UPPSALA UNIVERSITET

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1519

Study of biomarkers for improved diagnosis and therapy monitoring in young asthmatics

NIKOLAOS TSOLAKIS

ACTA UNIVERSITATIS UPSALIENSIS UPPSALA 2019

ISSN 1651-6206
ISBN 978-91-513-0517-2
urn:nbn:se:uu:diva-366892

Dissertation presented at Uppsala University to be publicly examined in Sal 42, Biomedicinskt centrum (BMC), Husargatan 3, Uppsala, Tuesday, 29 January 2019 at 09:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish. Faculty examiner: Docent Lennart Nilsson (Institution för klinisk och experimentell medicin, Linköpings universitet.).

Abstract

Tsolakis, N. 2019. Study of biomarkers for improved diagnosis and therapy monitoring in young asthmatics. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1519. 62 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0517-2.

Background: Type-2 asthma is often related to atopy and is characterized by elevated type-2 biomarkers. However, less is known about the pathophysiology of non-type 2 asthma, factors associated therewith, and the stability of different asthma phenotypes over time.

Aims: To identify an IgE antibody concentration and putative biomarkers that better separate non-type 2 from type-2 asthma. To study the association between longitudinal changes in inflammatory biomarkers and clinical outcomes. To investigate the pattern of IgE sensitization to different cat allergen components and its impact on type-2 biomarkers in young asthmatics.

Methods: The present thesis is based on the MIDAS asthma cohort, which includes asthmatics (n = 408) and healthy controls (n = 118), aged 10–35 years at baseline, with a follow-up visit 43{23-65} months later. All the subjects were characterized with regard to IgE sensitization, inflammation was assessed based on fractional exhaled NO (FeNO), blood eosinophil count (B-Eos) and other biomarkers, both type-2 and non-type 2, and lung function was evaluated with spirometry.

Results: FeNO and B-Eos maintained associations with clinical asthma outcomes in the IgE antibody concentration range 0.10–0.34 kU_A/L, but not below 0.10 kU_A/L. Non-atopic asthmatics with perceived cow's milk hypersensitivity had poorer asthma-related quality of life than those with atopic asthma, and were characterized by clinically significant non-type 2 inflammation. Furthermore, longitudinal increase in height-adjusted FeNO associated independently with decline in lung function. IgE sensitization to cat lipocalins and/or cat serum albumin were independently associated with FeNO and B-Eos.

Conclusions: Our findings demonstrated that a cut-off of 0.10 kU_A/L for IgE antibodies appeared to be useful for ruling out type-2 asthma in young subjects. A subgroup of non-atopic asthmatics was characterized by perceived cow's milk hypersensitivity and non-type 2 inflammation. Longitudinal changes in FeNO associated with lung function decline in asthmatics. IgE sensitization to minor cat allergen components may promote both local and systemic type 2 inflammation.

Keywords: Asthma phenotypes, IgE sensitization, atopy, cat allergen components, exhaled NO, airway hyper-responsiveness, lung function, asthma-related quality of life.

Nikolaos Tsolakis,

ISSN 1651-6206
ISBN 978-91-513-0517-2
urn:nbn:se:uu:diva-366892 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-366892)

To the four women of my life…

List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Tsolakis N, Malinovschi A, Nordvall L, Janson C, Borres MP, Alving K. The absence of serum IgE antibodies indicates non-type 2 disease in young asthmatics. *Clin Exp Allergy 2018. 48(6).*

II Tsolakis N, Nordvall L, Janson C, Rydell N, Malinovschi A, Alving K. Clinical and inflammatory characterization of a subgroup of non-type 2 asthma in young subjects. *Submitted for publication.*

III Tsolakis N, Janson C, Borres MP, Malinovschi A, Alving K. Relationship between longitudinal changes in type-2 inflammation and clinical outcomes in young asthmatics. *Manuscript.*

IV Tsolakis N, Malinovschi A, Nordvall L, Mattsson L, Lidholm J, Pedroletti C, Janson C, Borres MP, Alving K. Sensitization to minor cat allergen components is associated with type-2 biomarkers in young asthmatics. *Clin Exp Allergy 2018. 48(9).*

Contents

Abbreviations

ACT	Asthma Control Test
B-Eos	blood eosinophil count
CRP	C-reactive protein
Can f	*Canis familiaris*
Ecu c	*Ecuus caballus*
Fel d	*Felis domesticus*
FeNO	fractional exhaled nitric oxide
FEV_1	forced expiratory volume in one second
FVC	forced vital capacity
GLI	Global Lung Function Initiative
HNL	human neutrophil lipocalin
ICS	inhaled corticosteroid
IgE	immunoglobulin E
IL	interleukin
ILC2	innate-lymphoid cell type 2
iNOS	inducible NO synthase
LTRA	leukotriene-receptor antagonist
Mini-AQLQ	Mini Asthma Quality of Life Questionnaire
MIDAS	Minimally-Invasive Diagnostic Procedures in Allergy, Asthma, or Food Hypersensitivity Study
MMP	matrix metalloproteinase
NO	nitric oxide
PD_{20}	cumulative dose of methacholine causing a 20% reduction in FEV_1
S-ECP	serum eosinophilic cationic protein
Th2	T-helper cell type 2

Introduction

Asthma: definition and phenotypes

Asthma is described as a multifactorial disease with differences in intensity, comorbidities, and response to treatment. It is defined by a history of variable respiratory symptoms and by variable expiratory airflow limitation [1]. Mucous hypersecretion associated with airway hyper-responsiveness and inflammation are key features in the pathophysiology of asthma [2]. The prevalence of asthma has increased in the last few decades, particularly among children [3]. However, despite access to modern medication, asthma control in children remains poor worldwide [4]. This suggests that symptom-based treatment is not the way forward. Rather, the view is now emerging that patients must be segmented into subgroups.

The understanding of asthma has progressed and several attempts have been made to classify asthma based on age at onset, presence of atopy, clinical characteristics, and inflammatory background. Asthma phenotyping has mainly emphasized the dichotomy of allergic and non-allergic asthma, but many different asthma phenotypes have been identified, although future studies are needed to determine their clinical utility [5]. Allergic asthma is the dominating phenotype among school children. Type-2/eosinophilic inflammation is often present, in which inhaled corticosteroids (ICS) are the mainstay of treatment. In this phenotype, the level of airway inflammation is independently associated with IgE sensitization to both aeroallergens and food allergens [6, 7].

The non-allergic phenotype of asthma, also known as late-onset asthma, is less common in childhood and it is characterized by absence in the serum of specific IgE antibodies to common aeroallergens [8]. The key drivers of inflammation are unknown and this asthma phenotype includes multiple aspects, with nasal polyps, high total IgE, and hyper-eosinophilia at the severe side of its spectrum [9-11]. The importance of total IgE in this group of asthmatics is reflected by the positive effect of anti-IgE treatment, regardless of the presence of atopy [12].

The asthma phenotypes elucidate the clinical observable features of the disease and may predict the most appropriate treatment [13]. On the other hand, they do not provide information about the underlying pathophysiology of the asthma disease. Moreover, the lack of longitudinal data within the field of asthma has constituted an obstacle to studying the stability of pheno-

types over time. Therefore, molecular phenotyping, enabling measurement of biomarkers, has been incorporated into asthma research in an attempt to link the disease pathobiology to treatment interventions [14]. The term "biomarker" refers to a characteristic or a medical sign that can be measured objectively, accurately, and reproducibly, reflecting a biological process [15]. The development of asthma biomarkers with greater predictability is ongoing.

The newly described endotypes serve to define asthma entities by giving insights into cellular and molecular mechanisms [16]. Identifying detectable molecular biomarkers underlying asthma phenotypes may facilitate new therapeutic strategies and guide targeted treatment responses.

Type-2 inflammation and biomarkers

The sensitization process starts when an allergen is transported to a regional lymph node, where CD4+ cells are stimulated to develop into T-helper type 2 (Th2) cells. Cytokines produced by the Th2 cells stimulate B cells to form allergen-specific IgE antibodies [17]. These antibodies, in turn, attach to cell surfaces of mast cells and basophils, awaiting new contact with the allergen. Type-2 inflammation involves signaling pathways, including those of interleukins (IL)-4, 5, and 13. IL-4 is mainly involved in the primary sensitization process, while IL-13 is linked to airway changes during secondary airway exposure [18]. IL-4 and IL-13 can increase epithelial nitric oxide (NO) production through increased expression of inducible NO synthase (iNOS), producing the vast majority of the exhaled NO [19]–[20, 21].

At present, fractional exhaled nitric oxide (FeNO) is the most widely used biomarker in clinical practice. It has been seen as a trusted biomarker of type- 2 cytokine-driven inflammation in the bronchial mucosa, and is known for accurately predicting the response to ICS in asthma [22]. Furthermore, FeNO has been shown to be associated with the level of IgE antibodies, although this relationship seems to be disrupted by use of high doses of ICS [23]. Recently, a European Respiratory Society Task Force has considered FeNO to be an important non-invasive biomarker for guiding asthma treatment in children [24].

Blood eosinophilia is triggered by IL-5, although viral infections seem to cause eosinophil activation in allergic individuals [25, 26]. Moreover, a very recent study on mice has linked respiratory viral infections to the development of airway hyper-responsiveness and asthma disease, by involving the newly described type-2 innate lymphoid cells (ILC2) [27]. Systemic eosinophilic inflammation measured by the blood eosinophil count (B-Eos) is of importance for asthma, and B-Eos is recognized as a biomarker of allergic inflammation. It is known that B-Eos has clinical utility in reflecting disease activity, but the correlation to airway inflammation has been considered to

be moderate [28]. In addition, blood eosinophilia has been shown to be resistant to ICS, but appears to be more sensitive to systemic anti-inflammatory medicines like anti-leukotrienes [29, 30]

Studies have shown that the combined use of FeNO and B-Eos as biomarkers of both local and systemic inflammation offers independent and additive explanatory value for asthma morbidity [26, 31]. This could explain the large heterogeneity in the relationship between these two markers in patients with allergic asthma, particularly children.

IgE synthesis is a hallmark of type-2 inflammation and is stimulated by allergen exposure. However, to complicate the picture, ILC2 seem to play a pivotal role in this type of inflammation as part of the innate immune system (Figure 1) [32].

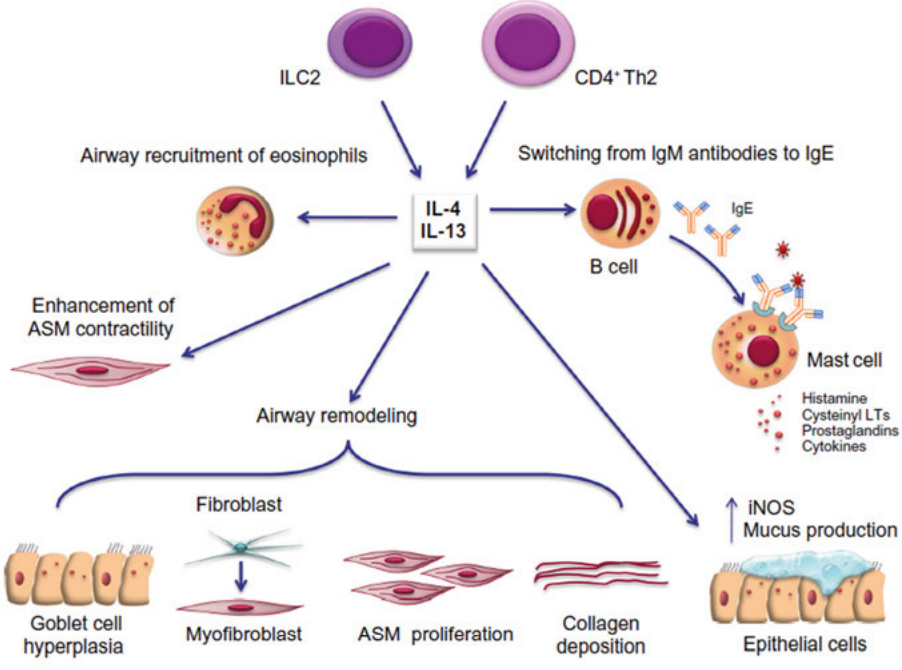

Figure 1. Role of ILC2 and Th2 cytokines in asthma pathophysiology [33] (reproduced with permission from the publisher). Abbreviations: ASM, airway smooth muscle; IL, interleukin; LTs, leukotrienes; iNOS, inducible nitric oxide synthase.

Atopy and asthma

Atopy is believed to be a strong risk factor for asthma, yet the interaction between the two is not completely understood [34]. Recent studies have hypothesized the presence of atopic sub-phenotypes underlying the development of allergic sensitization and asthma disease [35]. The traditional definition of atopy has been based on a serum IgE-antibody concentration above 0.35 kU_A/L or a skin prick test result with wheal diameter greater than 3 mm. Disagreement between skin prick tests and IgE measurements for confirming atopy has been reported previously [36], though detection of IgE antibodies in serum seems to have greater sensitivity [36, 37]. Further, elevated total serum IgE has been shown to be related to asthma irrespective of atopic status, reflected in that the role of atopy in asthma is a subject of debate [38, 39].

The presence of atopy in asthma is generally considered to indicate the existence of type-2/eosinophilic inflammation, and thus, corticosteroid-sensitive disease. It is well-known that patients with atopic/allergic asthma are characterized by elevated FeNO and that increased FeNO levels predict asthma exacerbations [40, 41]. However, in earlier studies, significantly higher FeNO appeared in non-atopic asthmatics than in healthy controls [42, 43] and unexpected corticosteroid responsiveness has been noted in patients with non-atopic asthma [44]. Further, these two subtypes have been shown to share common immuno-pathological mechanisms and are assumed to overlap in patterns of cellular inflammation [39, 45]. Interestingly, clinical features cannot be used as a tool for distinguishing the traditionally denoted non-atopic asthma from atopic/allergic asthma, making this asthma classification more complex [46, 47].

It has been a routine in the last decades to describe asthma as an atopic disease in children. However, the present definition of atopy appears to have limited clinical use and the importance of atopy as a causative agent of asthma may have been overestimated. Therefore, ruling out type-2 asthma based on the traditional criteria can lead to incorrect diagnoses.

Non-type 2 asthma

As mentioned above, type-2 asthma is often related to atopy and is characterized by elevated type-2 biomarkers. Less is known about the pathophysiology and factors associated with non-type 2 asthma, and validated biomarkers are lacking for this endotype. This type of asthma is primarily seen in adults, although its presence among young asthmatics has also been observed [48, 49]. Individuals with mild to moderate asthma, with no history of allergic features in childhood, may belong to this category [50].

It is likely that non-type 2 asthma includes more than one endotype, seemingly based on different pathogenetic mechanisms. Neutrophilic asthma, also known as "low Th2" asthma, has been reported [8]. This sub-entity of asthma belongs to the non-allergic asthma phenotype and is primarily characterized by corticosteroid resistance [51]. It is unknown whether neutrophil recruitment plays a role in asthma severity or whether it could possibly be the result of corticosteroid treatment targeting a resistant type-2 asthma [52]. The role of circulating ILC2 as an alternative source of type-2 cytokines has to be considered, since it might explain the existence of Th2 signals independent of atopy [32, 53]. A similar innate immune pathway has been suggested as a trigger factor for the co-occurrence of neutrophilia and eosinophilia in the individuals with the most severe asthma and the poorest asthma control [50, 54]. In terms of diagnostics, the absence of sputum eosinophilia combined with the presence of sputum neutrophilia has been used as a criterion for identifying neutrophilic asthma [55]. However, based on this sputum profiling, variations in the prevalence of the disease have also been reported, constituting an obstacle for defining the neutrophilic airway inflammation [56] [57]. Furthermore, sputum induction is an invasive, time-consuming method, difficult to apply in children [58].

To date, biomarkers of non-type 2 asthma have not been established. The controversial role of atopy creates a need for molecular stratification for better understanding of the mechanisms driving this kind of asthma. Such understanding might, in turn, be translated into new therapies for asthmatics whose asthma remains uncontrolled.

Changes of asthma biomarkers over time

The development of clinical and inflammatory phenotyping in asthma has aimed to address the complexity of the disease. The vast majority of the studies regarding asthma phenotyping are based on cross-sectional data, while only limited longitudinal data are available [9]. Whether these asthma characteristics are stable or tend to change over time remains to be determined [59].

It is well-known that increased levels of FeNO in terms of type-2 inflammation predict asthma exacerbations and may reflect the degree of airway inflammation, while it has been shown that FeNO measurements might be helpful in optimizing asthma control over time [40, 60]. Less is known about the relation between B-Eos, as a component of systemic type 2 inflammation, and longitudinal asthma control. Based on a prospective annual study, Price and co-workers have recently shown a count-response association between B-Eos and asthma exacerbation rates [61]. On the other hand, other studies have linked the eosinophilic airway inflammation, defined by induced sputum, to loss of lung function [62, 63].

With regard to lung function changes over time, longitudinal studies have demonstrated accelerated loss of lung function in older asthmatics compared with younger asthmatics, prior to the time of diagnosis [64]. Attempting to approach the association between lung function and asthma over time, atopy and total IgE have to be considered as two important contributing factors. Increased levels of total IgE have been reported to be inversely related to lung function earlier in life, when the incidence of allergic symptoms is higher [65] and the lungs are still maturing, though this relation weakens in elderly individuals [66]. Further, early allergic sensitization within the first year of life is suggested to be associated with lung function decline around 18 years of age [67]. However, it is important not to overlook the physiological age-related changes that the respiratory system undergoes [68].

As outlined above, the lack of longitudinal data on asthma outcome leads to limited knowledge about the stability of the existing asthma phenotypes over time, and this translates into difficulties in understanding and applying the concept of endotyping.

Asthma and cat allergy

Asthma is a heterogeneous disease with variable symptoms and more closely resembles a complex of clinical diseases than a single condition. Biomarkers of Th2-mediated inflammation such as FeNO and B-Eos have been shown to be sensitive in identifying children with high asthma morbidity [69]. Sensitization to furry animals is related to more frequent asthma symptoms as well as to increased markers of bronchial inflammation [70, 71]. Moreover, sensitization to indoor allergens has been associated with higher FeNO and B-Eos, resulting in more severe asthma [72].

It is well-known that asthma in terms of airway hyper-responsiveness is dependent on total IgE, but not on co-occurring atopic disease [73]. A recent study with a focus on dust mite and cat exposure showed that total IgE was associated with IgE antibodies to house dust mite but not to cat dander [74]. Cat is the major indoor inducer of allergic sensitization in Sweden and cat ownership has increased in the last few years [75]. However, indirect exposure to cat allergens can occur in schools, occupational environments, and outdoors, constituting a risk factor for subsequent asthma development [76, 77]. Early sensitization to cat, within the first three years of life, has been linked to lung function decline by school age [78]. Therefore, accurate diagnosis of sensitization to cats is important, irrespective of cat ownership. The study of cat allergen components has been a large step forward in the diagnosis of cat allergy.

Cat allergen components

Sensitization to cat allergen components has been shown to be of significant clinical importance for the development of allergic symptoms [79]. Fel d 1, known to be the major allergen component in cat dander extract, belongs to the secretoglobin family and is considered a marker for early cat sensitization in childhood [80]. In addition, other cat allergen components, such as serum albumin or lipocalins, appear to play an important role in improving cat allergy diagnostics [80]. Fel d 4 is a lipocalin cat allergen isolated from the cat submandibular gland, highly homologous to the major horse allergen Equ c 1 and the dog allergen Can f 6. It is an allergen with frequent binding of IgE (> 60%) from the sera of cat-allergic individuals [81]. Moreover, the cat lipocalin Fel d 7 has been detected in a tongue cDNA library, and has a prevalence of 38% among cat-allergic patients. Fel d 7 and Can f 1 have a high degree of structural identity (60%), implicating strong IgE co-sensitization contributing to symptoms in both cat- and dog-allergic patients [82, 83]. The serum albumin Fel d 2 is considered to be a minor cat allergen and is found in cat serum, urine and dander. It is mostly known as a cross-reactive allergen through its 88% sequence identity to dog serum albumin (Can f 3). The sensitization pattern of Fel d 2 can be quite complex; on the other hand not much is known about its clinical utility [84, 85].

Unfortunately, data on the relation between sensitization against cat allergen components and markers of type-2 inflammation and total IgE are scarce, since most of the earlier studies have tended to focus on the allergenic cross-reactivity between different mammalian species.

Further research must determine whether IgE antibodies to minor cat allergen components might contribute clinically in asthma diagnosis and asthma morbidity in individuals allergic to cats.

Allergen	Protein family
Fel d 1	**Secretoglobin**
Fel d 2	**Serum albumin**
Fel d 4	**Lipocalin**
Fel d 7	**Lipocalin**

Future molecular biomarkers

In the last years, component-resolved IgE antibody determination has been successfully used in establishing the connection between allergic sensitization and clinical allergic symptoms [86]. The continuous evolution within the field of molecular diagnostics has created a need for developing a combination of biomarkers with better specificity and sensitivity. A few large-scale protein studies based on the proteomic technique have attempted to add information for asthma phenotyping [87, 88]. A very recent study showed that proteomic analytes in serum and sputum, based on the Proseek Multiplex panels, could be used as a reliable tool in distinguishing between different asthma subtypes [89]. Proteomic analysis has aroused interest as it covers a broad range of applications, from biomarker discovery to verification and validation [90]. This method might offer an opportunity to identify new molecular fingerprints for capturing the biological complexities of the disease. Intervention studies are the next step for testing the predictive value of these molecular signatures.

Aims

The overall aim of my thesis was to increase the knowledge of predictive asthma biomarkers that can be used in children, and validate these biomarkers in a cohort of young asthmatics and healthy controls.

Study I: To identify an IgE-antibody concentration that better separates non-type 2 from type-2 asthma and to describe biomarker patterns in young asthma patients with high- and low-grade IgE sensitization.

Study II: To identify the presence of a distinct phenotype of non-type 2 asthma with perceived cow's milk hypersensitivity in a cohort of young asthmatics, by exploring a large set of biomarkers.

Study III: To give an overview of longitudinal changes in inflammatory biomarkers and asthma outcomes, and to determine the relationship between these changes.

Study IV: To study the pattern of IgE sensitization to different cat allergen components in young asthmatics and to assess the effect of this sensitization on type-2 biomarkers and asthma outcomes.

Material and methods

Study subjects: MIDAS asthma cohort

The four studies were based on subjects who participated in the **M**inimally-**I**nvasive **D**iagnostic Procedures in Allergy, **A**sthma, or Food Hypersensitivity **S**tudy (MIDAS) asthma cohort. A total of 411 asthmatic patients, all children or young adults (10–35 years), were recruited from primary and specialist care in Uppsala, Sweden. The subjects were characterized with regard to IgE sensitization to food and airborne allergens, as well as to local and systemic inflammation. Blood samples were collected from 408 subjects and the asthma control test (ACT) was performed in 405 of these 408 subjects. The inclusion criteria were physician-diagnosed asthma (according to medical records) and self-reported daily treatment with ICS and/or oral leukotriene-receptor antagonist (LTRA) during at least three months in the year before the study. Further, 121 non-asthmatic controls, age- and sex-matched, were randomly chosen from the population registry. Three subjects were excluded due to asthma diagnosis and/or lack of blood samples, leading to 118 non-asthmatic controls with complete datasets. Smokers were not excluded, but comprised less than 5% of the studied subjects. The baseline survey (MIDAS 1) was performed in 2010–2012, with the asthmatic subjects responding to questionnaires regarding asthma symptoms and attacks during the preceding year, and undergoing extensive clinical examinations (see below), while the use of anti-inflammatory medication was recorded in interviews [7, 91]. The follow-up survey was conducted at a median of 43 months later (MIDAS 2, 2014–2016) and 253 asthmatic subjects and 66 non-asthmatic controls could be recruited from MIDAS 1. The asthma characteristics were re-evaluated, excepting airway responsiveness to methacholine. Approximately the same proportion of asthmatic subjects (85%) were using ICS at baseline and follow-up visits.

Blood measurements and IgE sensitization

In the four studies, the presence of IgE antibodies was examined using two different multi-allergen tests, one with a mix of nine aeroallergens (birch, timothy, mugwort, *Dermatophagoides pteronyssinus, Dermatophagoides farinae, Cladosporium herbarum,* cat, dog, and horse; Phadiatop), and one

with a mix of six food allergens (egg white, cod fish, milk, soybean, wheat, and peanut; fx5) [92]. The ImmunoCAP system (Immunodiagnostics, Thermo Fisher Scientific, Uppsala, Sweden) was used for the measurement of total IgE, IgE antibodies, cat allergen components, serum eosinophilic cationic protein (S-ECP) and IgA/IgG antibodies against cow's milk protein. ELISA (Diagnostics Development, Uppsala, Sweden) was used for the quantification of human neutrophil lipocalin (HNL). Plasma C-reactive protein (CRP) was analysed using an immunoturbidimetric method (CRP Vario, Abbott, Illinois, USA), and blood cells were counted using a routine method (Cell-Dyn Sapphire, Abbott, Illinois, USA); both analyses were performed at Uppsala University Hospital.

A panel of 92 inflammation-related proteins (Proseek Multiplex Inflammation; Olink Bioscience, Uppsala, Sweden) was included in the analysis [93]. The inflammation panel is based on a multiplex proximity extension immunoassay (PEA), enabling analysis of 92 proteins across 96 samples simultaneously. The data were given in the form of relative quantifications and expressed as normalized protein expression (NPX).

Asthma symptoms, attacks, medication and allergic symptoms

The Asthma Control Test (ACT), a clinically validated measure, was used in all studies to assess asthma control [94]. Subjects older than 12 years of age responded to questions regarding obstructive symptoms, frequency of usage of reliever medication, opinions on asthma control, and impact of disease on everyday life, with 4-week recall. The total ACT score ranges from 5 to 25, with a lower score reflecting poorer asthma control. Well-controlled asthma was defined as an ACT score ≥ 20. In Studies II and III, the mini asthma-related quality of life questionnaire (mAQLQ) was also used. This questionnaire consists of 15 questions regarding asthma symptoms, emotional function, activity limitations and environmental stimuli, with 2-week recall. mAQLQ scores range from 1 to 7, with a lower score indicating a poorer quality of life [95]. Furthermore, asthmatic subjects were divided into groups based on whether or not they had had a recent asthma attack (last three months, self-reported). The use and dose of ongoing asthma medication (ICS, LTRA) was recorded in interviews, as well as asthma symptoms during the preceding 12 months [96].

In Study IV, allergic symptoms were defined as self-reported rhinitis and/or conjunctivitis during contact with cat.

Measurements of exhaled NO

In all four studies, FeNO measurements were performed in accordance with the recommendations of the American Thoracic Society/European Respiratory Society [97], using a chemiluminescence analyzer at an exhalation flow rate of 50 ml/s (NIOX Flex; Aerocrine AB, Solna, Sweden). Measurements were taken with the subject in a sitting position using a single-breath technique. The mean value from three exhalations (or two, if the first two NO measurements were within 10% of each other) was used for statistical analysis. The analyzer was calibrated every 14 days with certified NO/N_2 gas of 200 ppb. Subjects were asked to refrain from intense physical exercise and ingestion of nitrate-rich food on the day of the measurement.

In Studies I, II, and III, the percent of predicted FeNO (FeNO%) was calculated using the Malmberg algorithm, which adjusts for height in children and adolescents [98]. According to data from a large population-based study, height appeared to be an important independent variable explaining variations in FeNO [99].

Lung function

In all four studies, forced expiratory volume in one second (FEV_1) was measured using a Masterscope spirometer (Viasys Healthcare GmbH, Höchberg, Germany). Measurements were performed in accordance with the American Thoracic Society recommendations [97]. The highest value of FEV_1 after three acceptable trials was recorded. The subjects were further subdivided into groups, with reduced or normal lung function ($FEV_1 < 80\%$ or $\geq 80\%$ of predicted). The percent of predicted values for FEV_1 were calculated on the basis of Hedenström reference values for those ≥ 18 years of age [100, 101] and Solymar reference values for subjects < 18 years of age [102]. In Study IV, percentiles for FEV_1 and FEV_1/FVC (forced vital capacity) were calculated for each subject in the reference population in accordance with the GLI (Global Lung Function Initiative) reference values [103]. The GLI reference values are based on pre-bronchodilator measurements, and therefore only pre-bronchodilator values from the reference population were analyzed.

Methacholine challenge test

Details of the methacholine challenge test have been described previously [104]. In Studies I, III, and IV, a bronchial provocation test was performed with Aerosol Provocation System (Viasys Healthcare) using a single concentration of methacholine and increasing doses up to a maximal cumulative dose of 3.63 mg methacholine. Two minutes after each inhalation, FEV_1 was

measured and airway responsiveness was defined as normal if the cumulative dose causing a decline of 20% in FEV_1 (PD_{20}) was > 1.0 mg, borderline-to-mild at 0.3–1.0 mg, and moderate-to-severe at < 0.3 mg, in accordance with Schulze et al. [105]. In the present studies, airway hyper-responsiveness was defined as PD_{20} < 0.3 mg. Subjects were instructed to avoid taking asthma medication the day before the study.

Perceived food hypersensitivity symptoms

In Study II, subjects self-reported hypersensitivity reactions to the following food allergens: cow's milk, egg white, soy bean, wheat, peanut, and cod fish. The reported symptoms were grouped based on the organ affected: the upper airways (rhinitis, conjunctivitis), the oral cavity (oral allergy symptoms), the lower airways (asthma), the skin (urticaria, angioedema, and atopic eczema), the gastrointestinal tract (nausea, vomiting, diarrhea, abdominal pain), and self-reported anaphylaxis. Perceived symptoms that did not fit into any group were classified as "other". A total of 30 subjects with non-atopic asthma reported any food hypersensitivity symptoms, but the study focused on the 24 subjects who reported perceived symptoms to cow's milk. Of the remaining subjects, three reported wheat hypersensitivity, two peanut hypersensitivity and one cod fish hypersensitivity.

Study design

Study I was based on asthmatic subjects at the baseline visit. The asthmatic subjects were initially categorized according to IgE antibody concentrations (above or below 0.35 kU_A/L). The group with IgE < 0.35 kU_A/L was further divided into two groups (above or below 0.10 kU_A/L). Thus, three subgroups were created: elevated (≥ 0.35 kU_A/L), detectable (0.10–0.34 kU_A/L) and undetectable (< 0.10 kU_A/L) (Figure II).

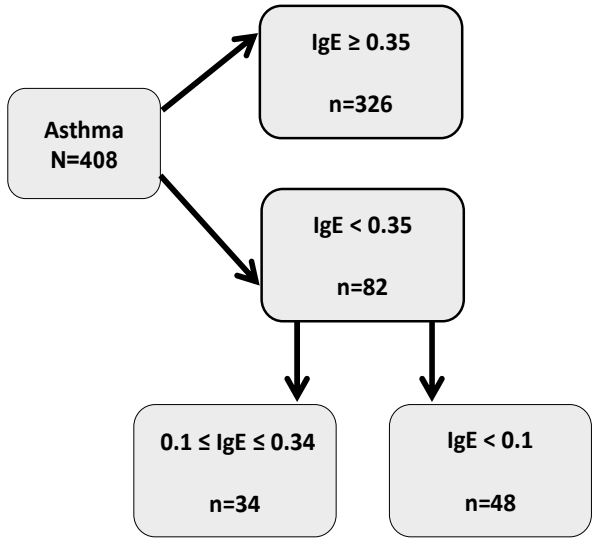

Figure II. Flow chart for the inclusion of subjects in Study I (IgE units in kU_A/L).

Study II was based on asthmatic subjects (n = 405) and non-asthmatic controls (n = 118) at the baseline visit. Three subgroups, age- and sex-matched, were created from this cohort, based on atopic status, presence of asthma, and food hypersensitivity. The first group consisted of all patients with non-atopic asthma (NAA; IgE sensitization to aeroallergens and food allergens < 0.35 kU_A/L) and perceived cow's milk hypersensitivity. This group was initially extracted from a slightly larger group of non-atopic asthmatics who reported any food hypersensitivity symptoms. The second group consisted of non-atopic healthy controls (NAC; IgE sensitization to aeroallergens and food allergens < 0.35 kU_A/L). The third group consisted of asthmatic subjects with atopy (AA; clear IgE sensitization to at least one aeroallergen or food allergen ≥ 0.35 kU_A/L) (Figure III). Seven non-atopic asthmatics with fx5 between 0.10 and 0.34 kU_A/L and three with Phadiatop between 0.10 and 0.34 kU_A/L were extracted from the NAA group and compared with the rest of the group.

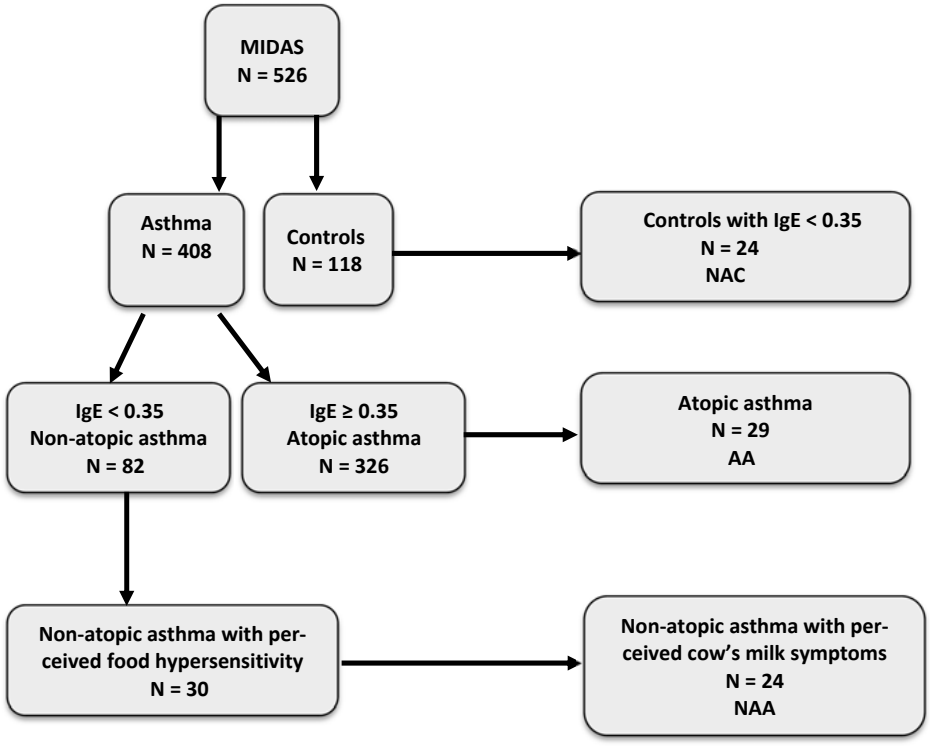

Figure III. Grouping of MIDAS subjects according to IgE sensitization status and history of perceived food hypersensitivity (IgE units in kU_A/L). The groups NAA, NAC, and AA were age- and sex-matched.

Study III was based on data from the baseline and follow-up visits for both asthmatic subjects and healthy controls. The dataset included IgE measurements and IgE sensitization was characterized at the baseline visit. Like Study I, three subgroups were formed based on the IgE antibody concentration (high: ≥ 0.35 kU_A/L, low: 0.10–0.34 kU_A/L, and undetectable: < 0.10 kU_A/L), though Study III included both asthmatic subjects and healthy controls (Figure IV).

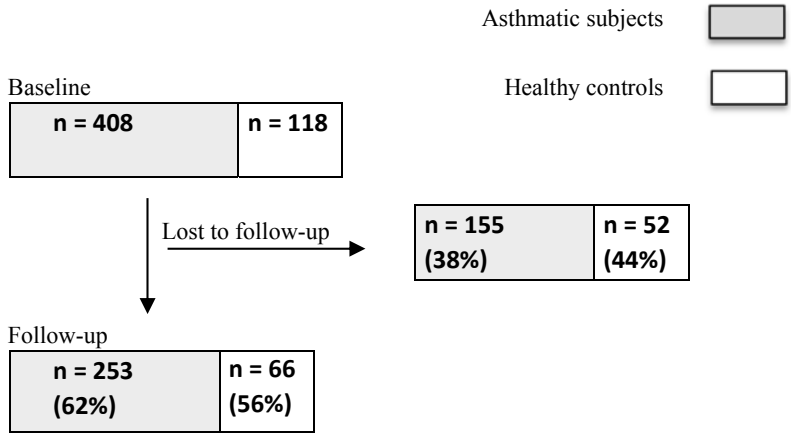

Figure IV. Flow chart for the inclusion of MIDAS participants at baseline and follow-up visits.

Study IV encompassed asthmatic subjects at the baseline visit; a total of 405 subjects with complete datasets were included. The presence of IgE antibodies was examined by using Phadiatop, a test including the most common indoor and outdoor allergens [92]. Subjects with levels of IgE for Phadiatop ≥ 0.35 kU_A/L were further characterized regarding IgE sensitization against different allergens included in the Phadiatop mix. Subjects sensitized to any of the furry animals included in the Phadiatop mix (cat, dog, or horse extract) were further characterized with regard to IgE sensitization to allergen components Fel d 1 (e1), Fel d 2 (e220), Fel d 4 (e228), and Fel d 7 (Figure V). A derived variable was created, named Fel d 4 + 7, by using the sum of Fel d 4 and Fel d 7 IgE- antibody concentrations.

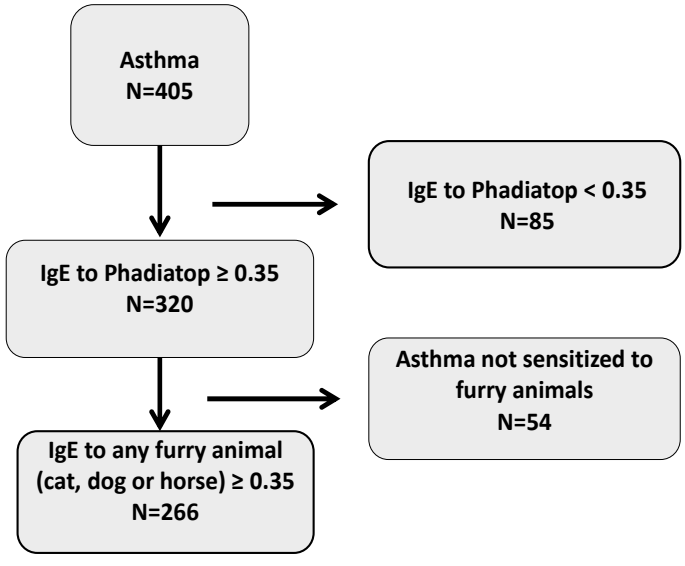

Figure V. Flow chart for the inclusion of subjects in Study IV (IgE in kU_A/L).

Statistical analyses

All statistical analyses were conducted using STATA/IC 13.1 (StataCorp LP, College Station, Texas, USA). Right-skewed variables (for continuous variables) were presented as geometric means (95% confidence interval) and logarithmic transformation was performed before further analyses. Unpaired t-tests (for continuous variables) and chi-squared tests (for categorical variables) were used for univariate analyses.

In Study I, multiple logistic regression analyses with outcome variables ACT < 20, FEV_1 < 80% predicted, recent asthma attacks, PD_{20} < 0.3 mg were performed to estimate odds ratios. FeNO%, B-Eos, age, sex, weight, smoking status, and ongoing asthma medication (ICS, LTRA) were used as independent variables. Due to the small number of subjects in some subgroups, multivariate analysis could only be performed in the entire group with Phadiatop and fx5 < 0.35 kU_A/L. The lower detection limit of the ImmunoCAP system (0.10 kU_A/L) was used as a cut-off for the analyses.

In Study II, univariate analyses were performed using the 3 out of the 92 Proseek-inflammations proteins with a crude p value < 0.05. Multivariate Analysis of variance (ANOVA) with adjustment for age, gender, Body Mass Index (BMI), and smoking status was used for candidate proteins and the robustness of the test was evaluated using bootstrapping. Multiple testing for p values was corrected using the Benjamin-Hochberg (BH) procedure. The principle of this approach is the control of the false discovery rate (FDR) and narrowing that to a predefined value (0.05) [106]. Spearman's test was used for correlation analyses between the significant Proseek-inflammation proteins and inflammatory (FeNO, B-Eos) and clinical biomarkers (ACT, mAQLQ, PD_{20}, FEV_1, FEV_1/FVC) in the three selected groups. The Mann-Whitney U test was used for group comparisons.

In Study III, univariate testing was performed for inflammatory (FeNO, B-Eos and total IgE) and clinical biomarkers (FEV_1, FEV_1/FVC, ACT, mAQLQ) at both baseline and follow-up visits. Δ values were defined as follow-up value minus baseline value for each variable. Pearson's test was used to estimate correlation coefficients between the Δ values of clinical biomarker variables. Paired t-test was used for estimating the Δ values between the two visits. Multiple linear regression models were used when examining inflammatory biomarkers as predictors using ΔFEV_1,ΔFEV_1/FVC, ΔACT and ΔmAQLQ as outcome variables, with concomitant adjustment for age change in months (median of 43 months), gender, changes in weight,

smoking, pet ownership, asthma medication and allergen-specific immunotherapy at follow-up. Multiple linear regression analyses were performed separately in asthmatics with IgE-antibody concentrations above or below 0.35 kU_A/L. Models based on asthmatics and controls with IgE ≥ 0.10 kU_A/L at baseline were also included in the analyses.

In Study IV, the asthmatics sensitized to cat were described using univariate analyses and the Bonferroni type adjustment was used for correction of multiple testing. Multiple linear regression analyses were performed using FeNO, B-Eos and total IgE as dependent variables. IgE to Fel d 1, Fel d 2, Fel d 4, Fel d 7, and cat dander were used as independent variables and the analyses were adjusted for age, sex, smoking status, height, and ongoing asthma medication. Zero values were replaced with the value 0.005 before log transformation. Predictors highly related to each other ($r > 0.50$, for example IgE to the two lipocalins, Fel d 4 and Fel d 7) were not included in the same multivariate analysis. The correlation coefficients were assessed using Pearson's test. Multiple linear regression analyses were also performed using the same outcome variables as in Study I. A p value < 0.05 was considered statistically significant and $p < 0.10$ indicated a trend.

Ethics

The parents of the subjects or the subjects themselves, if they were older than 18 years of age, gave written consent for participation in the studies. The protocols were approved by the Uppsala Regional Ethical Review Board (registration numbers 2009/349 and 2012/420).

Results

Study I

Characteristics of asthmatics in different IgE groups

Asthmatics with IgE antibodies < 0.35 kU_A/L had lower levels of type-2 biomarkers, including total IgE, and less airway responsiveness than asthmatics with IgE antibodies ≥ 0.35 kU_A/L. Asthmatics with IgE < 0.35 kU_A/L were more often female and used more LTRA than those with IgE ≥ 0.35 kU_A/L. Further subdivision of the group with IgE < 0.35 kU_A/L revealed that FeNO and total IgE were lower in subjects with IgE < 0.10 kU_A/L (undetectable IgE group) than subjects with IgE levels between 0.10 and 0.34 kU_A/L (detectable IgE group; Table I). Asthmatics with IgE < 0.10 kU_A/L used asthma medication more frequently, but PD_{20} and FEV_1 were not significantly different between these two subgroups.

Table I. Characteristics of asthmatics with IgE sensitization below 0.35 kU_A/L (IgE in kU_A/L). Mean ± SD, Geometric mean (95% CI).

	0.1 ≤ IgE ≤ 0.34 (n = 34)	IgE < 0.10 (n = 48)	p
FeNO (ppb)	12.6 (9.88, 16.1)	9.02 (7.50, 10.8)	0.032
FeNO (%)	105 (82.1, 134)	74.5 (62.4, 88.6)	0.050
B-Eos ($x10^9/L$)	0.132 (0.099, 0.177)	0.119 (0.095, 0.148)	0.387
PD_{20} (mg)	0.698 (0.344, 1.40)	0.887 (0.512, 1.53)	0.757
FEV_1 (%)	89.8 ± 14.4	91.6 ± 14.7	0.853
Total IgE	47.9 (35.4, 64.8)	12.4 (8.53, 18.2)	< .001
LTRA (%)	17.6	39.5	< .001
ICS (µg)	374 (295, 475)	422 (349, 510)	0.008
Females (%)	58.5	62.5	0.369

Associations between type-2 biomarkers and asthma outcomes in asthmatics with IgE ≥ 0.35 kU_A/L

In univariate analyses, statistically significant associations were noted between type-2 biomarkers and categorized clinical asthma outcomes (Table II) in the group with IgE-antibody concentrations ≥ 0.35 kU_A/L. Multivariate analyses with FeNO% and B-Eos as dependent variables were performed, since FeNO% and B-Eos were the inflammation biomarkers most closely associated with the clinical outcomes. An independent association between FeNO% or B-Eos and asthma outcomes was found in multiple logistic regression models adjusted for confounding factors. FeNO% remained significantly associated with poorly controlled asthma (ACT < 20) and B-Eos with reduced lung function (FEV_1 < 80% of predicted), whereas both biomarkers strongly related to airway hyper-responsiveness (PD_{20} < 0.3 mg) (Figure VI).

Table II. Normal and abnormal clinical asthma outcomes used in univariate and multivariate analysis in different IgE groups.

Normal asthma outcomes	Abnormal asthma outcomes
ACT ≥ 20	ACT < 20
FEV_1 ≥ 80% predicted	FEV_1 < 80% predicted
No recent asthma attacks	Recent asthma attacks
PD_{20} ≥ 0.3 mg	PD_{20} < 0.3 mg

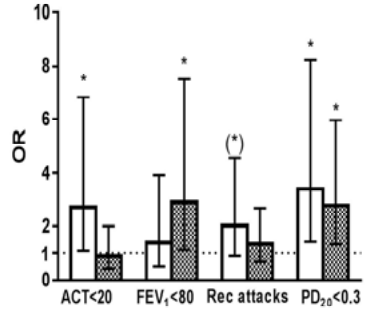

Figure VI. Adjusted odds ratios (OR) (95% CI) with four outcome variables (ACT, FEV_1 % predicted, recent asthma attacks, and PD_{20}) and two independent variables (open bars: FeNO%; filled bars: B-Eos) in asthmatics with IgE antibodies ≥ 0.35 kU_A/L. Results were also adjusted for confounders. *: $p < 0.05$, (*): $p < 0.10$.

Associations between Th2 biomarkers and asthma outcomes in asthmatics with IgE < 0.35 kU_A/L

In univariate analyses in subjects with IgE < 0.35 kU_A/L, FeNO% was related to reduced lung function, and B-Eos to recent asthma attacks (Table III). FeNO% maintained its relation to reduced lung function in subjects with IgE antibody concentrations between 0.10 and 0.34 kU_A/L. In multivariate analyses, an independent association between FeNO% and reduced lung function remained. A trend ($p < 0.10$) for an association between FeNO% and uncontrolled asthma was also observed. Ongoing asthma medication did not associate with the clinical outcomes in this group.

Table III. Differences in type-2 biomarkers between normal and abnormal clinical asthma outcomes (recent asthma attacks, FEV_1 % predicted in asthmatics with IgE antibodies < 0.35 kU_A/L).

	No recent asthma attacks (n = 44)	Recent asthma attacks (n = 38)	p value
FeNO (%)	79.9 (67.1, 95.2)	93.2 (72.7, 119)	0.299
B-Eos ($x10^9$)	0.124 (0.980, 0.157)	0.124 (0.095, 0.162)	0.018
	$FEV_1 \geq 80\%$ predicted (n = 67)	$FEV_1 < 80\%$ predicted (n = 15)	p value
FeNO (%)	78.6 (68.9, 89.6)	127 (73.5, 220)	0.010
B-Eos ($x10^9$)	0.122 (0.012, 0.147)	0.134 (0.081, 0.215)	0.721

Comparison of low and undetectable subgroups

In univariate analyses including the same variables as above, the relation between FeNO% and reduced lung function observed in asthmatics with IgE levels < 0.35 kU_A/L remained in the group with detectable IgE levels (between 0.10 and 0.34 kU_A/L). In the same group, B-Eos was found to relate to airway hyper-responsiveness. These associations disappeared when the same analyses were performed in the group with undetectable IgE antibodies (< 0.10 kU_A/L).

Study II

Subject characteristics

The group with non-atopic asthma (NAA) was characterized by increased airway responsiveness to methacholine and reduced FEV_1/FVC ratio compared with the group of non-atopic controls (NAC). Subjects with NAA displayed lower levels of type-2 biomarkers and scored lower on mAQLQ than atopic asthmatic subjects (AA) (Table IV). Asthma medication use, lung function, and airway responsiveness did not differ between NAA and AA subjects.10% of the group with AA reported milk-induced gastrointestinal symptoms, whereas such symptoms characterized the entire NAA group. Further, subjects with NAA did not report any anaphylaxis or symptoms from the upper airways, but more frequently reported symptoms from the lower airways than subjects with AA. Subjects with NAA had lower levels of IgA antibodies to casein compared with NAC. In the group with undetectable IgE antibodies (below 0.10 kU_A/L), the NAA group showed a trend toward lower mAQLQ ($p = 0.069$), compared with the non-atopic asthmatic group without perceived cow's milk hypersensitivity (n = 34).

Table IV. Characterization of the three groups included in the study. Comparison of non-atopic asthmatics (NAA) to healthy controls (NAC) and atopic asthmatics (AA).

	NAA (n = 24)	NAC (n = 24)	AA (n = 29)	p value NAA–NAC	p value NAA–AA
Females (%)	60	54.1	48.2	0.687	0.398
Height (cm)	163 ± 13	172 ± 11.5	166 ± 14.3	0.021	0.553
FeNO (ppb)	9.78 (8.15, 11.7)	10.5 (9.01 12.3)	29.2 (19.4, 44.2)	0.534	<.001
Total IgE (kU_A/L)	22.8 (14.1, 36.6)	15.6 (8.81, 27.7)	458 (334, 628)	0.300	<.001
ICS (µg)	438 (312, 615)	-	395 (307,509)	-	0.876
mAQLQ	5.17 ± 1.20	-	5.74 ± 0.821	-	0.045
FEV_1/FVC	82.6 (79.5, 85.8)	87.7 (85.4, 90.1)	80.5 (76.8, 84.2)	0.011	0.402
PD_{20} (mg)	0.677 (0.297,1.54)	4.56 (3.25, 6.41)	0.393 (0.130, 1.19)	<.001	0.422

Exploratory proteomic analyses

Based on multivariate ANOVA and correcting for multiple testing, MMP-1 was the only one out of the 92 inflammation-related proteins that maintained a trend when comparing mean group levels ($p = 0.10$). Therefore, correlation analyses were done between MMP-1 and clinical variables (see below). In univariate analyses, MMP-1 displayed higher levels in subjects with NAA than in either NAC or subjects with AA (Figure VII).

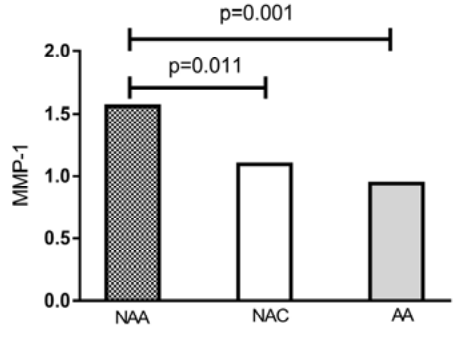

Figure VII. Levels of MMP-1 in the included study groups (concentrations in NPX).

Correlations between potential biomarkers and clinical outcomes

In subjects with NAA, HNL correlated negatively with airway responsiveness and CRP correlated negatively with both ACT and mAQLQ scores (Table V). In this group, type-2 biomarkers did not correlate with clinical outcomes. Instead, in subjects with AA, the type-2 biomarkers FeNO, B-Eos, and S-ECP all correlated negatively with PD_{20}, and total IgE was found to correlate negatively with FEV_1. Further, a negative correlation between MMP-1 and ACT score was found, whereas CRP correlated positively with PD_{20} in the group with AA (Table V).

Table V. Spearman's correlations between clinical outcomes and inflammatory biomarkers in groups with NAA and AA. * $p < 0.05$, ** $p < 0.01$, $^{(*)}$ $p < 0.10$.

NAA	ACT	mAQLQ	PD_{20}
CRP	-0.402*	-0.439*	-0.299
HNL	0.144	-0.030	-0.048*
MMP-1	-0.359$^{(*)}$	-0.320	-0.030
FeNO	-0.081	-0.003	-0.152
AA			
CRP	-0.089	-0.018	0.421*
HNL	-0.075	-0.142	0.213
MMP-1	-0.038*	-0.225	0.184
FeNO	-0.049	-0.011	-0.725**

Study III

Subject characteristics and changes in inflammatory biomarkers and clinical outcomes over time

A comparison was made of the clinical and inflammatory variables at baseline and follow-up, 43{23–65} months later, in asthmatic subjects. Higher absolute FeNO, lower total IgE, and reduced lung function were observed at follow-up. However, the increase in FeNO disappeared after adjusting for body height (FeNO%). Further, improvements in ACT and mAQLQ scores were noted, LTRAs were used more frequently, and higher ICS doses were used at the follow-up visit.

Longitudinal changes in inflammatory biomarkers and clinical outcomes according to baseline IgE-antibody levels

The changes in clinical and biomarker variables were studied after stratification of the asthmatics based on baseline IgE-antibody concentrations (Table VI). A significant decline in lung function was observed in the atopic group (IgE $\geq$ 0.35 kU_A/L). A lung function decline remained in the detectable IgE group (IgE 0.10–0.34 kU_A/L), but not in the undetectable IgE group (IgE < 0.10 kU_A/L). Total IgE was the only inflammatory variable with a significant longitudinal change (decline in the atopic group).

Table VI. Δ values of inflammatory biomarkers and clinical outcomes in asthmatic subjects in different groups based on IgE concentrations (kU_A/L). P values are presented for comparisons within each group.

Δ values	IgE ≥ 0.35 n = 202		IgE = 0.10–0.34 n = 22		IgE < 0.10 n = 29	
FEV_1/FVC (%)	-1.11	p 0.047	-3.43	p <.001	-1.20	p 0.413
mAQLQ	+0.145	p 0.016	+0.590	p 0.003	+0.254	p 0.217
Total IgE	-105	p 0.042	-5.20	p 0.558	-2.62	p 0.405

Association between longitudinal changes in clinical and inflammatory variables

In the unadjusted analyses, significant negative correlations between changes in B-Eos, FeNO% and $\Delta FEV_1/FVC$ were observed. Further investigations of the relationship between the change in FEV_1 and FEV_1/FVC, and inflammatory variables were done, using different multiple linear regression models adjusted for confounding factors. Changes in FeNO% related independently to changes in FEV_1 and FEV_1/FVC, respectively, in the entire asthma population (n = 253). Further, these associations remained in a different model including asthmatics with IgE level ≥ 0.10 kU_A/L (n = 224) (Figure VIII). Performing the same multivariate analyses in healthy controls (IgE ≥ 0.10 kU_A/L; n = 32) did not result in any significant associations between changes in lung function and inflammatory variables.

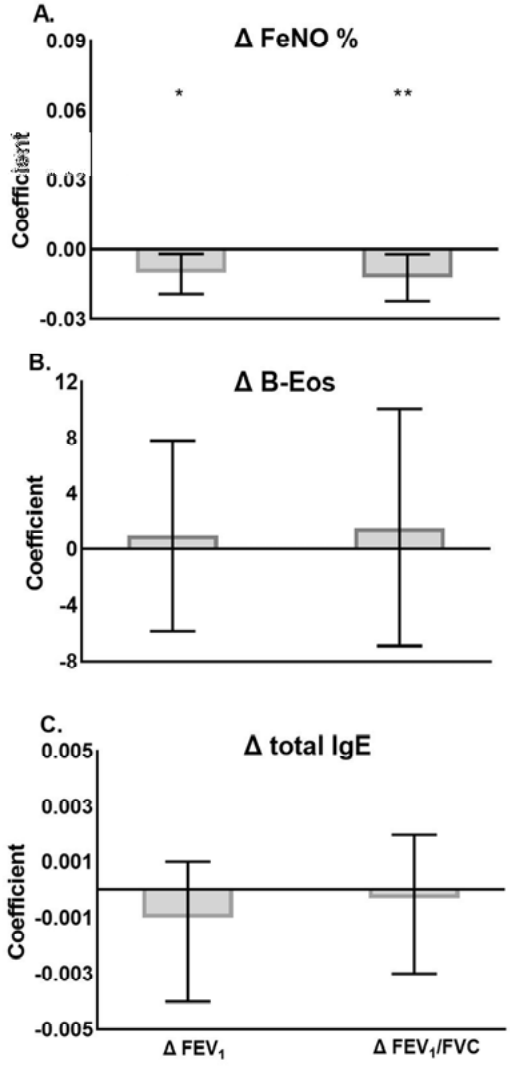

Figure VIII. Coefficient factors (beta(95% CI)) for Δ inflammatory biomarkers in relation to changes in FEV_1 and FEV_1/FVC, respectively, in multiple regression analysis performed in asthmatics (n= 224); (IgE ≥ 0.10 kU_A/L). Results were adjusted for confounders. * $p < 0.05$, ** $p < 0.01$.

The significant relationship between FeNO% and lung function in asthmatics with IgE antibodies below 0.35 kU_A/L, was a finding common to Studies I and III (Figure IX).

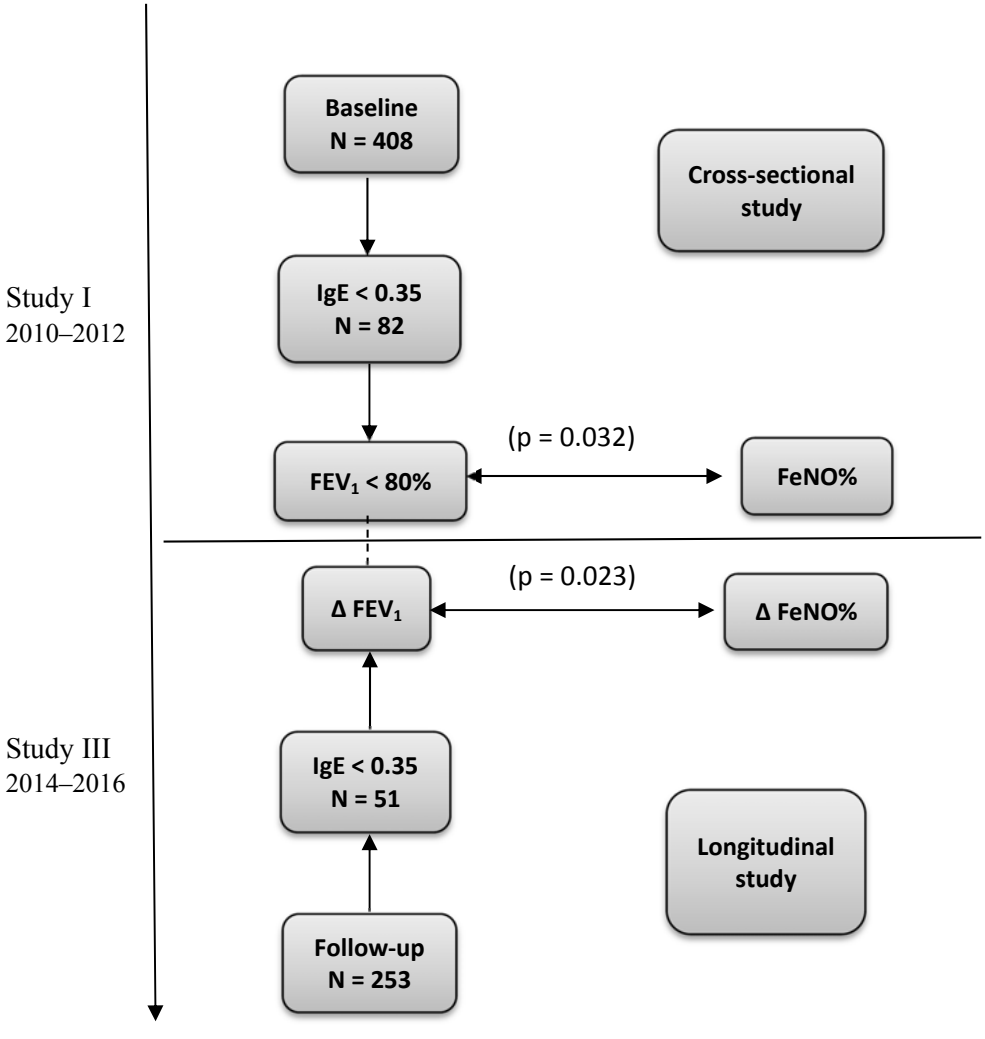

Figure IX. Illustration of the association between FEV_1 and FeNO% in asthmatics at baseline and follow-up visits (IgE in kU_A/L).

Study IV

Prevalence of IgE sensitization against cat allergens

In all, 96% (n = 255) of furry animal-sensitized asthmatics were sensitized to cat dander (Figure X). Fel d 1 was the most frequent and Fel d 2 the least frequent component that cat-sensitized asthmatics reacted to. Fel d 1 was the component with the highest prevalence of mono-sensitization (28%). Subjects co-sensitized to Fel d 1 and Fel d 2, or Fel d 1 and either Fel d 4 or Fel d 7, showed higher prevalence of recent asthma attacks compared with subjects mono-sensitized to Fel d 1.

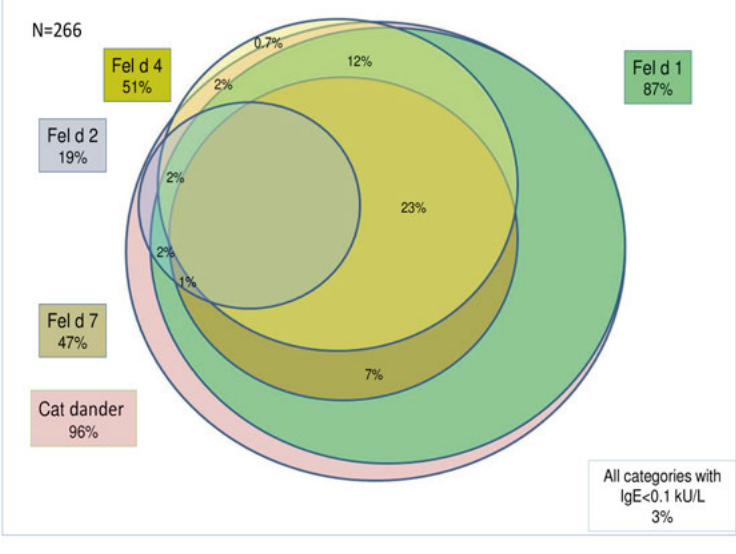

Figure X. Sensitization pattern of cat allergen components in young asthmatics (n = 266). Combinations of the following categories are not shown: Fel d 7+ Cat dander (1.8%) ; Fel d 2+ Fel d 4 (0.4%) ; and Cat dander + Fel d 4+ Fel d 7 + Fel d 2 (0.4%) (Reproduced with permission from the publisher).

Univariate analyses

Univariate analyses were performed of cat-sensitized asthmatics comparing IgE-antibody levels to different cat allergens, based on normal or elevated inflammatory asthma variables (FeNO, B-Eos), and clinical asthma outcomes (PD_{20}, ACT, FEV_1, asthma attacks). IgE to cat dander showed the highest concentrations, whereas IgE to Fel d 2 had the lowest concentrations. Weak correlations were noted between IgE levels to the different cat allergen components, and FeNO and B-Eos ($r = 0.18–0.29$, $p < 0.05$), whereas IgE to all different cat allergens related strongly to airway hyper-responsiveness.

Multivariate analyses

The relationship between sensitization to different allergen components and inflammatory variables was investigated using two different multivariate models. When introducing IgE to Fel d 1, Fel d 2 and Fel d 4 in the same model, a significant relationship was observed between IgE to Fel d 2 and FeNO, and between Fel d 4 and B-Eos (Figure XI). In a second model with IgE to Fel d 2 and the sum of IgE to Fel d 4 and Fel d 7 (Fel d 4 + 7) as independent variables, FeNO was significantly associated with both Fel d 4 + 7 and Fel d 2. Total IgE associated independently with all IgEs against cat allergens included in the two models, except Fel d 1, which did not associate with any of the outcome variables.

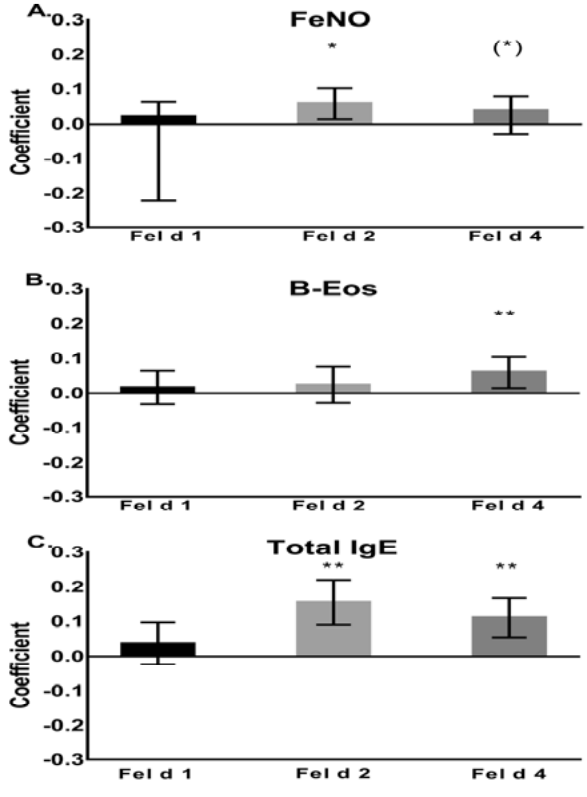

Figure XI. Multiple linear regression coefficient factors (95% CI) with three independent variables (Fel d 1, Fel d 2, Fel d 4) and three dependent variables (FeNO, B-Eos, total IgE) in cat-sensitized asthmatics (IgE ≥ 0.10 kU_A/L). *: $p < 0.05$, **: $p < 0.01$, (*): $p < 0.10$ (reproduced with permission from the publisher).

Discussion

Presence of type-2 and non-type 2 inflammation in relation to pattern of IgE sensitization in a cohort of young asthmatics

Study I revealed important findings regarding the relation between type-2 biomarkers and clinical outcomes in asthma patients with IgE-antibody concentrations found to be < 0.35 kU_A/L in conventional multi-allergen tests (Phadiatop and fx5). An association between type-2 inflammation and clinical outcomes was found also in subjects with IgE antibodies between 0.10 and 0.34 kU_A/L, but not in patients with IgE below 0.10 kU_A/L. The latter group of subjects seems to represent a distinct asthma phenotype. As expected, in the group with IgE ≥ 0.35 kU_A/L, traditionally defined as atopic, strong relationships were observed between two commonly used type-2 biomarkers, FeNO and B-Eos, and asthma outcomes. FeNO and B-Eos have been shown to associate independently with asthma morbidity, and provide additive information in relation to asthma outcomes [26]. When further dividing subjects based on IgE-antibody levels, these relationships were maintained in subjects with IgE antibodies ≥ 0.10 kU_A/L, but were virtually absent in subjects with IgE < 0.10 kU_A/L.

In Study II, subjects traditionally denoted non-atopic asthmatics, with perceived cow's milk hypersensitivity, were characterized by airway hyperresponsiveness and reduced lung function, and reported poorer asthma-related quality of life compared with subjects with atopic asthma, despite the absence of type-2 inflammatory signals. This is in line with a previous study where lower asthma-related quality of life was reported by non-atopic subjects with any food hypersensitivity [49]. Further, clinical outcomes such as airway responsiveness as well as asthma control and asthma-related quality of life, associated with neutrophil count, CRP, HNL, IL-8, and MMP-1 in blood, revealing clinically significant signals of non-type 2 inflammation in this group of non-atopic asthmatics.

Interestingly, MMP-1 was found to correlate with asthma control in the group with atopic asthma, in addition to the expected increased levels of type- 2 biomarkers. This suggested the existence of markers involved in both type-2 and non-type 2 inflammatory mechanisms. The observed findings in Study II strengthened the results from Study I, confirming the occurrence of

asthma in subjects with very low IgE levels but with a distinct inflammatory pattern.

Type-2 inflammatory biomarkers and so-called atopic asthma

The biomarker of local type-2 inflammation used in the present studies, i.e., FeNO, associated independently with reduced lung function in the group of asthmatics with IgE below 0.35 kU_A/L. Further, the group with detectable IgE antibody levels (0.10–0.34 kU_A/L) had significantly higher FeNO than the group with undetectable IgE antibodies (IgE below 0.10 kU_A/L). As previously reported, elevated FeNO was found among non-atopic (defined using traditional criteria) subjects with asthma compared with controls [42, 43]. Similar studies have shown trends for elevated FeNO in so-called non-atopic asthma [107-109]. In Study I, it was also demonstrated that B-Eos as a marker of systemic eosinophilic inflammation associated with recent asthma attacks in the group with IgE below 0.35 kU_A/L. This supported data from Karakoc *et al.* showing that blood eosinophilia was a risk factor for asthma, independent of atopy [110]. One could argue that eosinophilic inflammation implicates other factors than allergy, such as infections [19]. A previous study, based on the same material as that used in these studies, showed that a trend towards elevated S-ECP levels was associated with the presence of rhinovirus in the airways without ongoing respiratory symptoms [25]. Circulating ILC2 seem important in the control of eosinophilic inflammation [32], and it has been shown that ILC2 play a key role in the connection between rhinovirus infection and asthma exacerbations [53].

The measurement of total IgE revealed four times higher levels in the detectable IgE group than in the undetectable IgE group (based on IgE antibody levels), despite a minimal difference between absolute IgE-antibody concentrations calculated as the sum of results from the two multi-allergen tests. This finding reinforces evidence from population-based studies where total IgE has been considered a marker for asthma, regardless of the presence of atopy [11, 111]. It could be speculated that the two multi-allergen tests used in these studies failed to recognize and detect all IgE antibodies. An alternative explanation could be a possible induction of total IgE by bacterial enterotoxins, a finding that has been shown previously in asthmatics, and was unrelated to atopy [112]. However, the association between asthma and sensitization to these enterotoxins is clearer in adults than in children and adolescents [113].

The results emphasize the need to define a new cut-off for ruling out atopy. Since the described relationships were only seen in asthmatics with IgE antibodies ≥ 0.10 kU_A/L, while disappearing in the undetectable IgE group,

the suggestion was made to use of a cut-off of 0.10 kU_A/L for ruling out type-2 inflammation or IgE-mediated disease.

Clinical signals and biomarkers of non-type 2 inflammation among young asthmatics

A novel finding of Study II was that non-atopic asthmatics with perceived cow's milk hypersensitivity displayed significantly higher levels of MMP-1 than atopic asthmatics and non-atopic controls. Prior studies have shown that MMP-1 secretion may be regulated by Th2 cytokines, proposing MMP-1 as a mediator of airway hyper-responsiveness in allergen-induced asthma [114, 115]. Other interesting observations in the non-atopic group were the inter-correlations between FeNO, MMP-1, and C-X-C motif chemokine ligand (CXCL9). These were not seen in the group with atopic asthmatics. CXCL9 is a T-cell chemoattractant induced by IFN-γ [116] and may be considered a dual marker, as it is implicated in both type-2 and non-type 2 bronchial inflammation in asthma [117, 118]. Furthermore, IFN-γ has been suggested to be involved in the regulation of basal NO formation in the airway epithelium [19]. In atopic asthmatics, NO formation in the respiratory tract will be strongly influenced by Th2 cytokines, dominating the effect of IFN-γ. Thus, it could be speculated that the interrelationship between FeNO and CXCL9, and FeNO and MMP-1 in non-atopic asthmatics might be IFN-γ-dependent. However, this hypothesis could not be confirmed, since most of the observations were below the detection limit for IFN-γ.

Some correlations between clinical asthma outcomes and biomarkers were observed only in non-atopic asthmatics, indicating an asthma endotype distinct from the type-2 spectrum. For example, HNL correlated with PD_{20}, and CRP with both mAQLQ and ACT. HNL and CRP are well-known markers for distinguishing bacterial from viral infections [119-121]. However, these two markers have also been involved in the prediction of asthma severity [122, 123]. In addition, interesting data have been presented showing the potential role of neutrophilic inflammation in corticosteroid-resistant asthma, implicating IL-8 as a potent mediator [124, 125]. The non-atopic asthmatics were characterized by airway hyper-responsiveness and systemic inflammation, despite the use of asthma medication, However, since corticosteroids may fail to ameliorate neutrophilic asthma [126], and established biomarkers for systemic neutrophilia are lacking [127], advances need to be made for new treatment strategies. A common problem in studying the role of neutrophils in asthma is smoking among patients [128]. In this cohort, the prevalence of smoking was low (below 5%) and the multivariate analyses were adjusted for smoking.

Non-type 2 inflammation and associations between clinical asthma outcomes and IgE sensitization

Data from Study II confirmed the presence of asthma, including methacholine hyper-responsiveness and reduced lung function, in the non-atopic asthmatics with very low IgE-antibody concentrations. Further, 58% of the non-atopic asthmatics with perceived cow's milk hypersensitivity had undetectable IgE- antibody levels (below 0.10 kU_A/L). In Study II, the subgroup with detectable IgE-antibody levels was compared with the group with undetectable IgE, but no differences in clinical or inflammatory outcomes were seen. Therefore, it is reasonable to believe that this distribution of IgE levels resembles that in the normal population. However, the low IgE concentrations do not seem to account for mechanisms explaining the asthma disease in this subgroup of asthmatics, just as they do not in healthy controls.

Evaluating the role of non-IgE-mediated cow's milk hypersensitivity in non-type 2 asthma

Non-IgE antibodies (IgA and IgG) against different cow's milk proteins were studied, but no elevated levels were found in the group of non-atopic asthmatics. Instead, non-atopic asthmatics presented lower IgA levels against casein compared with non-atopic controls, a finding that could possibly be explained by avoidance of cow's milk due to the perceived symptoms. Despite these observations, the poorer asthma-related quality of life in this group remains an important clinical feature, indicating a need for reevaluation of asthma medication.

The longitudinal evolution of type-2 biomarkers and clinical outcomes in young asthmatics

In Study III, absolute FeNO was the only type-2 biomarker found to be elevated at a median of 43 months after baseline visit. However, FeNO values increase linearly in parallel with somatic growth, plateauing only after puberty [99]. Consistent with results of a previous study, where absolute FeNO associated independently with body height, the longitudinal increase in FeNO disappeared when adjusting absolute FeNO for height (FeNO%) [129]. The decrease seen in total IgE was in line with that in a large population-based study [130]. An age-related decline in T-cell function could hypothetically explain the fall in total IgE. However, since both B and T cells participate in the formation of total IgE, it is unclear to what extent the age-related decline in total IgE is T cell-dependent [131, 132].

There are very few longitudinal studies of asthma biomarkers and clinical outcomes. Silkoff *et al*. studied the variability in clinical characteristics and clinical asthma biomarkers over 12 months, demonstrating individual stability in low FeNO, but substantial variability in B-Eos [59]. In Study III, the

asthmatic subjects were characterized by lung function decline and higher use of asthma medication, although they had improved asthma control and reported fewer recent asthma attacks at follow-up. These findings will be further discussed below.

Longitudinal changes in asthma biomarkers after stratification into different IgE groups

The main finding derived from the stratification into different IgE groups was that a significant decline in lung function over time was present in groups with elevated and detectable IgE, respectively, but not in the undetectable IgE group. This observation is in agreement with Study I, underlining the presence of type-2 inflammation in patients with low but detectable IgE levels.

The investigation of type-2 biomarkers in the different IgE groups revealed that FeNO% tended to increase in both asthmatics and healthy controls with elevated IgE levels, but not in the other IgE groups. An earlier longitudinal study on asthmatics demonstrated that absolute FeNO maintained its stability over time despite varying baseline levels, and that FeNO is a biomarker indicating atopy regardless of asthma symptoms or ongoing asthma medication [133]. In Study III, the higher use of ICS among asthmatics at follow-up could be a reasonable explanation for the absence of association between FeNO and atopy, supporting previous reports on disruption of the relationship between FeNO and IgE sensitization due to high ICS doses [23].

Relationships between changes in type-2 biomarkers and clinical outcomes over time

The lack of correlation between change in height-adjusted FeNO, and ACT and mAQLQ scores, together with the fact that the asthmatics used higher ICS doses at follow-up, added support to the reported inability of FeNO to predict asthma control in subjects with high-dose ICS regimens [60]. In contrast, the longitudinal change in height-adjusted FeNO related independently to the decline in lung function in asthmatics with IgE $\geq$ 0.10 kU_A/L. This observation corroborated previous results from Study I, where FeNO% associated independently to reduced lung function in asthmatics with IgE below 0.35 kU_A/L. Together, the findings highlighted the existence and persistence of type-2 inflammation in asthmatics traditionally denoted non-atopic. Such inflammation is related to accelerated lung function decline. The significant correlation between longitudinal changes in lung function and B-Eos in well-controlled asthmatics may be an essential finding; this has scarcely been studied before. However, Brightling and George have discussed the absence of an association between eosinophilic inflammation and decline in lung function [134], and the correlation between B-Eos and lung function in Study III disappeared in multivariate analyses incorporating other type-2

biomarkers as well. It should be mentioned that in Study III, subject groups were not generated based on cut-off values of B-Eos, since the vast majority of the study sample consisted of well-controlled asthmatics with good asthma control. Such a classification could possibly identify subjects with severe eosinophilic asthma [135].

The relationship between longitudinal changes in total IgE and lung function development seems to be more complicated. A positive association between high levels of total IgE measured at baseline and lung function decline over time has been reported before, but there is a lack of data connecting the deterioration of lung function to changes in the concentration of IgE, and with regard to atopic status [66, 136]. A contributing factor to the lung function decline over time is the normal aging process in the lungs, including decreased respiratory muscle strength, reduction in chest wall compliance, and a lung growth rate which decelerates by the age of 20–25 years [68, 137]. Thus, it remains to be established whether or not changes in total IgE constitute an independent factor for longitudinal changes in lung function.

Still, this finding corresponds to previous data showing that high total IgE at baseline is related to impaired lung function at baseline but not to decline of lung function over time, at least in ever smokers [66]. It seems that no previous research has described the relationships between the change in lung function and local (FeNO) and systemic inflammation (B-Eos) in asthma.

The importance of sensitization to cat allergen components for type-2 inflammation and disease severity

Sensitization to minor cat allergen components might be clinically relevant, as IgE antibodies to Fel d 2, Fel d 4 and Fel d 7 were independently associated with FeNO, B-Eos, and total IgE, in a population of well-controlled young asthmatics. In contrast, IgE against Fel d 1 and cat dander failed to associate with type-2 biomarkers and clinical asthma outcomes. A novel and clinically important finding was that asthmatic subjects co-sensitized to Fel d 2 and/or Fel d 4, in addition to Fel d 1, showed higher prevalence of reduced lung function and reported more frequent asthma attacks. This finding is supported by a recent study assessing co-sensitization to Fel d 1 and Fel d 4 as a risk factor for asthma symptoms [138]. The impact of cat allergens on lung function in co-sensitized subjects confirms findings from Studies I and III, that lung function constitutes a reliable clinical asthma outcome for studying disease progression.

Unlike asthma, rhinoconjuctivitis symptoms upon cat exposure are primarily related to the major cat allergen Fel d 1 [79]. Further, the presence of Fel d 1 particles in indoor environments constitutes a sensitive marker for cat ownership and asthma worsening [70]. Moreover, the combination of pas-

sive exposure to cat particles and high prevalence of sensitization to cat in Sweden has been connected to poorer asthma outcome among furry animal-sensitized asthmatics [139, 140]. In Study IV, cat particle analysis in the home was not performed, but including cat ownership as a confounder in the multivariate analyses did not affect the results. The study showed that asthmatics presented with high IgE-antibody concentrations against Fel d 1, but could not confirm that these IgE antibodies were independently related to type-2 inflammation. A recent population-based study, where an independent association between IgE to Fel d 1 and both FeNO and bronchial responsiveness was seen, contrasts with Study IV [141]. However, it is worth noting that a multi-array technology with lower sensitivity was used in the other study (detection limit 1.0 kU_A/L), and it is clear from data in Study IV that IgE-antibody concentrations below 1.0 kU_A/L for Fel d 2 and Fel d 4/7 are important drivers of inflammation and asthma.

Pathophysiological implications

The complexities in the immunological backgrounds may also play a key role in the concentration difference of IgE antibodies to Fel d 1 and lipocalins (Fel d 4 and Fel d 7) that was observed in Study IV. The binding capacity of Fel d 1 to dendritic cells might explain the high allergenicity of this allergen [142]. On the other hand, lipocalin allergens activate the cellular immune system poorly, due to the existence of endogenous human lipocalins, thus resulting in lower IgE antibody responses [143]. Another possible explanation could be the activation of innate immune mechanisms, which lead to stimulation of dendritic and Th2 cells [144]. The finding in Study IV of high airway hyper-responsiveness in subjects with good asthma control may be the result of innate mechanisms targeting proteins with lower allergenicity, i.e., lipocalins and serum albumin.

Previous studies have reported alternative pathways for sensitization to minor cat allergens, increasing the risk for respiratory symptoms. For example, inflamed skin in cat allergic children with atopic eczema might be a pathway for sensitization towards Fel d 2 and Fel d 4, leading to wheeze development [145]. In addition, sensitization to furry animals' serum albumin, including Fel d 2, has been shown to associate with asthma severity [146]. This observation corresponds to results in Study IV, showing that Fel d 2 strongly relates to FeNO, in terms of type-2 inflammation. Since Fel d 2 is known for its cross-reactivity with porcine albumin, it may be hypothesized that pork ingestion can act as a trigger factor for driving type-2 inflammation [147, 148], although there was no information on allergic reactions to pork ingestion in the study.

It is well-known that elevated total IgE is related to asthma and asthma morbidity, independent of the degree of IgE sensitization [38]. The results showed no association between IgE to the major cat allergen Fel d 1 and total IgE, but instead strong independent associations between IgE to Fel d 2 or Fel d 4 and total IgE. A putative immunological explanation, which may

relate to innate cellular mechanisms in type-2 asthma, is that IgE to minor cat allergen components is part of increased polyclonal IgE formation, with both specific and non-specific IgE, leading to elevated levels of total IgE. This polyclonal IgE formation has been studied before, in an attempt to identify and compare two different IgE fractions within the paradigm of atopy [149]. On the other hand, Burrows and co-workers have presented data where IgE sensitization using crude allergen extracts associated with allergic rhinitis, independent of total IgE levels [11].

Clinical use of cat allergen components

A few studies have looked at the possibility of increasing the sensitivity in detection of cat allergy or IgE sensitization by using allergen components instead of crude allergen extract [76]. These studies reported lower sensitivity for Fel d 1 compared with extract using a cut-off of 0.35 kU_A/L. To our knowledge, no previous study had reported on IgE antibodies against cat allergen components below 0.35 kU_A/L. However, when using a cut-off of 0.10 kU_A/L, it was not possible to identify more IgE-sensitized subjects using cat allergen components than using cat dander extract.

In Study IV, we suggested that IgE to minor cat allergen components should be considered a biomarker primarily for endotyping innate cellular mechanisms, linked to persistent airway inflammation. The application of the measurement of IgE to minor cat allergen components for predicting asthma morbidity in clinical practice should be further evaluated in future studies.

The value of asthma medication in true non-atopic asthma and in the progress of asthma outcomes

In Study I, comparing the detectable and undetectable IgE groups revealed that the latter group used higher daily doses of ICS and more often used LTRA, although there were no significant differences in clinical outcomes between these two groups. The higher use of ICS may have contributed to the very low FeNO values noted in the undetectable IgE group. This observation suggests overtreatment of the undetectable IgE group, which seems to be relatively ICS-resistant. It was suggested that type-2 inflammation, and thus potentially ICS-responsive disease, cannot be ruled out by using the traditional cut-off of 0.35 kU_A/L. We have though recognized that the material was based on young asthmatics, in whom non-atopic asthma is considered to be relatively uncommon [48]. However, it should be noted that non-atopic asthmatics belonging to the undetectable IgE group comprised almost 12% of the asthma population.

In Study III, the asthmatic subjects used more asthma medication at the 43-month follow-up, despite a lower frequency of asthma attacks and improved asthma control, which is difficult to interpret. Hypothetically, im-

proved medication adherence as a study effect could explain these findings. Another reasonable theory could be that the transition from childhood to adulthood asthma as the study population aged led to higher ICS use, since guidelines recommend that adults take higher ICS doses for maintaining asthma control. At very least, this highlights the difficulties in understanding the asthma disease and its progress over time, especially in patients going from childhood to adulthood.

Study limitations

A limitation of Study I may be that the majority of the sample had well-controlled asthma, resulting in few recorded asthma attacks. Another potential limitation of this study is that the age range of patients and their early-onset of the disease meant that it was not possible to investigate the phenotype of late-onset asthma, which is characterized by high total IgE combined with absence of atopy [36].

In Study II, the small number of the subjects in the three age- and sex-matched groups may have been a limitation. The self-reported cow's milk hypersensitivity symptoms might also be considered a limitation. Furthermore, the symptoms interpreted as non-IgE-mediated cow's milk hypersensitivity can be unspecific and may have been due to other gastrointestinal disorders, such as lactose intolerance or irritable bowel syndrome. However, only a few subjects reported gastrointestinal symptoms not related to perceived food hypersensitivity. Still, it is acknowledged that the use of perceived food hypersensitivity symptoms may constitute a weakness, since there is a discrepancy between self-reported and objectively confirmed food hypersensitivity symptoms [150] [151].

A possible limitation of Study III was the loss to follow-up of the asthmatic subjects (38%) and healthy controls (44%) at the follow-up visit at 43 months, which may have led to selection bias. Further, the absence of a methacholine challenge test at the follow-up prevented investigation into the evolution of airway hyper-responsiveness over time.

In Study IV, the lack of longitudinal data did not allow for the inclusion of factors such as age change and cumulative cat allergen exposure, which may influence the observed associations over time. Another limitation may be the high cross-reactivity of lipocalins and serum albumins between different furry animals, which was not considered in the study.

Future research

We believe that the management of childhood asthma can be greatly improved by the introduction of endotype-specific biomarkers and thorough mapping of the IgE sensitization profile. We suggest that a combination of biomarkers should be used to better understand the pathogenesis and natural course of asthma. This will give further guidance for tailoring individual treatment approaches. Future studies will show if optimal (biomarker-guided) anti-inflammatory treatment, possibly in combination with allergen-avoidance regimens, may improve asthma control and long-term outcomes, such as better lung function and reduced allergen sensitivity. More specifically, longitudinal studies will help increase knowledge about the stability of the currently described phenotypes and thus help better define different endotypes. The undetectable IgE group signals the existence of true non-type 2 asthma, but constitutes a poorly investigated endotype. Clinical research could facilitate targeting of this asthma group and a substantial upgrade of future asthma care.

Proteomics, as a source of clinically assessable biomarkers, has the potential to add significant information related to asthma disease subtypes.

Conclusions

Important signs of type-2 inflammation were present in young asthmatic subjects with IgE-antibody levels below 0.35 kU_A/L. These signs were not seen in subjects with IgE-antibody levels below 0.10 kU_A/L. The following conclusions could be drawn:

1. A cut-off of 0.10 kU_A/L for IgE antibodies appeared to be useful in ruling out type-2 inflammation-driven disease and detecting true non-type 2 asthma. Airway hyper-responsiveness, reduced lung function, and low asthma-related quality of life characterized non-atopic young asthmatics with perceived cow's milk hypersensitivity. In these subjects, signs of type-2 inflammation were absent.

2. Newly explored biomarkers, such as MMP-1, HNL, CRP, and IL-8, indicated clinically significant non-type 2 inflammation in asthmatics with IgE below 0.35 kU_A/L.

3. Changes in height-adjusted FeNO over 43 months were associated with lung function decline in asthmatic subjects with IgE-antibody concentrations above 0.10 kU_A/L.

4. IgE sensitization to cat lipocalins and/or cat serum albumin ($\geq$ 0.10 kU_A/L) may promote both local and systemic type-2 inflammation. In contrast, the major cat allergen Fel d 1 did not relate to inflammatory and clinical outcomes in young asthmatics.

Acknowledgements

This work was carried out at the Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden. The financial support needed to perform these studies was obtained from: the Swedish Heart and Lung Foundation, Uppsala County Research Funding (FoU-medel), the Gillberg Foundation, the Swedish Foundation for Strategic research, the Hesselman´s Foundation, Thermo Fisher Scientific, and the Bror Hjerpstedt Foundation.

I would like to express my sincere gratitude to:

Kjell Alving, my main supervisor, for your endless patience and scientific guidance. I am greatly indebted for your continuous support, encouragement, and highly skilled supervising. Thank you for opening the door to research.

Andrei Malinovschi, my co-supervisor, for your valuable and indispensable contribution throughout this project. I am grateful for your fast reflections, advice, and excellent guidance in statistics.

Christophe Pedroletti, my co-supervisor and former Head of the Department of Children`s Hospital, for your inspiring ideas, and for sharing your expertise in the field of pediatric allergology.

Magnus Borres, Christer Janson, and Lennart Nordvall, co-writers, for your scientific advice, contributions, and important comments on the manuscripts you co-authored.

Pia Kalm-Stephens, for your contribution in building this cohort. It has been a pleasure working with you.

The current Heads of the Department, of Children´s Hospital, Klas Ekström and Gianni Celsi, for approving leave for clinical research. My colleagues, for their understanding and support during my research period.

Jonas Lidholm, Lars Mattsson, and Niclas Rydell, for your useful advice in the field of molecular allergology.

Carl Lindgren, my clinical supervisor during my residency years, for sharing your huge experience and valuable knowledge, for your friendship, and for helping me grow as pediatrician.

Staff at the lab and administrative staff for all your help.

Children and adults in the studied cohort, this project would have been impossible without your participation.

My family in Greece. For your endless love and belief in me. It is impossible to express my gratitude in words. Για το μεγάλο ευχαριστώ που δεν έχετε ακούσει ακόμη από μένα. Ο Θεός να με αξιώσει να προσφέρω στα παιδιά μου εστω και το ελάχιστο που πήρα από εσάς.

Lastly, and above all, my wife Anastasia: thank you for your love, encouragement and understanding even when I was physically at home but mentally doing research work. Thank you for taking care of our lovely daughters even when “ο μπαμπάς” was away. My beautiful girls, Aria, Anastasia and Stamatina, the pure meaning of my life, for your smiles and hugs, which made me forget every difficulty.

References

1. Reddel, H.K., et al., *A summary of the new GINA strategy: a roadmap to asthma control.* Eur Respir J, 2015. **46**(3): p. 622-39.
2. Bateman, E.D., et al., *Global strategy for asthma management and prevention: GINA executive summary.* Eur Respir J, 2008. **31**(1): p. 143-78.
3. Bacharier, L.B., et al., *Diagnosis and treatment of asthma in childhood: a PRACTALL consensus report.* Allergy, 2008. **63**(1): p. 5-34.
4. Gustafsson, P.M., et al., *Poor asthma control in children: evidence from epidemiological surveys and implications for clinical practice.* Int J Clin Pract, 2006. **60**(3): p. 321-34.
5. Corren, J., *Asthma phenotypes and endotypes: an evolving paradigm for classification.* Discov Med, 2013. **15**(83): p. 243-9.
6. Patelis, A., et al., *Population-based study of multiplexed IgE sensitization in relation to asthma, exhaled nitric oxide, and bronchial responsiveness.* J Allergy Clin Immunol, 2012. **130**(2): p. 397-402 e2.
7. Patelis, A., et al., *Aeroallergen and food IgE sensitization and local and systemic inflammation in asthma.* Allergy, 2014. **69**(3): p. 380-7.
8. Froidure, A., et al., *Asthma phenotypes and IgE responses.* Eur Respir J, 2016. **47**(1): p. 304-19.
9. Agache, I., et al., *Untangling asthma phenotypes and endotypes.* Allergy, 2012. **67**(7): p. 835-46.
10. Cordier, J.F., N. Freymond, and V. Cottin, *[Hypereosinophilic asthma].* Rev Prat, 2011. **61**(3): p. 325-6.
11. Burrows, B., et al., *Association of asthma with serum IgE levels and skin-test reactivity to allergens.* N Engl J Med, 1989. **320**(5): p. 271-7.
12. Gevaert, P., et al., *Omalizumab is effective in allergic and nonallergic patients with nasal polyps and asthma.* J Allergy Clin Immunol, 2013. **131**(1): p. 110-6 e1.
13. Campo, P., et al., *Phenotypes and endotypes of uncontrolled severe asthma: new treatments.* J Investig Allergol Clin Immunol, 2013. **23**(2): p. 76-88; quiz 1 p follow 88.
14. Szefler, S.J., et al., *Asthma outcomes: biomarkers.* J Allergy Clin Immunol, 2012. **129**(3 Suppl): p. S9-23.
15. Strimbu, K. and J.A. Tavel, *What are biomarkers?* Curr Opin HIV AIDS, 2010. **5**(6): p. 463-6.
16. Lotvall, J., et al., *Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome.* J Allergy Clin Immunol, 2011. **127**(2): p. 355-60.
17. van Ree, R., et al., *Allergic sensitization: host-immune factors.* Clin Transl Allergy, 2014. **4**(1): p. 12.
18. Kips, J.C., *Cytokines in asthma.* Eur Respir J Suppl, 2001. **34**: p. 24s-33s.
19. Alving, K., Malinovschi A, *Basic aspects of Nitric Oxide.* Eur Respir 2010. **49**: p. 1-31.

20. Guo, F.H., et al., *Continuous nitric oxide synthesis by inducible nitric oxide synthase in normal human airway epithelium in vivo.* Proc Natl Acad Sci U S A, 1995. **92**(17): p. 7809-13.
21. Lane, C., et al., *Epithelial inducible nitric oxide synthase activity is the major determinant of nitric oxide concentration in exhaled breath.* Thorax, 2004. **59**(9): p. 757-60.
22. Bjermer, L., et al., *Current evidence and future research needs for FeNO measurement in respiratory diseases.* Respir Med, 2014. **108**(6): p. 830-41.
23. Syk, J., A.L. Unden, and K. Alving, *Relationship between exhaled nitric oxide and IgE sensitisation in patients with asthma: influence of steroid treatment.* Clin Respir J, 2009. **3**(3): p. 143-51.
24. Moeller, A., et al., *Monitoring asthma in childhood: lung function, bronchial responsiveness and inflammation.* Eur Respir Rev, 2015. **24**(136): p. 204-15.
25. Ohrmalm, L., et al., *Presence of rhinovirus in the respiratory tract of adolescents and young adults with asthma without symptoms of infection.* Respir Med, 2016. **115**: p. 1-6.
26. Malinovschi, A., et al., *Exhaled nitric oxide levels and blood eosinophil counts independently associate with wheeze and asthma events in National Health and Nutrition Examination Survey subjects.* J Allergy Clin Immunol, 2013. **132**(4): p. 821-7 e1-5.
27. Han, M., et al., *The Innate Cytokines IL-25, IL-33, and TSLP Cooperate in the Induction of Type 2 Innate Lymphoid Cell Expansion and Mucous Metaplasia in Rhinovirus-Infected Immature Mice.* J Immunol, 2017. **199**(4): p. 1308-1318.
28. Strunk, R.C., et al., *Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children.* J Allergy Clin Immunol, 2003. **112**(5): p. 883-92.
29. Spahn, J.D., et al., *Effect of montelukast on peripheral airflow obstruction in children with asthma.* Ann Allergy Asthma Immunol, 2006. **96**(4): p. 541-9.
30. Currie, G.P., et al., *Effects of low dose fluticasone/salmeterol combination on surrogate inflammatory markers in moderate persistent asthma.* Allergy, 2003. **58**(7): p. 602-7.
31. Malinovschi, A., et al., *Simultaneously increased fraction of exhaled nitric oxide levels and blood eosinophil counts relate to increased asthma morbidity.* J Allergy Clin Immunol, 2016. **138**(5): p. 1301-1308 e2.
32. Liu, T., et al., *Type 2 innate lymphoid cells: A novel biomarker of eosinophilic airway inflammation in patients with mild to moderate asthma.* Respir Med, 2015. **109**(11): p. 1391-6.
33. Vatrella, A., et al., *Dupilumab: a novel treatment for asthma.* J Asthma Allergy, 2014. **7**: p. 123-30.
34. Lazic, N., et al., *Multiple atopy phenotypes and their associations with asthma: similar findings from two birth cohorts.* Allergy, 2013. **68**(6): p. 764-70.
35. Kurukulaaratchy, R.J., S. Matthews, and S.H. Arshad, *Defining childhood atopic phenotypes to investigate the association of atopic sensitization with allergic disease.* Allergy, 2005. **60**(10): p. 1280-6.
36. Frith, J., et al., *The complexities of defining atopy in severe childhood asthma.* Clin Exp Allergy, 2011. **41**(7): p. 948-53.
37. Schoos, A.M., et al., *Disagreement between skin prick test and specific IgE in young children.* Allergy, 2015. **70**(1): p. 41-8.
38. Sunyer, J., et al., *Total serum IgE is associated with asthma independently of specific IgE levels. The Spanish Group of the European Study of Asthma.* Eur Respir J, 1996. **9**(9): p. 1880-4.

39. Humbert, M., et al., *The immunopathology of extrinsic (atopic) and intrinsic (non-atopic) asthma: more similarities than differences.* Immunol Today, 1999. **20**(11): p. 528-33.
40. van der Valk, R.J., et al., *Daily exhaled nitric oxide measurements and asthma exacerbations in children.* Allergy, 2012. **67**(2): p. 265-71.
41. Dahlen, B., et al., *Effect of formoterol with or without budesonide in repeated low-dose allergen challenge.* Eur Respir J, 2009. **33**(4): p. 747-53.
42. Ekroos, H., et al., *Equally elevated concentrations of exhaled nitric oxide in nonatopic and low-sensitized atopic asthmatics.* Respir Med, 2009. **103**(1): p. 152-8.
43. Malmberg, L.P., et al., *Exhaled nitric oxide and exercise-induced bronchoconstriction in young wheezy children - interactions with atopy.* Pediatr Allergy Immunol, 2009. **20**(7): p. 673-8.
44. Dal Negro, R., et al., *Assessment of inhaled BDP-dose dependency of exhaled nitric oxide and local and serum eosinophilic markers in steroids-naive nonatopic asthmatics.* Allergy, 2003. **58**(10): p. 1018-22.
45. Pillai, P., C.J. Corrigan, and S. Ying, *Airway epithelium in atopic and nonatopic asthma: similarities and differences.* ISRN Allergy, 2011. **2011**: p. 195846.
46. Pekkanen, J., et al., *Analyzing atopic and non-atopic asthma.* Eur J Epidemiol, 2012. **27**(4): p. 281-6.
47. Sinisgalli, S., M.S. Collins, and C.M. Schramm, *Clinical features cannot distinguish allergic from non-allergic asthma in children.* J Asthma, 2012. **49**(1): p. 51-6.
48. de Nijs, S.B., L.N. Venekamp, and E.H. Bel, *Adult-onset asthma: is it really different?* Eur Respir Rev, 2013. **22**(127): p. 44-52.
49. Johnson, J., et al., *Perceived food hypersensitivity relates to poor asthma control and quality of life in young non-atopic asthmatics.* PLoS One, 2015. **10**(4): p. e0124675.
50. Wenzel, S.E., *Asthma phenotypes: the evolution from clinical to molecular approaches.* Nat Med, 2012. **18**(5): p. 716-25.
51. Wenzel, S.E., et al., *Bronchoscopic evaluation of severe asthma. Persistent inflammation associated with high dose glucocorticoids.* Am J Respir Crit Care Med, 1997. **156**(3 Pt 1): p. 737-43.
52. Robinson, D., et al., *Revisiting Type 2-high and Type 2-low airway inflammation in asthma: current knowledge and therapeutic implications.* Clin Exp Allergy, 2017. **47**(2): p. 161-175.
53. Jackson, D.J., et al., *IL-33-dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo.* Am J Respir Crit Care Med, 2014. **190**(12): p. 1373-82.
54. Hastie, A.T., et al., *Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes.* J Allergy Clin Immunol, 2010. **125**(5): p. 1028-1036 e13.
55. Schleich, F.N., et al., *Distribution of sputum cellular phenotype in a large asthma cohort: predicting factors for eosinophilic vs neutrophilic inflammation.* BMC Pulm Med, 2013. **13**: p. 11.
56. Moore, W.C., et al., *Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis.* J Allergy Clin Immunol, 2014. **133**(6): p. 1557-63 e5.
57. Schleich, F., et al., *Heterogeneity of phenotypes in severe asthmatics. The Belgian Severe Asthma Registry (BSAR).* Respir Med, 2014. **108**(12): p. 1723-32.

58. Wadsworth, S., D. Sin, and D. Dorscheid, *Clinical update on the use of biomarkers of airway inflammation in the management of asthma.* J Asthma Allergy, 2011. **4**: p. 77-86.
59. Silkoff, P.E., et al., *Longitudinal stability of asthma characteristics and biomarkers from the Airways Disease Endotyping for Personalized Therapeutics (ADEPT) study.* Respir Res, 2016. **17**: p. 43.
60. Michils, A., S. Baldassarre, and A. Van Muylem, *Exhaled nitric oxide and asthma control: a longitudinal study in unselected patients.* Eur Respir J, 2008. **31**(3): p. 539-46.
61. Price, D.B., et al., *Blood eosinophil count and prospective annual asthma disease burden: a UK cohort study.* Lancet Respir Med, 2015. **3**(11): p. 849-58.
62. Newby, C., et al., *Lung function decline and variable airway inflammatory pattern: Longitudinal analysis of severe asthma.* Journal of Allergy and Clinical Immunology, 2014. **134**(2): p. 287-+.
63. Ortega, H., et al., *Asthma Exacerbations Associated with Lung Function Decline in Patients with Severe Eosinophilic Asthma.* J Allergy Clin Immunol Pract, 2018. **6**(3): p. 980-986 e1.
64. Porsbjerg, C., P. Lange, and C.S. Ulrik, *Lung function impairment increases with age of diagnosis in adult onset asthma.* Respiratory Medicine, 2015. **109**(7): p. 821-827.
65. Barbee, R.A., et al., *Distribution of IgE in a community population sample: correlations with age, sex, and allergen skin test reactivity.* J Allergy Clin Immunol, 1981. **68**(2): p. 106-11.
66. Shadick, N.A., et al., *Relationship of serum IgE concentration to level and rate of decline of pulmonary function: the Normative Aging Study.* Thorax, 1996. **51**(8): p. 787-92.
67. Turner, S., et al., *A longitudinal study of lung function from 1 month to 18 years of age.* Thorax, 2014. **69**(11): p. 1015-20.
68. Sharma, G. and J. Goodwin, *Effect of aging on respiratory system physiology and immunology.* Clin Interv Aging, 2006. **1**(3): p. 253-60.
69. Konradsen, J.R., et al., *Predicting asthma morbidity in children using proposed markers of Th2-type inflammation.* Pediatr Allergy Immunol, 2015. **26**(8): p. 772-9.
70. Almqvist, C., et al., *Worsening of asthma in children allergic to cats, after indirect exposure to cat at school.* Am J Respir Crit Care Med, 2001. **163**(3 Pt 1): p. 694-8.
71. Langley, S.J., et al., *Exposure and sensitization to indoor allergens: association with lung function, bronchial reactivity, and exhaled nitric oxide measures in asthma.* J Allergy Clin Immunol, 2003. **112**(2): p. 362-8.
72. Nordlund, B., et al., *IgE antibodies to animal-derived lipocalin, kallikrein and secretoglobin are markers of bronchial inflammation in severe childhood asthma.* Allergy, 2012. **67**(5): p. 661-9.
73. Sears, M.R., et al., *Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children.* N Engl J Med, 1991. **325**(15): p. 1067-71.
74. Erwin, E.A., et al., *Contribution of dust mite and cat specific IgE to total IgE: relevance to asthma prevalence.* J Allergy Clin Immunol, 2007. **119**(2): p. 359-65.
75. Lindfors, A., et al., *Influence of interaction of environmental risk factors and sensitization in young asthmatic children.* J Allergy Clin Immunol, 1999. **104**(4 Pt 1): p. 755-62.

76. Eder, K., et al., *Usefulness of component resolved analysis of cat allergy in routine clinical practice.* Allergy Asthma Clin Immunol, 2016. **12**: p. 58.
77. Almqvist, C., et al., *Early predictors for developing allergic disease and asthma: examining separate steps in the 'allergic march'.* Clin Exp Allergy, 2007. **37**(9): p. 1296-302.
78. Illi, S., et al., *Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study.* Lancet, 2006. **368**(9537): p. 763-70.
79. Gronlund, H., et al., *The major cat allergen, Fel d 1, in diagnosis and therapy.* Int Arch Allergy Immunol, 2010. **151**(4): p. 265-74.
80. Saarne, T., et al., *Cat sensitization identified by recombinant Fel d 1 several years before symptoms--results from the BAMSE cohort.* Pediatr Allergy Immunol, 2010. **21**(2 Pt 1): p. 277-83.
81. Smith, W., et al., *Fel d 4, a cat lipocalin allergen.* Clin Exp Allergy, 2004. **34**(11): p. 1732-8.
82. Smith, W., et al., *Two newly identified cat allergens: the von Ebner gland protein Fel d 7 and the latherin-like protein Fel d 8.* Int Arch Allergy Immunol, 2011. **156**(2): p. 159-70.
83. Apostolovic, D., et al., *The cat lipocalin Fel d 7 and its cross-reactivity with the dog lipocalin Can f 1.* Allergy, 2016. **71**(10): p. 1490-5.
84. Cabanas, R., et al., *Importance of albumin in cross-reactivity among cat, dog and horse allergens.* J Investig Allergol Clin Immunol, 2000. **10**(2): p. 71-7.
85. Chruszcz, M., et al., *Serum albumins-unusual allergens.* Biochim Biophys Acta, 2013. **1830**(12): p. 5375-81.
86. Skamstrup Hansen, K. and L.K. Poulsen, *Component resolved testing for allergic sensitization.* Curr Allergy Asthma Rep, 2010. **10**(5): p. 340-8.
87. Houtman, R. and E. van den Worm, *Asthma, the ugly duckling of lung disease proteomics?* J Chromatogr B Analyt Technol Biomed Life Sci, 2005. **815**(1-2): p. 285-94.
88. Osei-Kumah, A., N. Hodyl, and V.L. Clifton, *Proteomics in asthma.* Expert Rev Clin Immunol, 2008. **4**(6): p. 713-21.
89. Kasaian, M.T., et al., *Proteomic analysis of serum and sputum analytes distinguishes controlled and poorly controlled asthmatics.* Clin Exp Allergy, 2018. **48**(7): p. 814-824.
90. Wiktorowicz, J.E. and M. Jamaluddin, *Proteomic analysis of the asthmatic airway.* Adv Exp Med Biol, 2014. **795**: p. 221-32.
91. Heijkenskjold-Rentzhog, C., et al., *Alveolar and exhaled NO in relation to asthma characteristics--effects of correction for axial diffusion.* Allergy, 2014. **69**(8): p. 1102-11.
92. Vidal, C., et al., *Evaluation of the phadiatop test in the diagnosis of allergic sensitization in a general adult population.* J Investig Allergol Clin Immunol, 2005. **15**(2): p. 124-30.
93. Assarsson, E., et al., *Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability.* PLoS One, 2014. **9**(4): p. e95192.
94. Nathan, R.A., et al., *Development of the asthma control test: a survey for assessing asthma control.* J Allergy Clin Immunol, 2004. **113**(1): p. 59-65.
95. Juniper, E.F., et al., *Development and validation of the Mini Asthma Quality of Life Questionnaire.* Eur Respir J, 1999. **14**(1): p. 32-8.
96. Siroux, V., et al., *Quality-of-life and asthma-severity in general population asthmatics: results of the ECRHS II study.* Allergy, 2008. **63**(5): p. 547-54.
97. *Standardization of Spirometry, 1994 Update. American Thoracic Society.* Am J Respir Crit Care Med, 1995. **152**(3): p. 1107-36.

98. Malmberg, L.P., et al., *Exhaled nitric oxide in healthy nonatopic school-age children: determinants and height-adjusted reference values.* Pediatr Pulmonol, 2006. **41**(7): p. 635-42.
99. Jacinto, T., et al., *Evolution of exhaled nitric oxide levels throughout development and aging of healthy humans.* J Breath Res, 2015. **9**(3): p. 036005.
100. Hedenstrom, H., P. Malmberg, and K. Agarwal, *Reference values for lung function tests in females. Regression equations with smoking variables.* Bull Eur Physiopathol Respir, 1985. **21**(6): p. 551-7.
101. Hedenstrom, H., P. Malmberg, and H.V. Fridriksson, *Reference values for lung function tests in men: regression equations with smoking variables.* Ups J Med Sci, 1986. **91**(3): p. 299-310.
102. Solymar, L., et al., *Nitrogen single breath test, flow-volume curves and spirometry in healthy children, 7-18 years of age.* Eur J Respir Dis, 1980. **61**(5): p. 275-86.
103. Quanjer, P.H., et al., *Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations.* Eur Respir J, 2012. **40**(6): p. 1324-43.
104. Chinn, S., et al., *Sensitization to individual allergens and bronchial responsiveness in the ECRHS. European Community Respiratory Health Survey.* Eur Respir J, 1999. **14**(4): p. 876-84.
105. Schulze, J., et al., *Methacholine challenge--comparison of an ATS protocol to a new rapid single concentration technique.* Respir Med, 2009. **103**(12): p. 1898-903.
106. Benjamini Y, H.Y., *Controlling the Face Discovery Rate: A Practical and Powerful Approach to Multiple Testing.* Journal of the Royal Statistical Society. Series B, 1995. **57**(1): p. 289-300.
107. Shim, E., et al., *The Association of Lung Function, Bronchial Hyperresponsiveness, and Exhaled Nitric Oxide Differs Between Atopic and Non-atopic Asthma in Children.* Allergy Asthma Immunol Res, 2015. **7**(4): p. 339-45.
108. Jouaville, L.F., et al., *Interrelationships among asthma, atopy, rhinitis and exhaled nitric oxide in a population-based sample of children.* Clin Exp Allergy, 2003. **33**(11): p. 1506-11.
109. Ludviksdottir, D., et al., *Exhaled nitric oxide and its relationship to airway responsiveness and atopy in asthma. BHR-Study Group.* Respir Med, 1999. **93**(8): p. 552-6.
110. Karakoc, F., et al., *The association between persistent eosinophilia and asthma in childhood is independent of atopic status.* Clin Exp Allergy, 2002. **32**(1): p. 51-6.
111. Freidhoff, L.R. and D.G. Marsh, *Relationship among asthma, serum IgE levels and skin test sensitivity to inhaled allergens.* Int Arch Allergy Immunol, 1993. **100**(4): p. 355-61.
112. Bachert, C., et al., *Total and specific IgE in nasal polyps is related to local eosinophilic inflammation.* J Allergy Clin Immunol, 2001. **107**(4): p. 607-14.
113. Sintobin, I., et al., *Is immunoglobulin E to Staphylococcus aureus enterotoxins associated with asthma at 20 years?* Pediatr Allergy Immunol, 2015. **26**(5): p. 461-5.
114. Rogers, N.K., et al., *Extra-cellular matrix proteins induce matrix metalloproteinase-1 (MMP-1) activity and increase airway smooth muscle contraction in asthma.* PLoS One, 2014. **9**(2): p. e90565.
115. Ohta, Y., et al., *Dual modulation of airway smooth muscle contraction by Th2 cytokines via matrix metalloproteinase-1 production.* J Immunol, 2008. **180**(6): p. 4191-9.

116. Takaku, Y., et al., *CXC chemokine superfamily induced by Interferon-gamma in asthma: a cross-sectional observational study.* Asthma Res Pract, 2016. **2**: p. 6.
117. Xie, M., et al., *IL-27 and type 2 immunity in asthmatic patients: association with severity, CXCL9, and signal transducer and activator of transcription signaling.* J Allergy Clin Immunol, 2015. **135**(2): p. 386-94.
118. Fulkerson P.C, R.E.M., *Chemokines, CXC|CXCL9 (MIG).* Encyclopedia of Respiratory Medicine, 2006: p. 398/402.
119. Olafsdottir, I.S., et al., *C reactive protein levels are increased in non-allergic but not allergic asthma: a multicentre epidemiological study.* Thorax, 2005. **60**(6): p. 451-4.
120. Venge, P., et al., *Human Neutrophil Lipocalin as a Superior Diagnostic Means To Distinguish between Acute Bacterial and Viral Infections.* Clin Vaccine Immunol, 2015. **22**(9): p. 1025-32.
121. Fjaertoft, G., et al., *Human neutrophil lipocalin (HNL) as a diagnostic tool in children with acute infections: a study of the kinetics.* Acta Paediatr, 2005. **94**(6): p. 661-6.
122. Serap Duru Akçali, B.K., Fattma Uçar, Esra Erdemir, Emin Alay, *Human neutrophil gelatinase-associated lipocalin: A new biomarker in asthma-COPD overlap syndrome.* European Respiratory Journal, 2016. **48**(60).
123. Monadi, M., et al., *Serum C-reactive protein in asthma and its ability in predicting asthma control, a case-control study.* Caspian J Intern Med, 2016. **7**(1): p. 37-42.
124. Jatakanon, A., et al., *Neutrophilic inflammation in severe persistent asthma.* Am J Respir Crit Care Med, 1999. **160**(5 Pt 1): p. 1532-9.
125. Ennis, M., *Neutrophils in asthma pathophysiology.* Curr Allergy Asthma Rep, 2003. **3**(2): p. 159-65.
126. Ray, A. and J.K. Kolls, *Neutrophilic Inflammation in Asthma and Association with Disease Severity.* Trends Immunol, 2017. **38**(12): p. 942-954.
127. Fricker, M., L.G. Heaney, and J.W. Upham, *Can biomarkers help us hit targets in difficult-to-treat asthma?* Respirology, 2017. **22**(3): p. 430-442.
128. Blidberg, K., et al., *Increased neutrophil migration in smokers with or without chronic obstructive pulmonary disease.* Respirology, 2012. **17**(5): p. 854-60.
129. Olin, A.C., et al., *Height, age, and atopy are associated with fraction of exhaled nitric oxide in a large adult general population sample.* Chest, 2006. **130**(5): p. 1319-25.
130. Amaral, A.F.S., et al., *Changes in IgE sensitization and total IgE levels over 20 years of follow-up.* J Allergy Clin Immunol, 2016. **137**(6): p. 1788-1795 e9.
131. Mediaty, A. and K. Neuber, *Total and specific serum IgE decreases with age in patients with allergic rhinitis, asthma and insect allergy but not in patients with atopic dermatitis.* Immun Ageing, 2005. **2**(1): p. 9.
132. Jackola, D.R., et al., *Robustness into advanced age of atopy-specific mechanisms in atopy-prone families.* Journals of Gerontology Series a-Biological Sciences and Medical Sciences, 2003. **58**(2): p. 99-107.
133. Elmasri, M., et al., *Longitudinal assessment of high versus low levels of fractional exhaled nitric oxide among children with asthma and atopy.* Lung, 2014. **192**(2): p. 305-12.
134. Brightling, C.E. and L. George, *Is the eosinophil a leading villain in lung function decline?* Chest, 2015. **148**(4): p. 844-846.
135. Soma, T., et al., *Implication of fraction of exhaled nitric oxide and blood eosinophil count in severe asthma.* Allergol Int, 2018. **67S**: p. S3-S11.
136. Ulrik, C.S., *Outcome of asthma: longitudinal changes in lung function.* Eur Respir J, 1999. **13**(4): p. 904-18.

137. Skloot, G.S., *The Effects of Aging on Lung Structure and Function.* Clin Geriatr Med, 2017. **33**(4): p. 447-457.
138. Bjerg, A., et al., *A population-based study of animal component sensitization, asthma, and rhinitis in schoolchildren.* Pediatr Allergy Immunol, 2015. **26**(6): p. 557-63.
139. Uddenfeldt, M., et al., *Sensitization to pets is a major determinant of persistent asthma and new asthma onset in Sweden.* Ups J Med Sci, 2013. **118**(2): p. 111-21.
140. Munir, A.K.M., et al., *Allergens in School Dust .1. The Amount of the Major Cat (Fel D I) and Dog (Can F I) Allergens in Dust from Swedish Schools Is High Enough to Probably Cause Perennial Symptoms in Most Children with Asthma Who Are Sensitized to Cat and Dog.* Journal of Allergy and Clinical Immunology, 1993. **91**(5): p. 1067-1074.
141. Patelis, A., et al., *Allergen extract vs. component sensitization and airway inflammation, responsiveness and new-onset respiratory disease.* Clin Exp Allergy, 2016. **46**(5): p. 730-40.
142. Emara, M., et al., *Recognition of the major cat allergen Fel d 1 through the cysteine-rich domain of the mannose receptor determines its allergenicity.* J Biol Chem, 2011. **286**(15): p. 13033-40.
143. Virtanen, T., T. Kinnunen, and M. Rytkonen-Nissinen, *Mammalian lipocalin allergens--insights into their enigmatic allergenicity.* Clin Exp Allergy, 2012. **42**(4): p. 494-504.
144. Papazian, D., et al., *Direct contact between dendritic cells and bronchial epithelial cells inhibits T cell recall responses towards mite and pollen allergen extracts in vitro.* Clin Exp Immunol, 2015. **181**(2): p. 207-18.
145. Wisniewski, J.A., et al., *Sensitization to food and inhalant allergens in relation to age and wheeze among children with atopic dermatitis.* Clin Exp Allergy, 2013. **43**(10): p. 1160-70.
146. Uriarte, S.A. and J. Sastre, *Clinical relevance of molecular diagnosis in pet allergy.* Allergy, 2016. **71**(7): p. 1066-8.
147. Matricardi, P.M., et al., *EAACI Molecular Allergology User's Guide.* Pediatr Allergy Immunol, 2016. **27 Suppl 23**: p. 1-250.
148. Konradsen, J.R., et al., *Allergy to furry animals: New insights, diagnostic approaches, and challenges.* J Allergy Clin Immunol, 2015. **135**(3): p. 616-25.
149. Jackola, D.R., M.N. Blumenthal, and A. Rosenberg, *Evidence for two independent distributions of serum immunoglobulin E in atopic families: cognate and non-cognate IgE.* Hum Immunol, 2004. **65**(1): p. 20-30.
150. Roehr, C.C., et al., *Food allergy and non-allergic food hypersensitivity in children and adolescents.* Clin Exp Allergy, 2004. **34**(10): p. 1534-41.
151. Osterballe, M., et al., *The prevalence of food hypersensitivity in an unselected population of children and adults.* Pediatr Allergy Immunol, 2005. **16**(7): p. 567-73.

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1519

Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title "Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine".)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-366892

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2019