24, 25). We extended these findings by associating these triggers with specific phenotypic characteristics and asthma outcomes.

"New sensitization" as the inciter of adult-onset asthma was reported in 11% of our patients. Although adult-onset asthma is more often non-atopic, atopy can apparently not be disregarded in this asthma subtype (26, 27). Patients with onset after new sensitization had the youngest age of onset, which is in line with a previous cluster analysis in adult-onset asthma that found an atopic phenotype with a relatively young age of asthma onset(28). Also, a study in adults which showed an association between lower age and onset of atopic asthma, but not non-atopic asthma(27). "Upper respiratory tract symptoms" (22%) and "pneumonia" (8%) as onset trigger was less reported than known from literature. In one study upper and lower respiratory tract infection 3 months preceding asthma diagnosis were reported in 44.4% and 9.4%, respectively(18). These infections were not explicitly related to disease onset, which could explain the discrepancy with our study.

"Stressful life events" as the inciting trigger of asthma was reported in 7% of our patients. In the literature, this association has been reported for specific life events such as war, divorce or personal conflicts(17, 24), but also for perceived stress in general(25). Not only is stress associated with asthma onset, also a higher asthma morbidity has been reported(29-31), which is in line with the absence of remission which we found in these patients.

The category "no trigger identified" was the largest, and probably included various factors of which patients were unaware, such as obesity, hidden sensitizers, air pollution and smoking(27, 32-38).

The proportion of "work-related asthma" was only 3% in our cohort, which is lower than the 16% which has been previously reported(39), but can be explained by the low incidence of occupational asthma in the Netherlands(40).
What can be the explanation for the relation between a trigger and the clinical and inflammatory characteristics, and moreover, for the remission rates that were observed? A substantial part of our patients developed asthma related to a "new allergic sensitization". With the youngest age of onset, no smoking history, high levels of total IgE and relatively mild asthma, this group seems to correspond with the atopic asthma phenotype(28, 41). Although atopy is generally associated with early onset asthma, a first sensitization in adulthood can still occur and elicit new onset disease. Another relatively large proportion of our patients had "upper respiratory tract symptoms" before the onset of asthma. These patients had the oldest age of onset, were relatively often non-atopic with signs of type-2 inflammation. Upper respiratory symptoms such as cough and nasal blockade could reflect a viral infection. A viral infection could be a true inciter, as has been described for RSV in children (42) and has also been suggested for atypical respiratory infections in adults (9). Another plausible explanation could be that the upper airway symptoms reflect (undiagnosed) chronic rhinosinusitis and nasal polyposis. Rhinosinusitis has been suggested as a precursor of asthma(43) and has also been associated with blood and sputum eosinophilia in patients with adult-onset asthma(44, 45) which can explain the elevated type 2 markers in this group. Thus, elevated type 2 markers seem to distinguish this group from the other 4 groups, suggesting that in this group inflammation is predominant(41). Inflammation predominant asthma, also called refractory eosinophilic asthma is often severe and persistent which is in line with the low percentage of patients whose asthma remitted in this particular group. Patients with "pneumonia" before asthma onset had less clear signs of type 2 inflammation and a relatively large proportion of them showed asthma remission. These patients probably experienced transient wheeze caused by bronchial hyperresponsiveness and/or slowly resolving symptoms after a lower respiratory tract infection. Interestingly this group had the highest percentage of ex-smokers (77%), and the highest number of pack years smoked, but no lung function impairment. Pneumonia was less often reported as the inciting trigger in the non-smoking patients. These patients reported "new sensitization" more often (data not shown). This suggests that smoking or ex-smoking patients represent a separate group, and are prone to develop asthma like symptoms following pneumonia. The largest group in our study contained

patients who did not identify a specific trigger for their disease onset, or did not recognise a hidden causative trigger. For example, smoking is a well-known contributing factor of adult-onset asthma (27, 38), but patients in our study only did not recognise current or ex-smoking as a trigger, and only reported smoking cessation, which is a momentary event. The "no trigger identified" category did not show any specific characteristics and therefore the precise mechanism is difficult to detect. Remarkably the remission rate was relatively high, which is not in line with a hidden trigger as the main contributing factor.

Perhaps the most intriguing group are the patients with a stressful life event as the inciter of their asthma. This group did not display an apparent inflammation pattern, suggestive for paucigranulocytic asthma. In addition, this group had relatively high symptom scores, was prescribed high dose of inhaled corticosteroids and did not report any disease remission. The relation between stress and asthma is complex(46). Possibly factors such as glucocorticoid insensitivity, the level of perceived stress or over-perception of dyspnoea could have contributed to the onset and persistence of asthma in this group(29, 47, 48).

The results of our study should be interpreted with caution. Firstly, several potentially important triggers such aspirin sensitivity, hormonal influences and occupational exposures could not be analysed properly because of small group sizes. Secondly, triggers in our study were reported by the patients and not objectively confirmed, which may have underestimated the role of hidden trigger factors. However, our study was not designed to detect all possible contributing factors of adult-onset asthma, but rather to help the practicing clinician to estimate the course of patient's asthma. The strength of our study was the 5 year follow-up period of a well-characterized cohort of adults with recently diagnosed asthma.

Our results has several implications. First, information with regards to the trigger of asthma onset is an important item in the medical history of adults with suspected new-onset asthma as it might provide information on disease prognosis. More importantly, the presence, but also the absence of a trigger might hint towards underlying mechanisms of asthma phenotypes. Second, it is important to realize that adult-onset asthma may have many different inciting triggers, associated with different expression of disease and prognosis. For example, it might be worthwhile to investigate whether removing or reducing the stressor might result in different outcome for these patients. Thirdly, a diagnosis new onset asthma following a pneumonia should be made with caution, as the asthma-like symptoms might still reflect a period of recovery, instead of true asthma. Lastly, it would be interesting

to investigate whether the different onset triggers can be associated with previously identified asthma phenotypes(28, 41, 49-52), as these triggers might be of value in guiding personalized treatment(53-55).

In conclusion, this study shows that patients with adult-onset asthma can be characterized based on the trigger that seemingly incited their asthma. Different triggers are likely to be a reflection of different underlying asthma mechanisms and are therefore important for the diagnosis and treatment of adults with new-onset asthma and for predicting disease outcome.

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Supplementary Material

Table EI. Baseline characteristics of patients reporting the remaining triggers

	Occupational exposure		Smoking cessation		Post-menopausal		Aspirin use	Other	
n	6		6		3		1	11	
Gender, % female	50		67		100		0	64	
Race (caucasic)	100		100		67		100	82	
Age (years)	53	13	47	8	60	3	35	49	15
BMI (kg/m^2)	26.6	3.2	28.9	4.9	32.7	0.85	25.2	28.8	5.4
Never/ex/current-smoker, %	50/33/17		0/100/0		100/0/0		0/100/0	55/36/9	
Pack Years	1	(0-23)	26	(19-33)	0	(0-0)	4	0	(0-9)
ACQ6-score	1.92	0.74	1.53	1.02	1.78	0.63	1.17	1.73	0.92
AQLQ-score	4.79	1.12	5.55	0.89	5.33	0.64	5.81	5.18	0.92
ICS dose, fluticasone equivalent(µg)	250	(250-500)	375	(188-563)	500	(0-..)	500	500	(250-1000)
Exacerbation (n in pervious year)	0	(0-1)	0	(0-0)	0	(0-..)	3	1	(0-1)
Total IgE	45	(6-66)	30	(15-43)	33	(31-..)	88	51	(19-192)
Atopy, %	17		33		0		0	36	
Nasal polyps, %	0		17		33		100	9	
Post-FEV_1 (% pred)	104.2	5.7	99.5	15.6	107.0	29.6	99	92	23
Post-FEV_1/VC (% pred)	100.3	5.3	87.5	8.7	96.0	8.7	88,0	91.5	14.7
RVTLC ratio (% pred)	86.3	23.2	87.0	18.4	92.7	18.5	84.0	99.4	25.3
PC_{20} methacholine (mg/mL)*	1.88	3.94	0.28	4.45	2.61	2.44	0.01	1.04	3.94
FeNO (ppb)	23,00	(13-42)	16,00	(13-17)	43,00	(19-..)	20,00	28,00	(16-51)
Blood eosinophils (x10^9/L)	0.11	(0.04-0.13)	0.11	(0.08-0.14)	0.39	(0.20-..)	0.11	0.18	(0.09-0.25)
Blood neutrophils (x10^9/L)	4.33	2.2	4.23	1.32	3.6	0.74	3.2	3.33	1.08
Sputum eosinophils (%)¥	0.1	(0.1-0.3)	0.1	(0.1-..)	3.3	(0.4-..)	0.1	0.2	(0.1-1.9)
Sputum neutrophils (%)¥	60.4	(55.5-84.6)	74.3	(70.5-..)	67.5	(57.3-..)	80.5	73.8	(71.0-87.8)

Data are mean ± SD, median (interquartile range), or percentage. Values in boldface are statistically significant. BMI, Body mass index; ACQ, asthma control questionnaire; AQLQ, asthma quality of life questionnaire; ICS, inhaled corticosteroids; post, post-bronchodilator; % pred, % predicted; FeNO, fraction of exhaled nitric oxide; ppb, parts/billion. *Geometric mean; ¥n=4/2/3/1/5, respectively

Table E2. Racial composition of the ADONIS-cohort.

Race	n	%
Caucasian	170	85
Negroid	23	12
Mongoloid	7	4

Chapter 9

Summary and General discussion

Summary

Chapter 1. In this chapter we have introduced the topic of this thesis: biomarkers in adult-onset asthma. Asthma is a common disease in both children and adults and the severity of the disease can range from mild to severe. In order to get more insight into the complexity of (severe) asthma and for the purpose of better treatment, phenotypes have been identified, which are based on clinical characteristic. Several studies have shown that the presence of eosinophilic inflammation and the age of asthma onset are important phenotypical characteristics.
Eosinophilic inflammation is associated with more severe disease. The presence of this type of inflammation (Type 2 inflammation) is often diagnosed with use of surrogate markers like blood eosinophils and/or fraction of exhaled nitric oxide (FeNO). However, current cut-offs are not optimal and often based on one single measurement. Therefore, in this thesis we focus on improving the diagnosis of eosinophilic inflammation.
Disease development in adulthood (so-called 'adult-onset asthma') is associated with several patient characteristics like absence of atopy and presence of nasal polyps, but also obesity. Adult-onset asthma is an important subtype as 50% of the disease is diagnosed in adults. Moreover, this subtype may be more severe than childhood-onset asthma. Yet prospective data is limited and knowledge on predictors for poor outcome is missing. To that end, this thesis deals with prognostic markers of adult-onset asthma.

Chapter 2 is a review focusing on the current methods to diagnose eosinophilic asthma. The diagnosis of eosinophilic inflammation is preferably based on sputum eosinophil levels. But execution of a sputum cell count is time consuming and requires specific technical expertise. For that reason blood eosinophils, FeNO, serum IgE and periostin are used as surrogate markers to detect airway eosinophilia. However, the diagnostic accuracy of these markers is not optimal and there is a need for other approaches. These alternate approaches include the use of high/low cut-off values of a surrogate marker in order to rule-in/out eosinophilic inflammation, or to combine known biomarkers to increase accuracy. On the other hand, novel 'omics' technology is also likely to provide new biomarkers for this purpose. In this review paper we conclude that for the upcoming years diagnosing eosinophilic *inflammation*, and not eosinophilic *asthma*, will be of importance. This fits in with personalized anti-eosinophilic treatment and the "treatable traits" approach.

In **chapter 3** we evaluate whether a single measurement of blood eosinophils or FeNO is sufficient to diagnose persistent blood eosinophilia. We found that based on repeated blood eosinophil measurements patients could either be classified as having "persistent blood eosinophilia", "fluctuating blood eosinophil levels" or "absent blood eosinophilia". A single blood eosinophil measurement with high and low cut-off values showed good accuracy to diagnose persistent eosinophilia or absent eosinophilia. FeNO measurements showed less accuracy. These results may be of help for clinical application of personalized treatments and implementation of the "treatable traits" approach.

Chapter 4 contains correspondence addressing the importance of age of disease onset in obese asthma patients when assessing the predictive ability of surrogate biomarkers to identify eosinophilic inflammation. The authors reported that they had found poor predictability of these surrogate biomarkers in a mixed group of obese asthma patients. This was in contrast with results previously published by our group and we replied that the ability of blood eosinophils and FeNO to predict eosinophilic inflammation is valid in adult-onset obese asthma, but possibly not in childhood-onset asthma.

Chapter 5 is a review of prognostic factors in adult-onset asthma associated with accelerated lung function decline, disease severity and asthma remission. There are only a few studies investigating the prognosis of adult-onset asthma and these studies are mostly performed in cross-sectional cohorts. With regard to the development of persistently impaired lung function, this is associated with absence of atopy and occurs more often in males. Smoking and poor lung function are both associated with accelerated decline in lung function and disease severity. Smoking is also a predictor of uncontrolled disease, whereas poor lung function is also a predictor of asthma exacerbations. Other predictors of asthma exacerbations include high levels of symptoms and eosinophilic airway inflammation. Lastly, asthma remission rates in adult-onset asthma are low. Patients showing asthma remission often have mild asthma and a short disease duration. Smoking, on the other hand, lowers the chance of remission. Thus, smoking and lung function seem to be important determinants for the prognosis of adult-onset asthma. However, the number of prospective longitudinal studies in adult-onset asthma is still limited, and more studies are needed to identify prognostic predictors in order to get more insight in the course of this important subtype.

In **chapter 6** we examined predictors of accelerated lung function decline in adult-onset asthma. Clinical, functional and inflammatory characteristics were assessed in 141 adult patients with recently diagnosed asthma. The results showed an overall decline in FEV_1 within normal limits (17,5 ml/year), but a significant association between a rapid decline in FEV_1 and high FeNO levels or low body weight. There was no association between the rate of decline in FEV_1 and severity of asthma symptoms, number of exacerbations or degree of airway hyperresponsiveness. These results suggested that high FeNO levels, even in patients with controlled symptoms, reflect subclinical, ongoing airway inflammation, eventually resulting in chronic lung function impairment.

Chapter 7 describes a study aiming at identifying predictors of remission and persistence of adult-onset asthma. Data on disease remission was available in 170 patients. Asthma remission was defined as no asthma medication and no symptoms for > 1 year. Five years after the initial diagnosis 27 patients (15.9%) were in clinical remission. Independent predictors of asthma persistence included the presence of nasal polyps and moderately severe airway hyperresponsiveness. If both characteristics were present in a patient, the chance of disease remission was close to 0%.

In the study described in **chapter 8** we hypothesized that the triggers which seemingly elicited asthma onset in adulthood were associated with different patient characteristics and different asthma outcomes. We compared patient characteristics between different "trigger groups" that contained 10 or more patients. Five trigger groups were identified, including "upper respiratory tract symptoms", "new (allergic or non-allergic) sensitization", "pneumonia", "stressful life event" and "no trigger identified". Patients with "upper respiratory tract symptoms" as onset trigger had high FeNO and high blood eosinophil levels, patients in the "new sensitization" group were mildly atopic and had a young age of disease onset, the "pneumonia" group contained patients with a smoking history and low IgE levels, patients in the "stressful life event" group had low inflammatory markers but used high dose of inhaled corticosteroids, and the group with "no trigger identified" did not show any specific characteristics. High remission rates were observed in the "pneumonia" and "no trigger identified" groups. No remission was seen in the group of patients with "stressful life event". The results of this study imply that the trigger of new-onset asthma in adults may be an important item in the history of the patient.

Discussion

The use of biomarkers and clinical markers in asthma is becoming more and more important in order to practise medicine tailored to an individual patient. In this thesis markers diagnosing eosinophilic asthma and markers for predicting the prognosis of adult-onset asthma are discussed.

In this thesis we showed that one single measurement of eosinophils in peripheral blood is sufficient to predict persistently elevated blood eosinophils as well as persistently low blood eosinophil levels, provided that high ($0.47x10^9$/L) or low ($0.17x10^9$/L) cut-off values are being used, respectively. This appeared not to be the case with single FeNO measurements. We chose to use two cut-off values for blood eosinophils (high and low) to increase our overall accuracy, rather than one cut-off value with a suboptimal accuracy, as has been suggested by Korevaar and colleagues[1]. Other studies that used a comparable approach to define cut-off values for blood eosinophils or FeNO to diagnose eosinophilia in sputum reported similar values[2, 3]. Whilst the correlation between blood and sputum eosinophil levels has been fairly well established, studies reporting the relationship between blood eosinophils and FeNO are less consistent[4, 5]. Our finding that one single FeNO measurement is not sufficient to diagnose persistent blood eosinophilia is in line with this inconsistency.

We also found several factors that predict the prognosis of adult-onset asthma. We showed that high FeNO levels and low BMI shortly after diagnosis predict accelerated lung function decline over the next five years. We also showed that the presence of nasal polyps and bronchial hyperresponiveness (BHR) predict new onset asthma to become chronic. In addition, we showed that asthma that started following upper airway disease was associated with signs of active type 2 inflammation and low remission rates, whereas other triggers of asthma onset (e.g. pneumonia) were associated with little eosinophilic inflammation and high(er) remission rates. These results are in agreement with earlier studies in childhood- or mixed-onset asthma populations, showing blood or sputum eosinophils to be predictors of poor asthma prognosis. In these studies high sputum eosinophil counts were associated with persistent airflow limitation[6], disease severity[7, 8] or exacerbation risk[9]. Other studies reported high FeNO levels to predict an increased exacerbation risk[10-13]. Likewise, the presence of nasal polyps, often regarded as a typical clinical characteristic of the eosinophilic asthma phenotype[14], has been associated with more severe disease[8, 15]. The predictors

that were identified in this thesis for the prognosis of adult-onset asthma are thereby confirming that markers of active type 2 inflammation are important predictors of poor prognosis, not only in classical childhood-onset asthma, but also in adult-onset asthma. With regard to BHR; this marker has been described previously as a predictor of accelerated lung function decline and asthma persistence[16-18], but in our studies in adult-onset asthma we could only confirm the latter.

Interpretation of the results

Our finding that one single measurement of FeNO isn't accurate enough to identify persistent blood eosinophilia could be explained by the fact that FeNO levels lag behind changes in sputum and blood eosinophils[19]. However, a more plausible explanation is that blood eosinophils and FeNO reflect different pathways of type 2 inflammation[20]. The uncoupling between these two markers has been clearly shown in trials with anti-interleukin (IL-)5, in which patients receiving the active drug showed a marked decrease in blood eosinophils but no change in FeNO levels[21]. Also, in trials blocking IL-13 or the IL-4R-alpha, FeNO levels showed to be significantly reduced, but no effect on blood eosinophils levels was observed[22, 23]. Additionally, it is important to recognize that exhaled NO is the result of complex processes, involving epithelial and inflammatory cells. Exhaled NO is thought to be synthesized from inducible NO synthases (iNOS), which is produced by epithelial cells[24, 25]. iNOS is not only induced by IL-4 and IL-13, but also by interferon gamma, IL-1-beta and tumour necrosis factor alfa[26]; all cytokines that are released from the epithelium upon stimulation. Thus, high FeNO levels might not only be a marker of type 2 activity leading to eosinophilic airway inflammation but also be a broader marker of dysfunctional epithelium[24].
In cross-sectional studies, high levels of FeNO, blood and sputum eosinophils, and the presence of nasal polyps have all been associated with accelerated lung function decline, but of these markers we found in this thesis that only a high FeNO level was an independent predictor of lung function decline[27]. Our explanation for this finding is that high FeNO levels reflect ongoing inflammation and airway remodelling. However, we cannot fully exclude that also high sputum eosinophils can predict an accelerated lung function decline, because in our study not all patients could produce adequate sputum samples, which led to a high number of missing values, and thereby insufficient statistical power. Blood

eosinophils do not seem to be strong predictors of accelerated decline in lung function, since the association between blood eosinophils and lung function decline was much weaker than the association between FeNO and lung function decline. Both markers reflect active type 2 inflammation, but FeNO levels are thought to reflect IL4/IL-13 activity, whereas blood eosinophils levels primarily reflect IL-5 activity[20]. Apart from inducing eosinophilic airway inflammation, IL-4 and IL-13 are thought to play a role in airway remodelling as well, through their (indirect) stimulatory effect on goblet cells, fibroblasts and airway smooth muscle cells[28, 29]. The role of IL-4 and IL-13 in airway remodelling is also supported by the results of recent trials with novel biological therapies in asthma. For example, therapies targeted at IL-4/IL-13 and IL-13 show significant increases in FEV_1, even when tapering oral corticosteroid doses[30, 31]. This is in contrast with treatment with anti-IL-5 monoclonal antibodies which show little to no effect on lung function[21, 32, 33]. In a recently published phase 3 trial with dupilumab (anti-IL4/IL-13) in patients with uncontrolled moderate-severe disease outcome parameters were stratified based on FeNO levels (<25ppb, 25-50ppb, >50ppb). Remarkably, the beneficial effect on FEV_1 increased per FeNO category[30]. Similar improvement in FEV_1 was obtained with an anti-IL-13 'only' monoclonal antibody, whereas this blockade had little effect asthma exacerbation rate[34, 35]. This suggests that IL-13 is an important cytokine regulating airway smooth muscle tone in asthma.

Low BMI was an unexpected predictor for accelerated lung function decline for which we proposed several underlying mechanisms (chapter 6). A high BMI (BMI > 30kg/m^2), on the other hand, tended to be associated with a more chronic disease course (chapter 7)[36]. Although both outcomes are associated with body weight, it is unlikely that they have shared underlying causal mechanisms. It rather emphasizes that obese and non-obese asthma patients represent different phenotypes both with regard to the underlying mechanisms, the type of inflammation and the prognosis[27]. In obese asthma patients airway inflammation is often neutrophilic[37] and BHR is more severe, although the latter finding is not consistent[38]. Yet, this might (partially) explain our finding of BHR as a marker for disease persistence.

Another important clinical predictor of asthma persistence was the presence of nasal polyps at disease diagnosis. Presence of nasal polyps was not only a predictor of a more chronic disease course, but also appeared to be associated with accelerated decline in lung function in univariate analysis. The inflammatory

process in nasal polyposis is not fully understood but several mechanisms may be involved including impairment of the epithelial barrier function in the nose[39, 40]. This impairment may result in a reduction of the physical barrier and host defence which leads to an increased exposure to inhaled pathogens and allergens[40]. Besides impaired epithelial barrier function, a dysregulation of the immune system with a shift towards more type 2 inflammation is thought to be involved[39]. Similar to Type 2 asthma, cytokines like IL-5, IL-13 and thymic stromal lymphopoietin (TSLP) are elevated, and eosinophils, ILC-2 and mast cells are part of the inflammatory process[40-43]. Altered inflammatory response in combination with increased exposure to allergens or pathogens may eventually lead to chronic inflammation in the upper airways[40]. The presence of upper airway symptoms at asthma onset may indicate that the eosinophilic airway inflammation initially started in the upper airways. This so called "united airways disease" concept is not new[44-46] and chronic rhinosinusitis with nasal polyposis has been long recognized as an important comorbidity in (adult) asthma patients[47, 48]. The combination of asthma and nasal polyposis might reflect a generalized eosinophilic inflammation in the whole airway mucosa of a patient. Apparently, this widespread inflammation makes it nearly impossible to achieve asthma remission, and might ultimately increase the risk of accelerated lung function decline.

Apart from the predictors discussed above, patient-reported triggers of asthma onset might also give important clues related to the pathogenesis and prognosis of adult-onset asthma. We observed distinctive characteristics in some patient groups reporting similar triggers of disease onset, and these characteristics fitted with well-defined asthma phenotypes. Surprisingly, patient- reported mental stress, seemed to influence the prognosis of asthma as well.

Implications and future directions

By using our high and low cut-offs values to predict or exclude persistent blood eosinophilia, patients with adult-onset asthma can be classified into the eosinophilic (Type 2) or non-eosinophilic (non-Type 2) phenotype. Blood eosinophils levels have already proved to be useful markers to select patients for anti-IL-5 treatments[30, 49, 50]. By using our cut-offs values asthma patients

may be diagnosed with persistent eosinophilic inflammation at an early stage and treated accordingly. Early treatment with anti-eosinophilic biologicals might even improve outcomes, but this has yet to be confirmed. Nonetheless, a substantial proportion of the patients in our study could not be classified by these cut-off values. For this "intermediate" eosinophilic phenotype there is still a need for new biomarkers. As described in chapter 2, new biomarkers might be found more upstream in the inflammatory cascade reflecting dysregulated epithelial response such as TSLP or IL-33 [20], or more downstream reflecting a specific part of a pathway such as dipeptidylpeptidase (DPP)-4 as a marker for IL-13 activity[51]. Alternatively, new biomarkers might be identified in genetic material, proteins in peripheral blood, sputum, or urine, or volatile organic compounds in exhaled breath. Until now, "omics" techniques, like genomics, proteomics and breathomics haven't produced new biomarkers for clinical practise[52]. However, current research looks promising and these new "omics" techniques are likely to provide new ways to classify asthma patients. For example, with the use of "breathomics" patients can already be distinguished by distinctive inflammatory characteristics[53]. Thus, the analysis of exhaled breath might become a helpful tool in phenotyping patients with airways disease and provide us with an easy-to-measure biomarker to improve personalized treatment.

The findings in this thesis are relevant for the whole adult-onset asthma population, since our cohort contained patients with mild, moderate and severe disease. Improving outcome, and eventually preventing accelerated lung function decline in these asthma patients is an important treatment goal. We found that high levels of FeNO, but not severe asthma symptoms were associated with accelerated lung function decline. How then convince patients, particularly with mild or moderate disease to take lifelong treatment if they don't experience symptoms all the time[54]? Another dilemma lies in the choice of treatment to be given to patients with adult-onset asthma. For example, anti-IL-5 treatment has shown to substantially reduce exacerbation rates and improve quality of life, but not to affect FeNO levels. Will this treatment then able to prevent accelerated decline in FEV_1 and improve asthma prognosis[54]? Can we be sure that asthma treatment is adequate (enough) when FeNO levels continue to be high or are we paying too little attention to other important processes which lead to decline in lung function and asthma persistence? We tend to consider high FeNO-levels, even without any other signs of active inflammation, a sign of ongoing inflammation and

airway remodelling. Future studies investigating FeNO-guided management with available biologics should be performed to investigate whether this may change the outcome with regard to lung function loss, but the first results with stratified anti IL-4/IL-13 response look promising[30].

Apart from high FeNO levels, the severity of BHR may also need more attention with regard to asthma prognosis. BHR is an important prognostic factor but is not affected by the current available biological treatments[55-57]. Is it time to expand our focus from the eosinophil towards the airway smooth muscle cell or the mast cell to further improve prognosis? The number of mast cells in airway smooth muscles cells has been associated with the severity of BHR[58]. However, the reduction of airway smooth muscle mass by bronchial thermoplasty doesn't seem to affect BHR[59]. On the other hand, the first trial with imatinib, an investigational drug targeting mast cells, showed promising results by reducing mast cells counts and BHR in asthma patients[60]. It would be interesting to see whether amelioration of BHR with this treatment results in higher asthma remission rates.

Increasing remission rates by treating nasal polyps is not likely to be successful, as a large proportion of our patients were already treated for this comorbidity and still had persistent asthma[36]. Nonetheless, it is important to assess the upper airways, as chronic rhinosinusitis with nasal polyposis is an important predictor of the prognosis of asthma. Moreover, treating the upper airways can improve the control of asthma symptoms[61, 62]. New biologicals, in particular anti IL-4/Il-13 treatment for nasal polyposis look promising[63, 64] and this treatment has also been shown to reduce FeNO levels. Whether this novel treatment will also improve asthma remission rates remains questionable, because its effect isn't likely to persist after cessation[65]. However, it could theoretically prevent onset of asthma in patients treated for nasal polyposis.

Finally, active eosinophilic airway inflammation and thereby elevated levels of FeNO, and blood and sputum eosinophils, are important diagnostic and prognostic biomarkers in adult-onset asthma. However they are not the only markers that can be used for phenotyping and choosing the right treatment for asthma patients. We showed that typical clinical characteristics like nasal polyps, body weight as well as the initial inciting trigger of asthma onset may also provide important information for daily practise. These clinical characteristics may help to apply personalised

medicine in patients with "inconclusive" inflammation, or reveal other traits to be treated in order to improve the prognosis of asthma[66, 67].

In summary, in this thesis we investigated diagnostic and prognostic markers in adult-onset asthma. We were able to show that one single blood eosinophil measurement is adequate to diagnose persistent blood eosinophilia. Besides this, we found that several type 2 inflammatory markers had prognostic value, and could predict lung function decline and asthma persistence. These markers provide new insight in asthma pathogenesis, have implication for daily practise, but also raise new research questions.

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Chapter 10

Nederlandse samenvatting

Nederlandse samenvatting

Astma is wereldwijd een veel voorkomende ziekte en treft mensen in alle leeftijdsgroepen. Vaak begint astma tijdens de kindertijd en kenmerkende symptomen voor astma zijn piepende ademhaling, kortademigheid en benauwdheid. Om meer inzicht te krijgen in de complexiteit van (ernstig) astma en om de behandelmogelijkheden te verbeteren zijn er fenotypen gedefinieerd. Verschillende onderzoeken hebben aangetoond dat de aanwezigheid van eosinofiele ontsteking en de leeftijd waarop astma begint belangrijke fenotypische kenmerken zijn.
Eosinofiele ontsteking in de luchtwegen wordt vaak gezien bij astma en is geassocieerd met een slechtere prognose. De aanwezigheid van dit type ontsteking wordt nu gediagnostiseerd met behulp van bloed eosinofielen en uitgeademde stikstofmonoxide (FeNO). Echter, de nauwkeurigheid van deze biomarkers om eosinofiele ontsteking te diagnosticeren is niet optimaal en vaak gebaseerd op één enkele meting. Daarom richten we ons in dit proefschrift op het verbeteren van de diagnose van eosinofiele ontsteking in astma patiënten.
De ontwikkeling van de ziekte op volwassen leeftijd (de zogenaamde 'laat astma') wordt geassocieerd met verschillende kenmerken van de patiënt, zoals afwezigheid van atopie en de aanwezigheid van neuspoliepen, maar ook obesitas. Tegenwoordig wordt bijna 50% van de astma diagnoses gesteld bij volwassenen en kan dit subtype ernstiger verlopen dan astma bij kinderen. Echter, de meeste gegevens astma dat ontstaat bij volwassenen komen uit cross-sectioneel onderzoek en kennis over voorspellers voor slechte(re) prognose ontbreekt. Daartoe houdt dit proefschrift zich bezig met prognostische markers van astma ontstaan bij volwassenen.

Hoofdstuk 2 is een review dat zich richt op de huidige technieken en manieren om eosinofiel astma te diagnosticeren. De diagnose van eosinofiel astma is bij voorkeur gebaseerd op een verhoogde aantal eosinofielen in sputum. Omdat het analyseren van sputum tijdrovend en expertise vereist, wordt er gebruik gemaakt van surrogaatmarkers zoals bloed eosinofielen, FeNO, serum-IgE en periostine. Er blijft echter behoefte aan andere methoden om eosinofiel astma te diagnosticeren. Een van deze methodes kan het gebruik van hoge / lage afkapwaarden zijn om eosinofiele ontsteking vast te stellen, dan wel uit te sluiten. Een andere optie is om bekende biomarkers te combineren en op die manier de nauwkeurigheid te vergroten. Aan de andere kant is het de verwachting dat

onderzoek naar nieuwe 'omics'-technologie waarschijnlijk ook nieuwe biomarkers voor eosinofiele ontsteking zullen opleveren. In dit review concluderen we dat voor dat in de komende jaren het diagnosticeren van eosinofiele ontsteking, en niet de diagnose van eosinofiel astma, van belang zal zijn. Dit in met het oog op de nieuwe, gepersonaliseerde anti-eosinofiele behandelingen en de "treatable traits" benadering.

In **hoofdstuk 3** wordt onderzocht of één enkele meting van bloed eosinofielen of FeNO gebruikt kan worden om persistente eosinofilie in het bloed te diagnosticeren. In dit onderzoek werden patiënten met meerdere bloed eosinofiel metingen geclassificeerd als zijnde "persistente eosinofilie", "fluctuerende bloedwaarden van eosinofielen" of "persisterende afwezigheid van eosinofilie". Een hoge en lage afkapwaarden van een bloed eosinofielen-meting had goede diagnostische nauwkeurigheid om persistente eosinofilie of afwezige eosinofilie te kunnen diagnosticeren. Een FeNO-meting vertoonde minder goede nauwkeurigheid. Deze afkapwaarden voor bloed eosinofielen kunnen van gebruikt bij de klinische toepassing van gepersonaliseerde behandelingen.

Hoofdstuk 4 bevat correspondentie over het belang van de leeftijd waarop astma ontstaat bij obese astmapatienten, bij het beoordelen van de surrogaatmarkers voor eosinofielen ontsteking. De auteurs rapporteerden dat ze een slechte voorspelbaarheid van deze surrogaat biomarkers hadden gevonden in een gemengde groep van obese astmapatiënten. Dit was in tegenstelling tot de resultaten van onze onderzoeksgroep en in onze correspondentie kaartten we aan dat het vermogen van eosinofielen en FeNO om eosinofiele ontsteking te voorspellen geldig is bij astma ontstaan bij volwassen met obesitas, maar mogelijk niet bij kinderen.

Hoofdstuk 5 is een review van de prognostische factoren bij 'laat astma'. Deze prognostische factoren hebben betrekking op de verslechtering van longfunctie, verergering van astma en astma remissie. Er zijn weinig studies die de prognose van dit subtype onderzoeken. Een slechtere longfunctie is geassocieerd met afwezigheid van atopie en komt vaker voor bij mannen. Roken en een slechte longfunctie aan het begin zijn beide geassocieerd met versnelde achteruitgang van de longfunctie en ernst van astma. Roken is ook een voorspeller voor slecht gecontroleerd astma en symptomen in de toekomst. Daarnaast is een lage

longfunctie ook een voorspeller voor het krijgen van astma exacerbaties. Andere voorspellers voor astma exacerbaties zijn veel astmasymptomen en eosinofiele ontsteking in de longen. Ten slotte zijn de remissies van astma bij astma bij volwassenen laag. Patiënten die astma-remissie vertonen, hebben vaak mild astma en een korte ziekteduur. Roken daarentegen verlaagt de kans op remissie. Roken en longfunctie lijken dus belangrijke factoren te zijn voor de prognose bij 'laat astma'. Niettemin is het aantal longitudinale onderzoeken in patiënten met 'laat astma' beperkt en zijn er studies nodig om voorspellers te identificeren om meer inzicht te krijgen in dit subtype.

In **hoofdstuk 6** zijn voorspellers voor versnelde longfunctiedaling onderzocht. Klinische, functionele en inflammatoire karakteristieken werden beoordeeld bij 141 volwassen patiënten met recent gediagnosticeerd astma. We observeerden een gemiddelde afname in geforceerd expiratie volume in 1 seconde (FEV_1) binnen de normale grenzen (-17,5 ml/jaar), en een significante associatie tussen een snelle afname van FEV_1 en hoge FeNO-waardes of een laag lichaamsgewicht. Er was geen verband tussen de mate van daling van FEV_1 en de ernst van de astmasymptomen, het aantal exacerbaties of de mate van hyperreactiviteit van de luchtweg. Deze resultaten suggereren dat hoge FeNO-waardes een aanhoudende luchtwegontsteking weerspiegelt die kan leiden tot chronische longfunctiestoornis, zelfs bij patiënten waarbij de astma onder controle lijkt.

Hoofdstuk 7 beschrijft een onderzoek gericht op het identificeren van voorspellers van remissie van astma. Gegevens over astma remissie waren beschikbaar bij 170 patiënten. Klinische remissie was gedefinieerd als: geen astma medicatie en geen symptomen gedurende> 1 jaar. Vijf jaar na de diagnose waren astma 27 patiënten (15,9%) in klinische remissie van astma. Onafhankelijke voorspellers voor de persistentie van astma waren de aanwezigheid van neuspoliepen en matig-ernstige bronchiale hyperreactiviteit. Als beide kenmerken aanwezig waren in een patiënt, was de verandering van de remissie van de ziekte bijna 0%.

De hypothese voor de studie beschreven in **hoofdstuk 8** was dat de uitlokkende factor (i.e. trigger) die ogenschijnlijk het begin van astma opwekten van invloed geassocieerd is met verschillende patiëntkarakteristieken en verschillende astma-uitkomsten. We vergeleken patiëntkarakteristieken tussen verschillende "triggergroepen" die 10 of meer patiënten bevatten. Er werden vijf triggergroepen

geïdentificeerd, “symptomen van de bovenste luchtwegen”, “nieuwe (allergische of niet-allergische) sensibilisatie”, “pneumonie”, “stressvolle levensgebeurtenis” en “geen trigger geïdentificeerd”. Patiënten met symptomen van de bovenste luchtwegen als trigger hadden hoge FeNO- en hoge bloed eosinofielen waarden, “nieuwe sensibilisatie” toonden patiënten met mild atopisch astma en een relatief jonge leeftijd waarop de ziekte ontstond, “pneumonie” bevatte patiënten met roken in de voorgeschiedenis en lage IgE waarde, patiënten met “stressvolle levensgebeurtenis” gebruikte hoge doses inhalatiecorticosteroïden en had lage inflammatoire markers, en” geen trigger geïdentificeerd” had geen specifieke kenmerken. Hoge remissie aantallen werden gezien in de categorieën “pneumonie” en “geen trigger geïdentificeerd”. Er werd geen remissie gezien in de categorie “stressvolle levensgebeurtenis”. Deze resultaten impliceren dat de trigger van ‘laat astma’ een belangrijk item is in de anamnese van een patiënt.

Chapter 11

Appendix
-Curriculum vitae
- List of publications
- Portfolio
- Dankwoord

Curriculum vitae

Hanneke Coumou werd op 10 augustus 1990 geboren in Enkhuizen. Na het behalen van haar VWO diploma aan het regionaal scholengemeenschap Enkhuizen ging zij geneeskunde studeren aan de Universiteit van Amsterdam. In haar tweede studie jaar werd zij geaccepteerd voor het Honours-programma en volgde zij haar eerste keuzestage als student-onderzoeker bij de ADONIS-studie onder leiding van prof. dr. E.H Bel. In het kader van het Honours-programma heeft zij oa een Summerschool “Introduction and Exploration of Issues in Clinical Medicine” in Praag gevolgd en vrijwilligerswerk gedaan bij Danyun fair tradein Kunming, China. Ze heeft plaatsgenomen als coassistent in de studentenraad en een keuze coschap gevolg bij de kinderlongziekten aan het Royal Brompton Hospital onder leiding van Prof. A. Bush. Na het behalen van haar artsen diploma in 2015 startte zij aan haar promotietraject bij de afdeling longziekten in het AMC Amsterdam onder leiding van prof. dr E.H.D Bel en prof. dr. P.J. Sterk. Sinds september 2019 is zij in opleiding tot longarts onder supervisie van dr. J. van den Aardweg en is momenteel bezig met haar vooropleiding interne geneeskunde bij prof. dr. S.E. Geerlings. In haar vakanties is ze graag te vinden in de bergen en ze heeft een cursus basis alpinisme gevolgd via de Koninklijke Nederlandse Klim- en Bergsport Vereniging. Daarnaast is ze als vrijwilliger actief bij stichting Junior Adventure om zomerkampen voor tieners te organiseren.

Gemaakt door: Menno Boermans

List of publications

1. **Coumou H**, Westerhof GA, de Nijs SB, Amelink M, Bel EH. New-onset asthma in adults; what does the trigger history tell us? J Allergy Clin Immunol Pract 2018.

2. **Coumou H,** Westerhof GA, de Nijs SB, Amelink M, Bel EH. Diagnosing persistent blood eosinophilia in asthma with single blood eosinophil or exhaled nitric oxide level. Respir Med 2018: 141: 81-86.

3. **Coumou H,** Westerhof GA, Bel EHD. Biomarkers in obese asthma: Age of asthma onset matters! J Allergy Clin Immunol 2018: 141(4): 1541.

4. **Coumou H**, Westerhof GA, de Nijs SB, Zwinderman AH, Bel EH. Predictors of accelerated decline in lung function in adult-onset asthma. Eur Respir J 2018: 51(2).

5. 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 2018: 141(1): 104-109 e103.

6. **Coumou H**, Bel EH. Improving the diagnosis of eosinophilic asthma. Expert Rev Respir Med 2016: 10(10): 1093-1103.

Portfolio

	Year	Workload ECTS
General courses		
• BROK-course	2015	1.0
• World of Science	2015	0.7
• Practical Biostatistics	2016	1.1
• Advanced Topics in Biostatistics	2017	2.1
• Advanced Immunology	2016	2.9
• Lung diseases and lung research	2017	5.0
• Infectious Diseases	2017	1.3
Seminars, workshops and master classes		
• Netherlands Respiratory Society, Young Investigator Symposium	2015-2017	0.75
• ERS Satellites: focus on severe asthma	2018	0.25
Presentations		
• Longdagen, Ermelo: "Predictors of accelerated decline in FEV_1 in a prospective new-onset asthma cohort (poster presentation)	2016	0.5
• European Respiratory Society International conference London: "Predictors of accelerated decline in FEV_1 in a prospective new-onset asthma cohort" (oral presentation)	2016	0.5
• American Thoracic Society International Conference Washington: "Predictors and Outcome of Persistent Airway Eosinophilia in Adults with New-Onset Asthma" (Thematic poster presentation)	2017	0.5
• European Respiratory Society International conference Milan: "Exhaled Nitric Oxide and blood eosinophilia: biomarkers of different adult asthma phenotypes" (poster discussion).	2017	0.5
• European Respiratory Society International conference Paris: "New-onset asthma in adults: what does the trigger tell us? " (poster discussion)	2018	0.5

(Inter) national conferences

• European Respiratory Society International conference Amsterdam	2015	1.25
• European Respiratory Society International conference London	2016	1.25
• Nederlandse longdagen	2016	0.50
• American Thoracic Society International Conference Washington	2017	1.25
• European Respiratory Society International conference Milan	2017	1.25
• European Respiratory Society International conference Paris	2018	1.25

Other

Journal club	2015-2018	6
Research meeting department	2015-2018	6

Dankwoord

Veel mensen hebben bijgedragen aan dit proefschrift, ik wil iedereen dan ook enorm bedanken. In het bijzonder wil ik graag bedanken:

Allereerst, de deelnemers aan de ADONIS-studie. Zonder jullie jaarlijkse bezoeken naar het AMC was dit proefschrift nooit tot stand gekomen. Bedankt voor jullie inzet!

Liesbeth, toen ik 10 jaar gelden als student-onderzoeker door jou begeleid werd, had ik niet gedacht dat dat zou leiden tot mijn promotietraject. Ik ben zo dankbaar voor al je adviezen over mijn onderzoek én daarbuiten. Je begeleiding en kritische noot hebben dit proefschrift en mijzelf op een hoger wetenschappelijk niveau gebracht. Ik bewonder je energie en drive om het beste uit mij, de manuscripten en jezelf te halen.

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Marijke, jij hebt mij in vele fases gezien en mij daarin kunnen begeleiden; student, coassistent en promovenda. Bedankt voor al je tijd, input, opbouwende woorden en duwtjes in de rug op het juiste moment.

De leden van mijn promotiecommissie; bedankt voor de tijd en energie die jullie hebben gestoken in het beoordelen van mijn proefschrift en om 4 juni een goede discussie over mijn proefschrift te voeren.

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