Tampere University

MINNA TOMMOLA

Adult-onset Asthma and Smoking

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ACADEMIC DISSERTATION
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PunaMusta Oy – Yliopistopaino
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To my family

ABSTRACT

Smokers and patients with smoking history have generally been excluded from previous studies on asthma, and therefore relatively little is known about the effect of smoking on asthma. Previous population-based and registry studies have suggested negative effects of smoking on asthma, but the results have been inconsistent. Asthma-chronic obstructive pulmonary disease overlap (ACO) has recently been recognized and included in guidelines but remained rarely studied. Identification of ACO has nevertheless been considered important, because of the modern, personalized therapy options. The diagnostic criteria for ACO are not confirmed, but some criteria have been previously suggested.

The aim of the present study was to evaluate the effect of smoking on asthma and to investigate the differences between asthma and ACO. Further aims were to evaluate the usability and validity of the proposed criteria for ACO, and to investigate the role of occupational exposures in developing of ACO.

The present study investigated adult-onset asthma patients in the Seinäjoki Adult Asthma Study (SAAS) but also data on patients entitled to asthma medication reimbursement in Finland, and data on Cohort for Reality and Evolution of Adult Asthma (COREA) cohort were used in some analyses. The Seinäjoki Adult Asthma Study is a real-life cohort of patients with asthma diagnosed at adult age. The diagnosis was based on objective lung function measurements and respiratory specialist evaluation and the guidelines were followed. Smokers were included in the SAAS cohort. Smoking history of ≥ 10 pack-years was associated with increased loss of lung function in adult-onset asthma. The accelerated loss of lung function continued even after smoking had stopped if 10 pack-years had been reached. The pack-year history was dose-dependently associated with increased disease burden and multimorbidity when measured by hospitalizations, symptoms and comorbidities. A pack-year history of ≥ 20 pack-years was independent of other factors associated with hospitalizations for any respiratory reason. ACO differed from asthma by showing lower diffusing capacity, higher blood neutrophil and IL-6 levels and higher remaining bronchial reversibility. Differences were also found between ACO and obstructive asthma, suggesting that the obstructive asthma driven by smoking is not the same as the one caused by ongoing asthma inflammation.

Occupational exposures to vapors, gases, dusts or fumes (VGDF) were associated with higher ACO prevalence, and the results suggested an additive effect between smoking and occupational exposures in development of ACO. Previous suggestions for ACO criteria state that in a patient with fixed airway obstruction and significant smoking history, asthma should be diagnosed before the age of 40 years or high bronchial reversibility (>400mL in FEV_1) should be shown. In reflection to the previously proposed criteria for ACO, age and bronchodilator response (BDR) at asthma diagnosis were evaluated. The majority of asthma was shown to be diagnosed after 40 years of age, especially among women. The BDR at diagnosis of asthma was shown to be stable despite the age at diagnosis of adult-onset asthma. Thus, the need for re-evaluation of the previously proposed criteria for ACO was suggested.

The results of the current study show that the adverse effects of smoking on asthma are significant and may take place already at an early phase of a patient's smoking history. Thus, early intervention towards smoking cessation and strong preventive actions regarding smoking are recommended. Significant differences between asthma and ACO were found, and more research on ACO is needed.

TIIVISTELMÄ

Tupakoitsijat ja aiemmin pidempään tupakoineet ihmiset on yleensä poissuljettu aikaisemmista astmatutkimuksista, ja siksi tietomme astman ja tupakoinnin yhteyksistä on hyvin vähäistä. Aikaisempien väestö- ja rekisteripohjaisten tutkimusten tulokset ovat viitanneet siihen, että tupakoinnilla on haitallisia vaikutuksia astmaan, mutta tutkimustulokset eivät ole olleet täysin yhteneväisiä. Astman ja keuhkoahtaumataudin (COPD) yhtäaikainen esiintyminen potilaalla (astma-COPD overlap, ACO) on hiljattain tunnistettu ja sisällytetty hoitosuosituksiin, mutta sitä on tutkittu vasta hyvin vähän. ACO:n tunnistaminen on kuitenkin todettu tärkeäksi, sillä nykyään on käytettävissä uusia, yksilöllisiä hoitomuotoja. ACO:n diagnostisia kriteerejä ei ole vielä tarkkaan sovittu, mutta ehdotuksia kriteereiksi on aiemmin tehty.

Tämän tutkimuksen tavoite oli selvittää tupakoinnin vaikutuksia astmassa sekä löytää kliinisesti merkittäviä eroavaisuuksia astman ja ACO:n välillä. Lisäksi tavoitteena oli arvioida aiemmin ehdotettujen ACO-kriteereiden käytettävyyttä ja luotettavuutta potilailla, sekä selvittää ammattialtistuksen yhteyttä ACO:n kehittymiseen.

Tutkimus toteutettiin käyttäen Seinäjoki Adult Asthma Study –kohortissa kerättyä tietoa. Lisäksi käytettiin kansaneläkelaitokselta saatuja tietoja astmalääkkeiden erityiskorvausoikeuden saaneiden potilaiden määristä, sekä kliinisen COREA-kohortin (Etelä-Korea) potilaiden tietoja. Seinäjoki Adult Asthma Study on aikuisena astmaan sairastuneiden potilaiden kohorttitutkimus. Potilaiden astmadiagnoosi pohjautui objektiivisiin keuhkojen toimintakokeiden mittauksiin, erikoislääkärin arvioon sekä hoitosuositusten ohjeisiin. Tupakoitsijat ja aiemmin tupakoineet potilaat otettiin tutkimusjoukkoon mukaan. Tutkimustulosten mukaan vähintään 10 askivuoden tupakointihistoria oli yhteydessä nopeampaan keuhkofunktion laskuun aikuisena alkavassa astmassa. Keuhkojen toiminnan lasku jatkui kiihtyneenä kymmenen askivuoden jälkeen, vaikka tupakointi oli jo loppunut. Arvioitaessa sairaalahoitojen, oireiden ja liitännäissairauksien määrää, askivuosihistoria oli annosvasteisesti yhteydessä lisääntyneeseen astmaan liittyvään sairastavuustaakkaan sekä liitännäissairastavuuteen. Vähintään 20 askivuoden tupakkahistoria oli muista tekijöistä riippumatta itsenäinen selittäjä lisääntyneille

hengityselimistön ongelmista johtuville sairaalahoidoille. ACO:n todettiin eroavan astmasta matalampien diffuusiokapasiteettiarvojen, korkeamman veren neutrofiilisten valkosolujen määrän, korkeampien IL-6 arvojen, sekä suuremman obstruktion palautuvuuden osalta. Eroja todettiin myös ACO:n ja obstruktiivisen astman välillä. Tulokset viittaavat siihen, että tupakan astmaatikolle aiheuttama pysyvä keuhkoputkien ahtauma ei ole samanlainen tila kuin astmatulehduksen aiheuttama obstruktio. Ammattialtistumisen höyryille, kaasuille tai pölyille nähtiin olevan yhteydessä korkeampaan ACO:n vallitsevuuteen, ja tulokset viittaavat siihen, että tupakoinnin ja ammattialtistumisten välillä voi olla summautuva vaikutus ACO:n kehittymisessä. Aiemmin ACO-kriteereiksi on ehdotettu, että pysyvää keuhkoputkien obstruktiota sairastavalla potilaalla, jolla on merkittävä tupakkahistoria, astma tulisi olla todettuna ennen 40 ikävuotta, tai tulisi olla osoitettuna suuri keuhkoputkien reversibiliteetti (>400mL FEV_1:ssa). Näiden aiemmin ehdotettujen kriteerien tutkimiseksi potilaiden ikä ja bronkodilataatiovaste astman diagnoosihetkellä analysoitiin. Tulosten perusteella suurin osa astmasta todetaan vasta 40 ikävuoden jälkeen, erityisesti naisilla. Bronkodilataatiovaste astmadiagnoosin aikaan pysyi samanlaisena huolimatta siitä, missä iässä aikuisastman diagnoosi tehtiin. Tulosten perusteella aiemmin ehdotetut ACO-kriteerit tulee arvioida uudelleen.

Tutkimustulosten perusteella tupakoinnin haitalliset vaikutukset astmassa ovat merkittäviä ja saattavat alkaa jo hyvin aikaisessa vaiheessa tupakointihistoriaa. Siksi aikainen puuttuminen potilaiden tupakointiin, tupakoinnin lopettamiseen tähtäävät toimenpiteet sekä tehokkaat tupakointia ehkäisevät toimet ovat suositeltavia. ACO:n ja astman välillä nähtiin merkittäviä eroavaisuuksia, ja tulevaisuudessa tarvitaankin lisätutkimuksia ACO:n suhteen.

CONTENTS

ABBREVIATIONS

ACO	Asthma-COPD overlap
ACOS	Asthma-COPD overlap syndrome
ACQ	Asthma control questionnaire
ACT	Asthma control test
ANOVA	Analysis of variance test
AQLQ	Asthma quality of life questionnaire
AQ20	Airways questionnaire 20
BDR	Bronchodilator response
BMI	Body mass index
CAT	COPD assessment test
COPD	Chronic obstructive pulmonary disease
COREA	Cohort for Reality and Evolution of Adult Asthma in Korea
FeNO	Fraction of exhaled nitric oxide
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
GINA	Global Initiative for Asthma
GOLD	Global Initiative for Obstructive Lung Disease
HR	Hazard ratio
hsCRP	High sensitivity C-reactive protein
ICS	Inhaled corticosteroids
IgE	Immunoglobulin E
IL-6	Interleukin 6
ILC2	Innate lymphoid cells group 2
LABA	Long-acting $beta_2$-agonist
LTRA	Leukotriene receptor antagonists
$Max_{0\text{-}2.5}$	The point where the maximum lung function in FEV_1 during the first 2.5 years after the diagnosis of asthma was achieved
mL	Millilitre
MMP	Matrix metalloproteinase
NSAID	Non-steroidal anti-inflammatory drug

OR	Odds ratio
PC20FEV1	Provocative concentration causing a 20% fall in forced expiratory volume in one second
PEF	Peak expiratory flow
ROS	Reactive oxygen species
SAAS	Seinäjoki Adult Asthma Study
SABA	Short-acting beta$_2$-agonist
T2	Type 2
Th2	T-helper 2 lymphocytes
TNF-α	Tumor necrosis factor α
VGDF	Vapors, gases, dust and fumes
WHO	World Health Organisation

ORIGINAL PUBLICATIONS

This thesis is based on the following original communications, referred to in the text by their Roman numerals (I-VI). In addition, some unpublished data are presented.

I The effect of smoking on lung function: a clinical study of adult-onset asthma. Tommola M, Ilmarinen P, Tuomisto LE, Haanpää J, Kankaanranta T, Niemelä O, Kankaanranta H. Eur Respir J. 2016;48:1298-1306.

II Differences between asthma-COPD overlap syndrome and adult-onset asthma. Tommola M, Ilmarinen P, Tuomisto LE, Lehtimäki L, Haanpää J, Niemelä O, Kankaanranta H. Eur Respir J. 2017;49. pii: 1602383. doi: 10.1183/13993003.02383-2016.

III Concern of underdiagnosing asthma-COPD overlap syndrome if age limit of 40 years for asthma is used. Tommola M, Ilmarinen P, Tuomisto LE, Kankaanranta H. Eur Respir J. 2017;50. pii: 1700871. doi: 10.1183/13993003.00871-2017.

IV Cumulative effect of smoking on disease burden and multimorbidity in adult-onset asthma. Tommola M, Ilmarinen P, Tuomisto LE, Lehtimäki L, Niemelä O, Nieminen P, Kankaanranta H. Eur Respir J. 2019. pii: 1801580. doi: 10.1183/13993003.01580-2018.

V Occupational exposures and asthma-COPD overlap in a clinical cohort of adult-onset asthma. Tommola M, Ilmarinen P, Tuomisto LE, Lehtimäki L, Kankaanranta H. ERJ Open Res. 2019 Oct 21;5(4). pii: 00191-2019. doi: 10.1183/23120541.00191-2019.

VI Relationship between age and bronchodilator response at diagnosis in adult-onset asthma. Tommola M, Won HK, Ilmarinen P, Jung H, Tuomisto LE, Lehtimäki L, Niemelä O, Kim TB, Kankaanranta H. Respir res. In Press.

1. INTRODUCTION

Asthma is a common disease affecting 1-18% of the population worldwide (GINA 2019). Up to 26% of patients with asthma are shown to be active cigarette smokers, with smoking prevalence being similar among asthmatics and the healthy population (Cerveri et al. 2012; Polosa & Thomson 2013). Little is known, however, about the effects of smoking on asthma. Smokers have commonly been excluded from previous studies on asthma, and therefore a lack of clinical studies among real-life patients with asthma has persisted. Population-based and registry studies have previously suggested tobacco smoking to have negative effects on asthma: accelerated lung function decline (Aanerud et al. 2015; Apostol et al. 2002; Colak et al. 2015; Hancox et al. 2016; James et al. 2005; Lange et al. 1998), increased risk for hospitalisations (Eisner & Iribarren 2007; Kauppi et al. 2014; Thomson et al. 2013), and increased severity of asthma (Eisner & Iribarren 2007; Polosa et al. 2011; Westerhof et al. 2014). However, the effect of life-long smoking history in pack-years has rarely been evaluated. In addition, there has remained a considerable need for confirmation of the previously suggested adverse effects of smoking on asthma in clinical studies.

Asthma may overlap with chronic obstructive pulmonary disease (COPD) in a single patient, and recently asthma-COPD overlap (ACO) has been recognized and included in clinical guidelines (GINA/GOLD 2017; Kankaanranta et al. 2015; Miravitlles et al. 2013; Miravitlles et al. 2016). However, a consensus on the diagnostic criteria for ACO is still missing, and even the definition of ACO has not been clearly described because of the lack of knowledge and studies on ACO (Kostikas et al. 2016; Postma & Rabe 2015; Sin et al. 2016; Tho et al. 2016). The prevalence of ACO has been proposed to be up to 55% among patients with asthma (Gibson & McDonald 2015; Wurst et al. 2016); thus ACO has been suggested to affect a large proportion of patients. Previously, some diagnostic criteria for ACO have been suggested, but the validity of the proposed criteria has not been evaluated. Considering the modern options for targeted therapy on asthma and COPD, the diagnostics of the overlap of these two diseases has been recognized as highly important.

The present series of studies aimed to investigate the effects of tobacco smoking on asthma in a clinical setting of real-life patients with adult-onset asthma. In addition, the study aimed to recognize how ACO differs from asthma and to evaluate the validity of the proposed criteria for ACO.

2. REVIEW OF THE LITERATURE

2.1. Asthma

2.1.1 Description of asthma

Asthma is a chronic, inflammatory disease of the airways, characterized by bronchial obstruction leading to airflow limitation (GINA 2019). Asthma is estimated to affect 1-18% of the population worldwide (GINA 2019) and in Finland, the prevalence of doctor-diagnosed asthma is shown to be up to 11% (Honkamäki et al. 2019). Typical symptoms of asthma are wheezing, cough, shortness of breath and bronchial mucus production (GINA 2019; McCracken et al. 2017). Reversibility or variability of the airway obstruction is commonly present in asthma, and the degree of airway limitation, as well as the severity of symptoms may vary over time (GINA 2019). There may be long periods of time without symptoms or bronchial obstruction in asthma, followed by worsening of these features or even severe exacerbation. The variable bronchial obstruction and asthma symptoms may be triggered by several factors, e.g., respiratory infections, exposure to irritant inhaled particles or allergens, cold air and exercise (GINA 2019; McCracken et al. 2017).

T-helper 2 (Th2) lymphocytes play a significant role in the asthma inflammation and thus, asthma inflammation is commonly addressed as eosinophilic, but mast cells and neutrophils may also contribute to the inflammatory process. Th2 cells release cytokines such as interleukin (IL)-4, IL-5, IL-9 and IL-13, which promote immunoglobulin E (IgE) production and induce eosinophilic inflammation (McCracken et al. 2017; Quirt et al. 2018). Recently, the terminology has changed from using "Th2 high" to "T2 high" inflammation, because cells other than the classic Th2 CD4+ cells, such as the innate lymphoid cells group 2 (ILC2), have also been identified to participate in the inflammation process (Sze et al. 2019). The asthmatic inflammation leads to hyperreactivity of the smooth muscle surrounding the bronchial wall, and contracting of the hyperreactive muscle causes an airway obstruction (McCracken et al. 2017). Recently, the importance of non-T2 asthma has also been increasingly understood. In non-T2 asthma, features of T2 asthma are lacking, and the inflammation is suggested to be more neutrophilic or paucigranulocytic (Sze et al. 2019). Absence, or normal levels of eosinophils and other T2 markers is described as characteristic to non-T2 asthma (Sze et al. 2019).

Currently, no generally agreed definition exists for non-T2 asthma; thus, the prevalence of non-T2 asthma is not exactly known (Sze et al. 2019). However, it has been proposed that non-T2 asthma is not uncommon, and up to two thirds of asthma patients may actually have non-T2 type of inflammation (Sze et al. 2019).

Over time, the ongoing bronchial inflammation may cause permanent changes in the airway through a process called remodeling (Al-Muhsen et al. 2011). In the remodeling process, the inflammation eventually causes cellular and structural changes in the bronchi, which will lead to increased smooth muscle mass and thickening of the bronchial wall (Al-Muhsen et al. 2011). This causes a further narrowing of the airway. In addition, the activation of fibroblasts/myofibroblasts leads to subepithelial fibrosis, which causes fixed bronchial obstruction and permanent loss of lung function (Al-Muhsen et al. 2011).

2.1.2 Asthma diagnosis and therapy

Asthma can be diagnosed on a patient with a history and symptoms suggestive for asthma by using lung function measurements showing significant reversibility or variability of the airway obstruction (GINA 2019). Spirometry is the most commonly used lung function measurement for asthma diagnosis but the diagnosis can also be made with peak expiratory flow (PEF) monitoring, by showing bronchial obstruction in response to challenge with an allergen or exercise, or by presenting reversibility of obstruction with steroid therapy (GINA 2019). In addition to direct bronchial challenge tests, such as methacholine- or histamine challenge test, also indirect tests are used to assess the airway hyperresponsiveness in asthma diagnostics. Indirect bronchial challenge tests are, for example, exercise challenge test, eucapnic voluntary hyperpnoea test, cold air challenge test, hypertonic saline challenge and mannitol challenge tests (Hallstrand et al. 2018; Koskela et al. 2003; Koskela et al. 2004; Purokivi et al. 2007; Purokivi et al. 2008)

The aims of asthma therapy are to achieve good control of asthma symptoms, maintain normal activity levels, and minimize the risks of exacerbations, loss of lung function and asthma-related deaths (GINA 2019; McCracken et al. 2017). Asthma medication commonly consists of anti-inflammatory medication controlling the inflammation, and of a symptom-relieving medication. The most commonly used controllers are the inhaled corticosteroids (ICS), and relievers, the short-acting beta$_2$-

agonists (SABA). A combination of ICS and long-acting beta$_2$-agonist (LABA) medication can be used for patients with persistent symptoms despite an adequate dose and use of ICS. Add-on therapies can also be considered for patients with severe asthma and remaining lack of asthma control with ICS and LABA use, and a reliever medication in use. The most commonly used add-on therapies are tiotropium and leukotriene receptor antagonists (LTRA), but for the most severe asthma also theophylline, azithromycin, low dose oral corticosteroids or the biologic medications, such as anti-immunoglobulin E (anti-IgE), anti-interleukin 5 (anti-IL5) or anti-interleukin 4 (anti-IL4) drugs, are the options for treatment. Allergen-specific immunotherapy may be considered in patients with allergy playing a significant role in asthma (GINA 2019). The need for medication changes is individually evaluated for every patient during the follow-up visits based on the patient's symptoms and asthma control (GINA 2019; Quirt et al. 2018).

In addition to pharmacological treatment, non-pharmacological therapy is also very important in asthma. This includes, for example, smoking cessation, physical activity, breathing exercises, avoidance of any medication that may worsen asthma, e.g., non-steroidal anti-inflammatory drugs (NSAIDs), a healthy diet, weight reduction for obese patients and avoidance of occupational exposures, indoor allergens and air pollutions (GINA 2019). It has been recommended that smoking patients with asthma should strongly be advised at every visit to quit smoking (GINA 2019; Quirt et al. 2018). Patient education on how to recognize and respond to asthma worsening and exacerbations, a self-management plan, and skills training on how to use the inhaler devices are preferred (GINA 2019; Haahtela et al. 2001).

2.1.3. Lung function in asthma

Lung volumes normally increase from childhood to adolescence along with growth, and peak lung function is achieved in early adulthood. The peak in lung function is obtained for some years during a period known as a plateau phase. After that, lung function starts physiologically to decline along with aging (Figure 1) (McGeachie et al. 2016). Several factors are reported to affect both the development and the decline of lung function. For example, maternal exposure to tobacco smoke during pregnancy, early life infections and exposures to toxic inhaled particles, and low birth weight are shown to have negative effects on lung function development in early life (GOLD 2019; Svanes et al. 2010).

The inflammatory process of asthma has been shown to lead to both short- and long-term effects on lung function. Thus, the measurement of lung function is an important step in asthma diagnostics and in assessment of future risk of exacerbations (GINA 2019). The most relevant values when assessing lung function in spirometry are Forced Vital Capacity (FVC), Forced Expiratory Volume in one second (FEV_1), and their ratio (FEV_1/FVC), which reflects the degree of airway obstruction. The physiological, annual decline in FEV_1 in healthy persons is estimated to be on average 22mL, and in persons with asthma the decline is suggested to be accelerated to 38mL/year (Lange et al. 1998). However, when evaluating the effect of asthma or any other factor on the rate of decline in lung function, confounding factors may also play a role. Recently, different lung function trajectories and factors affecting those trajectories have been described, especially in COPD research. It has been proposed that low values of FEV_1 in early adulthood may be an important factor in the development of COPD later in life, and an accelerated decline in FEV_1 is not an obligate feature of COPD (Lange et al. 2015). However, it should be noted that the study actually described lung function trajectories leading to airflow limitation, not to COPD disease. A large proportion of the subjects were never-smokers, no other exposures to inhaled particles were assessed, and the study population also included subjects with asthma (Lange et al. 2015).

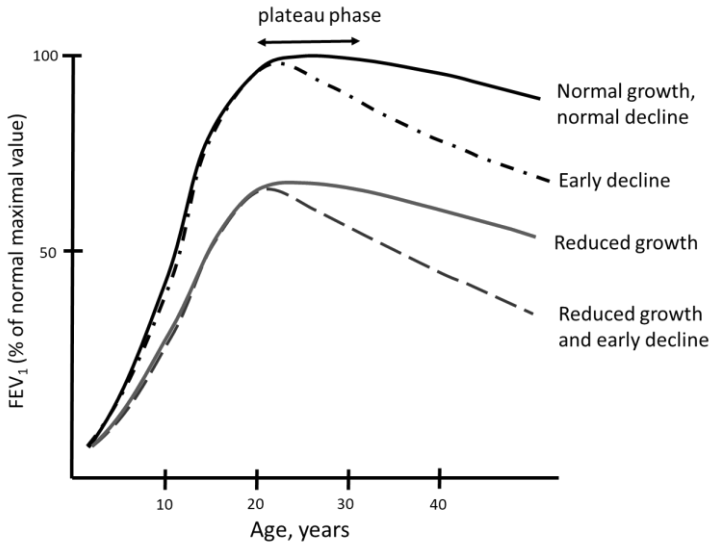


Figure 1. Lung function development trajectories (Modified from McGeachie et al. 2016)

2.1.4. Use of spirometry in asthma

Spirometry is an objective and commonly used laboratory examination of the lung function and is a recommended tool for asthma diagnostics (GINA 2019). Bronchial reversibility in $FEV_1 \geq 12\%$ and ≥ 200 mL after administration of bronchodilator medication is considered one of the most relevant diagnostic findings in asthma (GINA 2019). However, population-based studies have shown a severe underuse of spirometry for asthma diagnostics, with up to 57% of asthma patients actually being left without lung function testing at the time of diagnosis (Gershon et al. 2012). Underuse of lung function testing in asthma diagnostics has been shown to lead to overdiagnosing asthma (Aaron et al. 2018; Gershon et al. 2012).

2.1.5. Asthma phenotypes

Asthma is a heterogeneous disease with several different clinical features, demographic and pathophysiologic factors. Asthma has commonly been considered to start in childhood and have a strong association with allergic conditions. However, many distinct clinical asthma phenotypes have recently been recognized in cluster analyses assessing different asthma features (Amelink et al. 2013; Ilmarinen et al. 2017; Kim et al. 2013; Miranda et al. 2004, Moore et al. 2010). Age at onset of asthma has been shown to be an important factor separating asthma phenotypes, and cluster analyses have recognized adult-onset asthma as one separate phenotype of asthma (Amelink et al. 2013; GINA 2019; Ilmarinen et al. 2017; Miranda et al. 2004). Other most commonly reported asthma phenotypes are obesity-related asthma, atopic or allergic asthma and smoking asthma (Amelink et al. 2013; GINA 2019; Ilmarinen et al. 2017; Miranda et al. 2004; Wenzel 2012) (Figure 2).

Adult-onset asthma starts at adult age and is less associated with allergic conditions as compared with childhood-onset asthma (Ilmarinen et al. 2017; Miranda et al. 2004). Previously, the common perception has been that asthma starts in early childhood. However, the importance of adult-onset asthma has increasingly been recognized recently, and the majority of asthma has actually been shown to be diagnosed at adult age, especially among women (Honkamäki et al. 2019; Kankaanranta et al. 2017; Sood et al. 2013). The prognosis of adult-onset asthma has been shown to clearly differ from that of childhood-onset asthma. Three out of four children achieve remission of asthma by adolescence and adulthood (Bisgaard et al.

2010; Burgess et al. 2011; de Nijs et al. 2013). Conversely, remission is found to be rare in adult-onset asthma, and only 1.5-5% of adult-onset asthma patients are shown to obtain remission (Rönnmark et al. 2007; Tuomisto et al. 2016). A recent study of adult-onset asthma reported one of six patients to experience remission during the first five years of asthma, and remission rate among patients with moderate to severe hyperresponsiveness and nasal polyposis to be close to zero (Westerhof et al. 2018) Even more recently, a paper reporting a remission rate of adult-onset asthma of 16 % was published, although the study was performed in an asthma cohort that excluded patients with 10 pack-years of smoking (Kauppinen et al. 2019). Different phenotypes among adult-onset asthma patients have been suggested, such as exercise-induced, late-onset eosinophilic (often severe), obesity-related, non-rhinitic, female asthma, early-onset atopic adult asthma and smoking-related neutrophilic asthma (Ilmarinen et al. 2015; Ilmarinen et al. 2017).

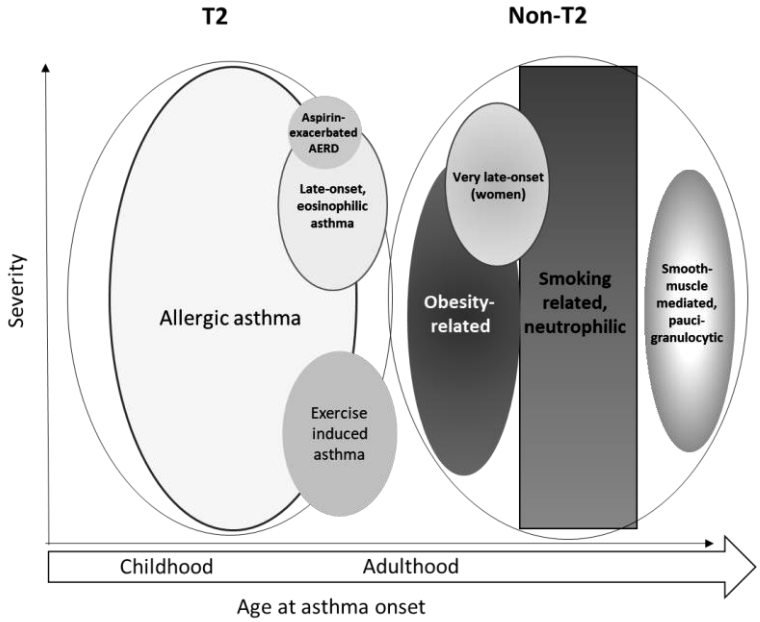


Figure 2. Different asthma phenotypes and the theoretical predominant inflammation type (Modified from Wenzel 2012)

2.2. Tobacco smoking

2.2.1 Impact of smoking on health

Tobacco use has been evaluated to cause 6 million deaths across the world annually, 600,000 of those being due to second-hand smoke (WHO 2015). Smoking is reported to be the leading cause of preventable deaths in the European Union, causing 700,000 deaths annually (European Comission 2015; Jayes et al. 2016). Smokers are estimated to lose 14 years of life and 50% of smokers die prematurely (European Comission 2015; Jayes et al. 2016). Tobacco smoke contains more than 5,000 chemicals, many of those toxic and carcinogenic (Talhout et al. 2011). Tobacco use has been widely shown to increase the risk for cardiovascular diseases, respiratory diseases and various types of cancer (Jayes et al. 2016). Passive smoking is also shown to be a significant hazard to health, especially in children (Jayes et al. 2016). Tobacco-prevention strategies have been actively developed and carried out, but smoking prevention has still remained the most important issue for increasing health worldwide (Jayes et al. 2016). Tobacco smoking is strongly linked on a molecular level to oxidative stress, systemic inflammation, and release of cytokines, which are associated with increased morbidity (Arnson et al. 2010; Ilmarinen et al. 2016). Tobacco smoking also has several adverse effects on the immune system and therefore causing increased risk of infections and relative immunodeficiency (Arnson et al. 2010).

Tobacco contains nicotine, a highly addictive chemical, which is reported to cause several psychoactive effects. The instant psychoactive effects of nicotine are often experienced as positive, primarily because nicotine relieves withdrawal symptoms, such as restlessness, anxiety and irritability (Benowitz 2010). In addition to the physical addiction caused by nicotine, the psychological addiction via the habit of smoking and conditioned behavior has also been shown to increase the tobacco addiction (Benowitz 2010). Based on findings in animal models, it has been proposed that nicotine may cause permanent changes in the brain, especially if smoking is started in childhood or adolescence when the risk of developing dependence is also reported to be the highest (Benowitz 2010).

2.2.2 Smoking and asthma

People with asthma have been shown to smoke tobacco almost as frequently as the general population, with 26% of asthmatics being active smokers (Cerveri et al. 2012; Polosa & Thomson 2013). Smoking has been suggested to increase the risk of developing asthma (Nakamura et al. 2009; Piipari et al. 2004). Patients with allergies have particularly been reported to have a higher risk for developing asthma and smoking increases the risk 2.7-fold (Polosa et al. 2008). Tobacco smoking has also been shown to alter the inflammation type in asthma towards non-eosinophilic, more neutrophilic and macrophage predominant (Polosa & Thomson 2013; Thomson 2017). Macrophages, if exposed to tobacco smoke, are shown to produce pro-inflammatory molecules, tumor necrosis factor-α (TNF-α), tissue proteinases, such as matrix metalloproteinases (MMPs), and reactive oxygen species (ROS) that are associated with lung damage, and chemokines associated with longer survival of neutrophils (Polosa & Thomson 2013). Furthermore, tobacco smoke has been suggested to have direct effects on bronchial epithelial cells, leading to further release of pro-inflammatory molecules affecting the remodeling of the airway (Polosa & Thomson 2013).

Previous studies on asthma have generally excluded smoking patients and patients with a smoking history. Similarly, COPD studies have commonly excluded patients with asthma or a history of asthma. Therefore, the effects of smoking on asthma are still relatively unstudied. Generally, the existing previous studies have mainly been executed as population- and registry-based studies with a short follow-up or no follow-up at all, as shown in Table 1. (Colak et al. 2015; Eisner & Iribarren 2007; Kauppi et al. 2014; Thomson et al. 2013; Westerhof et al. 2014). The previous studies with a follow-up have not started the follow-up at the diagnostic moment of asthma. For example, in a 2-year follow-up study on the severity of new-onset adult asthma, the recruitment of patients actually occurred within a year after the asthma diagnosis (Westerhof et al. 2014). The common method of the previous studies using mostly self-reported or self-reported, doctor-diagnosed asthma leads to marked limitations considering the reliability of the asthma diagnosis (Aanerud et al. 2015; Colak et al. 2015; Hancox et al. 2016; James et al. 2005; Lange et al. 1998). Moreover, also commonly seen in the previous studies, the subjects were evaluated during ongoing asthma medication, or the medication information was not available in the study. (Aanerud et al. 2015; Apostol et al. 2002; Colak et al. 2015; Hancox et al. 2016; James et al. 2005; Kauppi et al. 2014; Lange et al. 1998; Polosa et al. 2011; Thomson et al. 2013). Therefore, there is a lack of knowledge of effects of smoking

on therapy naïve patients with asthma. Additionally, the baseline of some previous studies reaches back to the years before inhaled corticosteroids became widely available and used, leading to limitations in the applicability of the results in modern clinical work (James et al. 2005; Lange et al. 1998). Therefore, a lack of real-world clinical studies and studies on the long term effects of smoking on asthma, especially in adult-onset asthma, has persisted. Furthermore, the effects of smoking duration and the dose-dependent effect of smoking on clinical patients with confirmed asthma have remained mainly unreported in the previous studies (Table 1) (Eisner & Iribarren 2007; Kauppi et al. 2014; Thomson et al. 2013; Polosa et al. 2011).

Despite the considerable limits of the previous pioneering studies on asthma and smoking, several adverse effects of smoking on asthma have been proposed. Smoking has been suggested to alter the type of airway asthma inflammation towards more neutrophilic (Boulet et al. 2006; Chalmers et al. 2001; Thomson et al. 2013). Among a small cohort of patients with mild asthma and no ICS medication in use, asthmatic smokers were reported to have higher proportional sputum neutrophil counts of 47% when compared to asthmatic nonsmokers with 23 % ($p=0.003$) (Chalmers et al. 2001). Moreover, the response to corticosteroid medication has been proposed to be decreased in asthmatics who smoke (Chalmers et al. 2002; Dijkstra et al. 2006; Lazarus et al. 2007; Tomlinson et al. 2005). Tobacco smoking has also been suggested to increase the burden of asthma and decrease the lung function, which chapters 2.2.3 and 2.2.4 discuss in more detail.

2.2.3 Smoking and disease burden in asthma

The findings of population-based studies and registry studies on the effect of smoking on disease burden of asthma have previously suggested smoking to be associated with a greater risk for hospitalization and unscheduled healthcare visits for asthma (Eisner & Iribarren 2007; Kauppi et al. 2014; Thomson et al. 2013) and with a decreased asthma-specific quality of life (Eisner & Iribarren 2007; Thomson et al. 2013). In a registry-based study with a cross-sectional evaluation of patients with severe asthma, current smokers were suggested to have poorer asthma control, more need for oral corticosteroid courses and more anxiety and depression symptoms when compared to ex- or never-smokers (Thomson et al. 2013). In addition, current smokers with severe asthma were reported to have more unscheduled healthcare visits during the past year with a median of 6 visits when compared to never-smokers with 4 visits (Thomson et al. 2013). However, since only

3.7% of asthma patients are estimated to have a severe disease (GINA 2019; Hekking et al. 2015; Ilmarinen et al. 2019), the previously reported findings do not necessarily reflect the common clinical situation of the majority of asthma patients. Furthermore, the evaluation of the use of healthcare and hospitalizations in the previous study was based on information from a time period of one year (Thomson et al. 2013). A one year period is a very short time for assessing hospitalizations, because asthma is a variable disease; thus, longer follow-ups are needed. Another retrospective, registry-based study on asthma, proposed current smoking to be an independent risk factor for emergency department visits, with a hazard ratio (HR) of 3.6 (Kauppi et al. 2014). Smoking has been suggested to be associated with an increased severity of asthma (Eisner & Iribarren 2007; Polosa et al. 2011; Westerhof et al. 2014). In a retrospective registry-based study among patients with allergic rhinitis, 62.5% of those subjects who developed asthma during 10 years of follow-up and had >20 pack-years of smoking history, were reported to have a severity of moderate or severe asthma, when in non-smokers only 25.6 % had moderate severity or severe disease (Polosa et al. 2011). The study also suggested a dose-dependent relationship between smoking duration and increased asthma severity, as well as between smoking duration and poorer asthma control. However, the original study design of non-asthmatic patients with allergic rhinitis, and the small number of patients with >10 pack-years and poorer asthma control lead to limitations in interpretation of the results (Polosa et al. 2011).

Previous existing studies (Table 1) on asthma and smoking have mostly reported the effect of momentary smoking status (never/ex/current smoker) on the disease burden of asthma but the impact of lifelong smoking history and smoked pack-years has rarely been evaluated (Eisner & Iribarren 2007; Kauppi et al. 2014; Polosa et al. 2011; Thomson et al. 2013). The importance of assessing patients' cumulative smoking history in pack-years has been recognized, and the evaluation of lifelong smoking history is reported to have even more value than evaluating a patient's momentary smoking status (Polosa et al. 2011). However, only few studies have previously reported the negative impact of smoked pack-years on asthma (Hancox et al. 2016; Polosa et al. 2011; Tuomisto et al. 2016; Westerhof et al. 2014). A dose-dependent effect of smoking on loss of asthma control and increase in asthma severity have been previously proposed (Polosa etl al. 2011; Tuomisto et al. 2016; Westerhof et al. 2014). Predictors for increasing asthma severity were analyzed in a study with a two-year follow-up of patients with adult-onset asthma. Every 10 pack-years of smoking was reported to be independently associated with an increase of asthma severity with an odds ratio (OR) of 1.4, and thus, a dose-dependent effect of

smoking on asthma severity was proposed (Westerhof et al. 2014). However, a relatively short follow-up period of two years in the previous study does not answer the question of long-term effects of smoking on the disease burden in asthma. Additionally, the results of the effect of smoking history on asthma disease burden in the previously published studies have not been concordant. In another study among ex-smoking patients with severe asthma, no significant differences were reported in healthcare use, asthma related questionnaires (ACQ and AQLQ) or medication use when patients were categorized based on smoked pack-year history (Thomson et al. 2013). Thus, the impact of smoked pack-years on asthma has rarely been evaluated and is controversial. Furthermore, no clinical studies among actual patients with confirmed asthma, reporting the long-term impact of smoking on disease burden of asthma, have previously been published.

Table 1. Descriptions of the previous studies on asthma and smoking

Study	Subjects with asthma (+smoking history)	Study cohort description	Asthma diagnosis based on	Follow-up	Pack-years assessed	Onset of asthma	Asthma medication under study	Main finding
Ulrik et al. Thorax 1992	143 (60)	Clinical	Doctor's diagnosed asthma based on significant reversibility in FEV_1 with bronchodilator	10 years (1976-1988)	yes	unclear	Not clearly described. 50% had received corticosteroid medication during past 12 months.	Lung function decline was greater among patients with intrinsic asthma than in those with extrinsic asthma. No relation between the rate of lung function decline and number of smoked cigarettes was found.
Lange et al. N Engl J Med 1998	1095 (550)	Population-based	Self-reported	15 years, (1976-1994)	no	n.a.	No data available until 1991. Follow-up started before widespread use of ICS.	Subjects with asthma had greater decline in FEV_1 compared to those without asthma. Smoking accelerated the decline.
Grol et al. Am J Respir Crit Care Med 1999	101 (59)	Clinical, at baseline asthmatic children	Physician diagnosed asthma	1966-1996	yes	childhood	ICS not available at baseline. 11 subjects used ICS between 1983-1996	Low lung function in childhood and hyperresponsiveness were independent risk factors for low FEV_1 in adulthood. Smoking did not increase the rate of annual lung function decline.
Chalmers et al. Chest 2001	67 (31)	Clinical	According to ATS 1987 definition. Metacholine provocation test	no	no	n.a.	Only bronchodilator during past 2 months	Eosinophilic inflammation was observed in patients with asthma. Smoking induces neutrophilic airway inflammation.
Apostol et al. Am J Respir Crit Care Med 2002	608 (n.a.)	Population-based	Self-reported doctor or nurse diagnosed asthma, or receiving asthma medication	10 years, (1985-1995)	no	n.a.	Treatment methods not available	Early smoking initiation was associated with accelerated decline in FEV_1. Combination of asthma and heavier smoking had synergistic effect on decline.

Piipari et al. Eur Respir J 2004	521 (279)	Population-based	Reversible obstruction in lung function measurements. According to national consensus on asthma criteria	2.5 years	cigarette-years	n.a.	Only therapy-naïve patients included. Follow-up medication not described	The risk of developing asthma was higher among current and ex-smokers compared with never smokers.
James et al. Am J Respir Crit Care Med 2005	1301 (633)	Population-based	Self-reported doctor-diagnosed or self-reported	(1966-1994)	no	n.a.	Medication use was not recorded until in 1981 (=beginning of widespread use of ICS), and 1994	Asthma was associated with reduced lung function and accelerated decline during adulthood. Smoking and asthma had additive effect on lung function decline.
Dijkstra et al. Thorax 2006	122 (62)	Clinical	Unclear, mild hyper-responsiveness	23 years, (1963-1999)	yes	Median age at onset 6 years	Age at start of ICS 40-45 years.	ICS therapy was associated with smaller decline in FEV_1, except in those with ≥ 5 pack-years of smoking.
Boulet et al. Chest 2006	49 (22)	Clinical	According to ATS 1987 criteria. All had >12% increase in FEV_1 after bronchodilator	no	no	childhood or adulthood	Corticosteroid naïve patients	Smokers had more respiratory symptoms, lower lung function and diffusing capacity, and higher sputum neutrophil counts compared with nonsmokers.
Eisner and Iribarren Nicotine Tob Res 2007	865 (564)	Registry based, hospitalized for asthma	ICD-9 codes 493.00-493.99, and self-reported doctor-diagnosed asthma	4 years, beginning in 2000	no	n.a.	Not evaluated or described	Current smoking was associated with increased severity of asthma and greater risk of hospitalization for asthma.
Polosa et al. J Allergy Clin Immunol 2008, and Respir Res 2011	152 (91)	Registry based, at baseline non-asthmatic subjects with allergic rhinitis	According to ATS 1987 definition. Bronchodilator test not assessed	10 years (1990-2000)	yes	adulthood >18 years of age	31.6% of the subjects with asthma at follow-up had used ICS during follow-up	Smoking was related to the risk of incident asthma. A dose-response association for pack-years and risk of new-onset asthma was observed. Pack-year history was associated to uncontrolled asthma dose-dependently.

O'Byrne et al. Chest 2009	2924 (492)	Clinical, post hoc analysis, mild persistent asthma	Spirometry and reversibility test, exercise test or PEF follow-up	3 years	no	n.a.	Randomised to have: Placebo, n=1478 or Budesonide 400ug x1, n=1446	Smoking was associated with accelerated decline in FEV_1. ICS therapy prevented lung function decline similarly in smokers and nonsmokers.
Thomson et al. J Allergy Clin Immunol 2013	740 (279)	Registry based, severe asthma	ATS definition of severe asthma 2000: lung function measurements recommended	no	yes	n.a.	ICS dose described. Maintenance oral steroid in use in 42% of the subjects.	Current smokers have poorer clinical and health care outcomes, and different inflammatory profile in sputum and blood compared with ex-smokers and never smokers.
Kauppi et al. BMC Pulm Med 2014	344 (133)	Registry based	Spirometry and reversibility test, PEF follow-up, exercise test or hyperreactivity test	10 years (1995-2006)	no	age at onset 42.6-43.7 years	Not evaluated	Smoking, poor health related quality of life and poor lung function are independent risk factors for ER visits.
Westerhof et al. J Allergy Clin Immunol 2014	128 (80)	Clinical, asthma diagnosis <1 year prior to enrollment	Spirometry and reversibility test or methacholine provocation test	2 years	yes	adulthood >18 years of age	Median ICS dose/day 375ug fluticasone equivalent	Pack-year history predicted an increase in asthma severity dose-dependently.
Aanerud et al. Eur Respir J 2015	2116 at baseline (n.a.)	Population-based	Self-reported	8.9 years, (1991-2002)	yes	early- and late-onset (cut-off 10 years of age)	Not evaluated or described	Asthma was associated with increased risk of adult airway obstruction. Smoking increased the risk among those with asthma onset after 10 years of age.
Colak et al. Am J Respir Crit Care Med 2015	5691 (3387)	Population-based	Self-reported	4.5 years, beginning in 2003	yes	n.a.	Any kind of asthma medication in use in 69% of the subjects	Smokers with asthma had more symptoms, airflow limitation, higher inflammatory and allergic biomarkers, higher risk for lung cancer, cardiovascular comorbidities and death than those without asthma.
Hancox et al. Am J Respir Crit Care Med 2016	268 (142)	Population-based	Parent-reported or self-reported and compatible symptoms and medication use	From 3 to 38 years of age, beginning in 1975	yes	childhood or adulthood	Not clearly described. ICS use reported by 62% of those with childhood-persistent asthma	Smoking was associated with lower FEV_1/FVC ratios among subjects with late-onset asthma but not among subjects with childhood-onset persistent asthma.

ICS: inhaled corticosteroids, ATS: American Thoracic Society, PEF: peak expiratory flow, ER: emergency department

2.2.4. Effect of smoking on lung function in asthma

The effect of smoking on lung function in asthma patients has remained mainly unknown because of the lack of clinical studies with smoking asthma patients (Table 1). Population-based studies have previously suggested active smoking to have a negative effect on lung function in persons with asthma (Aanerud et al. 2015; Apostol et al. 2002; Colak et al. 2015; Hancox et al. 2016; James et al. 2005; Lange et al. 1998). In a population-based study on data from the European Community Respiratory Health Survey, current smokers with late-onset asthma were suggested to have more rapid annual decline in FEV_1 when compared to never-smokers. The mean adjusted FEV_1 decline was reported to be 34mL/year among current smokers and 30mL/year in never-smokers (Aanerud et al. 2015). However, in the study, the definition of asthma was based on subjects' self-reporting, which may lead to a considerable possibility of miscategorizing asthma. Moreover, a low age cut-off of 10 years for asthma-onset when categorizing subjects as having late-onset disease was used; thus child-onset asthma patients in the study may be miscategorized (Aanerud et al. 2015). Another study in a population-based birth cohort of the Dunedin Study in New Zealand has reported cumulative smoking to be associated with lower FEV_1/FVC ratios (1.4%) per every 10 pack-years smoked among subjects with late-onset asthma. However, using self-reported asthma diagnoses might lead to bias and actual inclusion of subjects with COPD as asthmatics. Additionally, the study does not describe the use of any medication or the number of exacerbations of the subjects, which is a severe limitation (Hancox et al. 2016). The adherence to asthma medication is commonly shown to be poor (Engelkes et al. 2015), and asthma exacerbations have been shown to predict excess lung function decline in asthma (Bai et al. 2007). Thus, such information is extremely necessary when reporting the factors associated with lung function decline.

No clinical studies with long-term follow-up showing the negative impact of smoking on lung function have previously been published (Table 1). Only short follow-up studies, or studies with cross-sectional lung function evaluation of clinical patients with asthma without any follow-up, have previously been published (Boulet et al. 2006; O´Byrne et al. 2009). In a post hoc analysis of a three-year follow-up study among patients with mild asthma, smoking was reported to be associated with accelerated decline in lung function. The study reported that smokers lost a mean of 192mL and non-smokers 134mL of lung function in FEV_1 during the 3-year follow-

up (O´Byrne et al. 2009). In the previous population-based studies, the follow up was not started at the diagnosis of asthma, and the use of self-reported asthma or self-reported, doctor-diagnosed asthma may lead to misclassification of asthma (Aanerud et al. 2015; Apostol et al. 2002; Colak et al. 2015; Hancox et al. 2016; James et al. 2005; Lange et al. 1998). In a pioneering, general population study of Copenhagen City Heart study, the annual lung function decline in different age, sex and smoking groups has been previously reported (Lange et al. 1998). The annual change in FEV_1 among 40-59 year-old male subjects was reported to be -58mL in smokers with asthma and -33mL in non-smokers with asthma. In asthmatic women in the same age group, the decline was reported to be 38mL in smokers, and 31mL in non-smokers, annually (Lange et al. 1998). However, the diagnosis of asthma in the study remains uncertain due to the use of the subjects' self-reporting and lack of objective confirmation of the diagnosis. More importantly, the follow-up of the study has started years before widespread use of inhaled corticosteroids, similarly as in also another pioneering study (James et al. 2005; Lange et al. 1998). Therefore the results do not reflect the situation among modern clinical patients with treated asthma.

Some negative studies on the effect of smoking on lung function have also been published, showing no relationship between lung function decline and tobacco smoking (Grol et al. 1999; Ulrik et al. 1992). Thus, the effect of tobacco smoking on lung function in patients with objectively confirmed asthma has remained controversial, and the long-term effect of smoking on adult-onset asthma is especially unknown.

2.3. Asthma-COPD overlap

2.3.1. Description of COPD

Chronic obstructive pulmonary disease (COPD) is a preventable disease characterized by persistent bronchial obstruction causing respiratory symptoms (GOLD 2019; Miravitlles et al. 2014). The cause of persistent airflow limitation is chronic bronchial inflammation due to long term exposure to noxious inhaled particles of the air (Hogg & Timens 2009). The most common exposure leading to COPD in western countries is tobacco smoking but other risk factors for COPD have also been recognized. For example, some occupational exposures and burning

biomass fuels may increase the risk for developing COPD. In addition, some genetic factors (e.g., alpha-1 antitrypsin deficiency) as well as childhood disadvantage factors (e.g., respiratory infections, low birth weight and maternal smoking) may increase the risk for developing COPD (GOLD 2019; Lange et al. 2015). The inflammatory mechanisms in COPD are not completely understood, but oxidative stress and an excess of proteinases are likely to modify the inflammation (Barnes 2016). The inflammation in the lung may persist even after smoking cessation (GOLD 2019). The presence of comorbid conditions is common in COPD, and systemic inflammation has been proposed to play a role in the comorbid conditions (Cavaillès et al. 2013; Miller et al. 2013). COPD causes persistent airway obstruction and/or lung parenchymal emphysema, and the symptoms usually consist of dyspnea, cough and sputum production (GOLD 2019; Hogg 2004). COPD can be diagnosed in a patient with a history and symptoms suggestive for COPD by measuring lung function with spirometry. Additional investigations, such as diffusing capacity measurements, may be used to evaluate the presence of emphysema (GOLD 2019; Rossi et al. 2017). A FEV_1/FVC ratio less than 0.7 after administration of a bronchodilator is considered to confirm persistent airflow limitation typical for COPD (GOLD 2019). The aims of COPD therapy are to reduce symptoms, to minimize the risk of exacerbations and to improve the health status and exercise tolerance of patients. The therapy commonly consists of bronchodilator medications, inhaled corticosteroids for some patients with exacerbations, increasing the physical activity of the patient, vaccinations and most importantly, smoking cessation (GOLD 2019; Riley & Sciurba 2019).

2.3.2. Definition of asthma-COPD overlap

Asthma and chronic obstructive pulmonary disease (COPD) have previously been considered as two different diseases with completely different clinical features (GINA 2019; GOLD 2019). However, a novel clinical phenotype, asthma-COPD overlap (ACO), has recently been described and included in several national guidelines (GINA/GOLD 2017; Kankaanranta et al. 2015; Miravitlles et al. 2013, Miravitlles et al. 2016). An ACO patient is considered to have several features of both diseases, asthma and COPD but no consensus exists on what these features exactly are and how to differentiate between ACO, asthma and COPD

(GINA/GOLD 2017; Kankaanranta et al. 2015; Miravitlles et al. 2013; Postma & Rabe 2015).

The term "asthma-COPD overlap syndrome (ACOS)" has also been previously used in addition to ACO. Use of the term ACOS is no longer advised, because ACO is currently not considered to represent a single disease or a syndrome but two diseases overlapping (GINA/GOLD 2017). However, both the terms ACO and ACOS may still be seen in the literature when describing the overlapping of asthma and COPD.

The overall need for the label and phenotype of ACO has also been debated, and it has even been suggested that no categorizing labels should be used among obstructive airway diseases because of the heterogeneity of the patients (Agusti et al. 2016; Cazzola & Rogliani 2016; Gibson & McDonald 2015). There is some fear that blurring the line between asthma and COPD might lead to overuse of inhaled corticosteroid medication (Postma & Rabe 2015). However, the need for better recognition of the underlying mechanisms, biomarkers, endotypes and phenotypes among obstructive airway diseases has been accordantly acknowledged (GINA 2019; Martin et al. 2019; Reddel 2015). Additionally, considering the modern opportunities for highly personalized medical treatment in obstructive lung diseases, the identification of ACO among patients with asthma or COPD is considered to be highly important. Recognition of ACO in COPD patients most commonly affords the opportunity to benefit from inhaled corticosteroids or furthermore from biologic medication. The recognition of ACO among patients with asthma will similarly affect the choice of therapy towards more targeted treatments, such as roflumilast and long-acting muscarinic antagonists (LAMA). Better identification of the patients leads to higher quality and more cost-effective treatment of the patients.

2.3.3. Development of asthma-COPD overlap

Asthma-COPD overlap may develop on different pathways. The most commonly studied pathway is when a patient has a previous diagnosis of COPD and later develops asthma-like symptoms and high bronchial reversibility or variability visible in the lung function measurements. Another but less often recognized pathway is when a patient with a previous asthma diagnosis continues smoking and develops persistent obstruction of the airway compatible with the COPD diagnostic criteria $FEV_1/FVC < 0.7$ (Barrecheguren et al. 2015; Wurst et al. 2016). A third pathway has also been suggested in which an asthma patient without significant smoking history

develops persistent, non-reversible bronchial obstruction (Wurst et al. 2016). However, the exposure to noxious inhaled particles, most commonly tobacco smoke in western countries, has been considered a major requirement for COPD diagnosis (GOLD 2019). Therefore, a significant smoking history has been further considered a necessary factor when categorizing patients as having ACO (Barrecheguren et al. 2015; Sin et al. 2016).

2.3.4. Diagnosis of asthma-COPD overlap

Specific diagnostic criteria and an exact definition of ACO are still lacking. No specific biomarker, clinical feature or spirometric finding has been recognized to separate ACO from asthma (Kostikas et al. 2016; Postma & Rabe 2015; Sin et al. 2016; Tho et al. 2016). Currently, the most widely suggested major features for ACO are an asthma-like reversible airflow limitation shown in objective lung function measurements, a persistent airway obstruction $FEV_1/FVC < 0.7$, and a history of tobacco smoking, all in the same patient (GINA/GOLD 2017). However, it has also been suggested that patients who do not have high bronchial reversibility in FEV_1 (>400mL), asthma or atopy should have been diagnosed before the age of 40 years in order to fulfill ACO diagnosis (Sin et al. 2016).

The lack of previous studies on asthma-COPD overlap results mostly from excluding asthma patients from COPD studies and vice versa: excluding smoking patients from studies on asthma. Therefore, an urgent need has been recognized for specific identification of ACO biomarkers and characteristics (Kostikas et al. 2016; Postma & Rabe 2015; Reddel 2015).

2.3.5. Spirometry in diagnostics of ACO

No specific spirometric criteria for ACO diagnostics are available (Kostikas et al. 2016; Postma & Rabe 2015; Sin et al. 2016; Tho et al. 2016). Suggestions for major characteristics and findings favoring the diagnosis of ACO have been suggested, but the suggestions are under debate and no consensus is established. The persistent bronchial obstruction in spirometry, i.e. $FEV_1/FVC < 0.7$ after a bronchodilator test, has been considered as one major criterion for ACO, because it is also a requirement for COPD diagnosis (GINA 2019; GOLD 2019). In addition, a significant bronchodilator response (BDR) of at least 12 or 15 % and 400 mL has been suggested as compatible with a diagnosis of ACO among patients with smoking

history and fixed airway limitation (GINA/GOLD 2017; Kankaanranta et al. 2015; Miravitlles et al. 2012; Miravitlles et al. 2016). For patients without a previous asthma diagnosis but with fixed airway obstruction and significant smoking history, a higher reversibility of > 400 mL in FEV_1 has been proposed as a criterion to fulfill an ACO diagnosis, if the patient has no previous asthma diagnosis before the age of 40 years (Kankaanranta et al. 2015; Miravitlles et al. 2016; Sin et al. 2016; Soler-Cataluña et al. 2012).

The use of a bronchodilator test in spirometry has been considered as the gold standard in the diagnostics of obstructive lung diseases (GINA 2019; GOLD 2019). However, the current evidence shows that the correct FEV_1 bronchodilation cut-off in asthma diagnostics is not widely studied, and even less is known about the ability of a bronchodilation test to differentiate between asthma, COPD and ACO (Tuomisto et al. 2019). A recent population-based study on subjects with asthma or COPD reported a similar proportion of patients fulfilled the limit of bronchodilator response ≥ 12% and ≥ 200 mL in FEV_1; 17.3% of subjects with asthma and 18.4% of subjects with COPD reached the limit (Janson et al. 2019). However, the asthma diagnosis in the study was defined based on the subjects' self-reported, physician-diagnosed asthma, which leads to uncertainty about the correct diagnosis (Janson et al. 2019). Furthermore, the evaluation was executed on subjects with ongoing therapy; thus, the result reveals nothing about the therapy-naïve situation at the diagnostic moment (Janson et al. 2019). The sensitivity and specificity of any previously suggested cut-off values for BDR in FEV_1 have not been clearly shown, and therefore, it has recently even been proposed that bronchodilator test in spirometry may not be a very sensitive tool in asthma and COPD diagnostics (Janson et al. 2019; Tuomisto et al. 2019). A study among hospital outpatient clinic patients with respiratory symptoms and measured lung function proposed that the bronchial reversibility of patients categorized as having asthma and those with ACO was similar. In the study, asthma patients were reported to have a mean of 375mL and the ACO patients 382mL of response to the bronchodilator in FEV_1 (Ozkaya et al. 2016).

2.3.6. Asthma-COPD overlap vs. COPD

The prevalence of ACO among patients with COPD has been proposed to be 12-55% (Gibson & McDonald 2015; Wurst et al. 2016). When compared to patients with COPD alone, ACO patients are reported to have a poorer quality of life

(Miravitlles et al. 2013) and more frequent hospitalizations (De Marco et al. 2013; De Marco et al. 2015; Menezes et al. 2014). In a population-based study of the European Community Respiratory Health Survey, subjects with asthma-COPD overlap were reported to have five-fold greater, and subjects with COPD a two-fold greater risk of reported hospital/emergency department admissions over the follow up of 9 years when compared to healthy subjects (De Marco et al. 2015). However, 36% of the subjects in the ACO group and up to 28% in the COPD group were reported to be non-smokers; thus, the ACO diagnosis was not based on smoking history (De Marco et al. 2015). ACO patients have also been suggested to have more frequent exacerbations (De Marco et al. 2013; Hardin et al. 2014; Menezes et al. 2014), reduced physical activity (Miravitlles et al. 2013), and increased dyspnea and wheezing (De Marco et al. 2013; Miravitlles et al. 2013), when compared to COPD alone. In the COPDGene study, subjects with self-reported, doctor-diagnosed asthma before the age of 40 years were categorized as having asthma-COPD overlap. In the study, subjects with ACO were reported to have a mean 1.2 exacerbations during the year prior to enrolling in the study, whereas subjects with COPD had only 0.7 exacerbations (Hardin et al. 2014). A considerable limitation of the study, however, is the age limit of 40 years for an asthma diagnosis, because a majority of asthma has already been shown to be diagnosed at adult age (Honkamäki et al. 2019; Kankaanranta et al. 2017; Sood et al. 2013).

2.3.7. Asthma-COPD overlap vs. asthma

Asthma-COPD overlap among patients with asthma is far less studied. A major need for clinical studies with actual, real life patients still exists, although some registry-based and epidemiological studies have been previously published. The prevalence of ACO is proposed to be 13-61% among subjects with asthma, depending on the ACO-criteria used (Gibson & McDonald 2015; Wurst et al. 2016). It has been suggested by the previous studies that ACO patients have a poorer quality of life (Kauppi et al. 2011), increased symptoms of dyspnoea (Milanese et al. 2014), and more frequent exacerbations (Menezes et al. 2014; Milanese et al. 2014) when compared to patients with asthma alone. In an Italian study among elderly asthma patients >65 years old, 43% of ACO patients were reported to have experienced a severe exacerbation during the previous year, whereas 18% of patients with asthma alone had experienced a severe exacerbation (Milanese et al. 2014). The same study reported ACO patients to have poorer control of the disease, with Asthma Control

Test (ACT) score a mean of 18 when compared to asthma patients with an ACT score of 21 (Milanese et al. 2014). However, the criterion for ACO in that study was a presence of chronic bronchitis or a declined diffusing capacity test result. A total of 56% of ACO patients were never-smokers; thus, it is very likely that the ACO group actually included mainly patients with asthma. In addition, the patients in the ACO group were reported to less frequently have ICS/LABA medication in use when compared to asthma patients; therefore the results may not reflect the true differences between ACO and asthma (Milanese et al. 2014). Another, population-based study of obstructive lung disease identified asthma by using lung function tests or self-reporting of the subjects (Menezes et al. 2014). ACO patients have been reported to have impaired lung function (Kauppi et al. 2011; Menezes et al. 2014; Milanese et al. 2014) when compared to patients with asthma alone, which is logical because the definition of ACO usually includes declined lung function with airway obstruction. Furthermore, an increased rate (Andersén et al. 2013; Menezes et al. 2014) and duration of hospitalization (Andersén et al. 2013) among patients with ACO have been proposed when compared to patients with asthma alone. In a previous Finnish study, based on data from the hospital discharge registry, ACO patients were reported to have, on average, 6.0 treatment days in the hospital during the years 2000-2009, whereas patients with asthma diagnosis alone were treated, on average, for 2.1 days (Andersén et al. 2013). The limitations of the study, however, are the lack of clinical data, such as lung function measurements, smoking history and use of medication, and the uncertainty about the correctness of the diagnosis reported in the registry (Andersén et al. 2013). In addition, the mortality of ACO patients (Lange et al. 2016; Sorino et al. 2016), and the proportion of patients having comorbidities (Milanese et al. 2014) have been reported to be higher among ACO patients as compared with asthma patients. The life expectancy of individuals with ACO with late-onset asthma has been suggested to be up to 12.8 years shorter than that of healthy never-smokers (Lange et al. 2016). The finding indicates the importance of ACO, although the results can be considered only suggestive, because of the population-based study design, the asthma diagnosis being based on the subjects' self-reports, and the ACO definition not being based on smoking history (Lange et al. 2016).

The definition of ACO has varied considerably in previous studies, which has led to inconsistent results and conclusions. Some previous studies among persons with asthma have categorized patients with airway obstruction *per se* as having ACO, even without any smoking history or significant exposure to other harmful particles in the air. Furthermore, the previous perspective of asthma being merely an allergic disease

is also reflected also in the ACO studies. Thus, smoking adult patients with fixed airway obstruction but without allergic conditions have not been categorized as having ACO in some studies, even with a significant reversibility shown in spirometry. The use of spirometry in diagnostics of obstructive lung diseases, especially asthma, varies in different countries. Up to 57 % of asthma patients have been reported not to have their lung function objectively measured at the time of diagnosis (Gershon et al. 2012), a result reflected in the previous ACO studies. Coexistence of a reported diagnosis of asthma and COPD in the same patient still does not reliably describe the basis of the diagnoses, a limitation that should be considered, especially when evaluating the findings of registry- and population-based studies. Thus, there has still remained a lack of studies in well-described, real-world cohorts of patients with objectively confirmed asthma.

2.3.8. Occupational exposures in developing asthma-COPD overlap

Tobacco smoking is considered the main cause for developing COPD among the western population. Occupational exposure to inhaled noxious particles, most commonly vapors, gases, dusts and fumes (VGDF), is also a recognized risk factor for COPD (GOLD 2019; Sadhra et al. 2017). It has been estimated, that up to 15% of COPD is attributable to occupational exposures (Balmes et al. 2003). Occupational exposures and smoking have previously been suggested to have an additive effect in development of COPD (Blanc et al. 2009). Occupational exposure alone is reported to double the odds for developing COPD, whereas smoking alone increased the risk to 7-fold. The combination of smoking and occupational exposure increased the risk for COPD up to 14-fold (Blanc et al. 2009). A previous population-based study reported a similar proportion of subjects with asthma and with ACO to have occupational exposure history when defined based on whether subject ever had worked in an occupation with exposures (De Marco et al. 2015). However, the use of self-reported diagnosis of asthma, or the presence of airway hyperreactivity as the basis of asthma diagnosis in the study may lead to selection bias: subjects without actual asthma disease, or subjects with COPD and mild hyperreactivity may have been included as asthmatic subjects (De Marco et al. 2015). A previous study on the predictors of ACO after World Trade Center dust exposure did not assess the effect of smoking at all, and the diagnosis of ACO was not based on smoking history (Singh et al. 2018). Another previous study evaluated clinical and inflammatory

characteristics of ACO among subjects with occupational asthma, reporting older age and higher ICS dose to be independently associated with ACO (Ojanguren et al. 2018). However, the considerable limitation of the study was, that smoking was not included in the model when predictors for ACO were evaluated (Ojanguren et al. 2018). Thus, the role of occupational exposures in development of asthma-COPD overlap among real-life patients with asthma has remained unknown and unstudied.

2.3.9. Biomarkers in asthma-COPD overlap

Identifying specific inflammatory biomarkers in ACO would not only help in the diagnostics but would also provide a pathway for personalized and targeted therapy options. Despite the widely recognized, urgent need for better characterization of ACO patients, no specific biomarkers have yet been found to identify ACO (GINA 2019; GINA/GOLD 2017; Kostikas et al. 2016; Tho et al. 2016). The results of the previous studies have been variable however, due to different definitions of ACO in the studies and the heterogeneity of obstructive lung diseases overall (Gibson & McDonald 2015). Asthma inflammation is commonly addressed as Th2-mediated; thus, FeNO, IgE, sputum eosinophils and blood eosinophils have been suggested to be usable in distinguishing ACO among patients with COPD (Cosio et al. 2016; Kitaguchi et al. 2012; Kobayashi et al. 2016). However, the usability of sputum eosinophil measurements in clinical practice is poor, and up to 10-40% of COPD patients are reported to have eosinophilic inflammation (Brightling & Geening 2019; George et al. 2019; Yun et al. 2018). Furthermore, smoking is reported to decrease FeNO levels, whereas several infectious conditions may lead to increased FeNO results (Bjermer et al. 2014; Schleich et al. 2010; Thomson et al. 2013). Based on the association between COPD and systemic inflammation (Barnes 2016), it has also been suggested that measurements of CRP or IL-6 could be used to identify ACO patients (Fu et al. 2014). Higher sputum and serum IL-6 levels, but no differences in CRP levels have been reported among overlap patients when compared to asthma patients, although the ACO diagnosis was not based on smoking history in some studies (Fu et al. 2014; Gao et al. 2016).

More recently, in addition to phenotyping airway diseases, an increasingly active field of research has been identifying treatable traits among obstructive lung diseases (Agusti et al. 2016; McDonald et al. 2019). Treatable traits have been defined as clinically relevant, measurable, identifiable and treatable factors, and biomarkers

(e.g., blood eosinophils) have been most commonly proposed as treatable traits. It has also been suggested that a questionnaire result or a simple patient characteristic (e.g., obesity) could be used as a treatable trait (McDonald et al. 2019).

3. AIMS OF THE STUDY

The aim of the present study was to evaluate the effects of smoking on adult-onset asthma.

The detailed aims were:

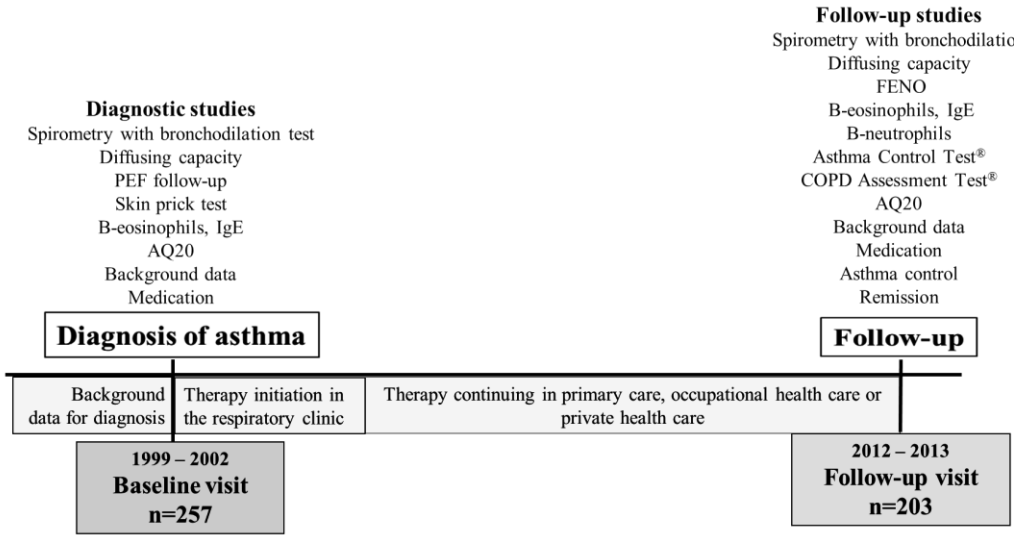
1. To study what is the effect of smoking on lung function, morbidity and disease burden in patients with adult-onset asthma. (I and IV)
2. To study what are the differences between asthma-COPD overlap (ACO) and adult-onset asthma, and how to recognize ACO patients in clinical work. (II)
3. To study what is the role of occupational exposures in developing ACO. (V)
4. To study whether the bronchodilator response in adult-onset asthma diagnosis differs according to age, and whether the suggested diagnostic features for ACO are valid and usable. (III and VI)

4. SUBJECT AND METHODS

4.1. Study design and setting of SAAS (I-II, IV-VI)

The present study is a part of the Seinäjoki Adult Asthma Study (SAAS, Figure 3.) (Kankaanranta et al. 2015). In the Seinäjoki Adult Asthma Study, a total of 257 patients were diagnosed with new-onset adult asthma in Seinäjoki Central Hospital, Finland, during the years 1999-2002. Patients were referred to specialized care by primary care physicians due to asthma suspicion. The diagnosis of asthma was made by a respiratory physician and the existing guidelines were followed. Consecutive patients with new-onset adult asthma were recruited to the study from the diagnostic visit and a written informed consent was obtained. Patients were aged ≥ 15 years in the diagnostic phase, and thus, in the enrolment into the study. The exclusion criteria of the Seinäjoki Adult Asthma Study were a previous diagnosis of asthma in childhood or an inability to give an informed consent. Diagnosis of asthma was based on typical symptoms and objective lung function measurements showing significant bronchial reversibility or variability. A histamine bronchoprovocation test or an exercise provocation test were performed on some patients at the diagnosis if considered necessary. Ex- and current smoking patients were included in the study. The majority of the patients were therapy-naïve at the diagnosis and inhaled corticosteroid medication was started immediately after the diagnostic visit as advised by the Finnish Asthma Program (Haahtela et al. 2001).

After 12 years of follow-up, 203 patients (79%) returned to a control visit during the years 2012-2013. The "lost to follow-up" reasons and the number of patients are described in Figure 4. During the control visit, lung function was measured, blood samples were collected, and patients were asked for background information and medication use by a structured questionnaire. During the follow-up period, patients were actively treated according to the Finnish Asthma Programme Guidelines and the Finnish Current Care Guidelines for asthma (Haahtela et al. 2001; Current Care Guidelines 2000 & 2012). Body mass index (BMI) was evaluated both at the baseline and at the follow-up visit. The number of patients included in studies from the original SAAS cohort are described in Table 2.



PEF: peak expiratory flow, B: blood, IgE: immunoglobulin E, AQ20: Asthma Questionnaire 20, FENO: fraction of exhaled nitric oxide, COPD: chronic obstructive pulmonary disease

Figure 3. A Schematic presentation of the Seinäjoki Adult Asthma Study (SAAS) (Modified from Kankaanranta et al. 2015)

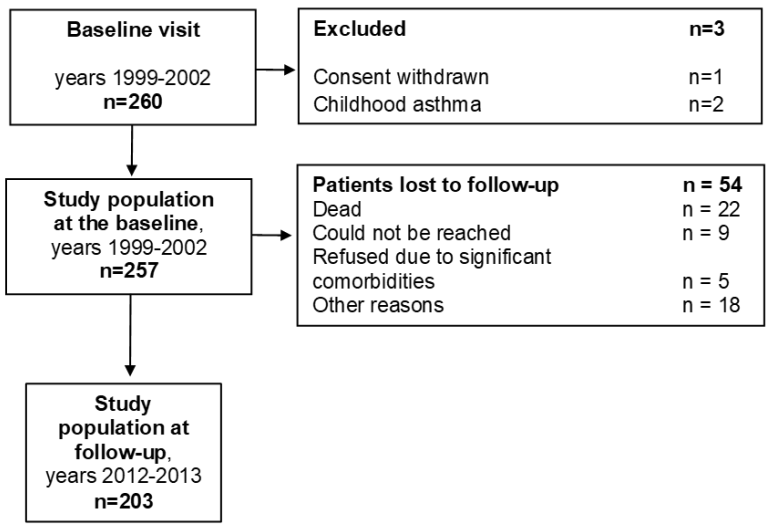


Figure 4. Included and excluded patients of SAAS cohort in a flow diagram (Modified from Tuomisto et al. 2016)

Table 2. Patients included from the original SAAS cohort in studies I, II and IV-VI (n=257)

Study	Included from the original cohort	n
I	Patients who returned to follow-up visit	203
II	Patients with information on pack-years at follow-up. Current smokers with smoking history <10 pack-years excluded	188
IV	Patients with detailed information on pack-years at follow-up	193
V	Patients with information on pack-years at follow-up	194
VI	Patients with BDR tested at baseline	245

SAAS: Seinäjoki Adult Asthma Study, BDR: bronchodilator response

4.1.1. Lung function measurements

Lung function measurements on every patient were performed with a spirometer (Vmax20C, Vmax22 or Vmax22D; Viasys Healthcare, Palm Springs, CA, USA or M9426 Spirometer, Medikro Oy, Kuopio, Finland) that was calibrated daily. Finnish reference values were used (Viljanen et al. 1982). At the baseline, a bronchodilator test with 200 μg of salbutamol was performed and an increase of at least 200 mL and 15 % in FEV_1 or FVC was considered significant. In the follow-up, bronchodilator test was performed with 400 μg of salbutamol. Lung function measurements at the baseline, when asthma diagnosis was made (1999-2002), and at the follow-up (2012-2013) were chosen for analyses. In addition, the maximum achieved lung function in FEV_1 during the first 2.5 years after the asthma diagnosis (i.e., the baseline visit) was assessed ($Max_{0\text{-}2.5}$). The point $Max_{0\text{-}2.5}$ describes the best achievable lung function on every patient after initiation and use of asthma medication, and it was achieved, on average, 0.6 years after the diagnosis (Figure 5). Lung function measurements after the baseline visit were performed without pauses or withholding of the asthma therapy and medication. Diffusing capacity of the lung was measured at the baseline and follow-up. Peak expiratory flow (PEF) monitoring was performed for two weeks at the baseline, and the response to bronchodilator medication was also measured during the PEF follow-up.

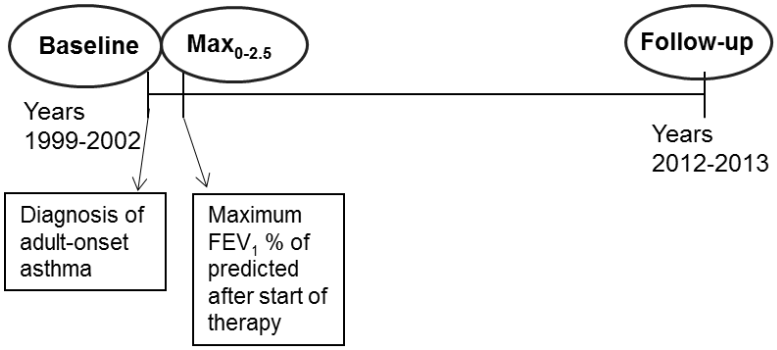


Figure 5. Measurement points of lung function.

4.1.2. Blood samples

Venous blood samples were collected at the baseline and follow-up visits. Laboratory assays were performed in the Seinäjoki Central Hospital accredited laboratory (SFS-EN ISO/IEC 17025:2005 and ISO 15189:2007). White blood cell differential counts were performed, immunoglobulin E (IgE) levels were determined (ImmunoCAP), and serum levels of IL-6 were assessed by ELISA (R & D Systems, Minneapolis, MN, USA). HsCRP was measured with the particle-enhanced immunoturbidometric method on the Roche Cobas 8000 automated clinical chemistry analyzer (Roche Diagnostics, Basel, Switzerland).

4.1.3. FeNO

The fraction of exhaled nitric oxide (FeNO) was measured with a portable rapid-response chemiluminescent analyzer (flow rate 50 mL·s-1; NIOX System, Aerocrine, Solna, Sweden). The American Thoracic Society standards were followed (ATS/ERS 2005).

4.1.4. Allergy testing

Atopy was tested by skin-prick tests towards common aeroallergens at the baseline visit. At least one positive reaction towards an allergen was considered significant and the patient was considered atopic.

4.1.5. Background data, symptoms and use of medication

A structured questionnaire to collect background information was used. The use of medication at the time of follow-up visit was reported by patients on the questionnaire. Data on symptoms were collected by using the questionnaires of the Asthma Control Test (ACT) (Nathan et al. 2004), COPD Assessment Test (CAT) (Jones et al. 2009), and Asthma Questionnaire 20 (AQ20) (Barley et al. 1998).

4.1.6. Healthcare use

The data on healthcare use, hospitalizations and emergency department visits were retrospectively collected from the patient records. All visits in the primary care, occupational care, private clinics and public hospitals during the 12 year follow-up were collected and in-patient hospital days were calculated.

4.1.7. Occupational data

Occupational data were retrospectively collected by using a questionnaire at the follow-up visit. The patients' occupation was asked and if already retired, the year of retirement and the occupation retired from were asked. To evaluate the duration of the patients' profession, the working information and reported occupation at the time of diagnosis (i.e., baseline) was confirmed from the patient records among the patients with VGDF exposure.

4.1.8. Comorbidities

The assessment of comorbidities was based on patients' self-reporting comorbidities or on self-reported medication in use. Unclear cases were confirmed from the patient

records. Conditions included as comorbidities were bronchiectasis, cancer, hypertension, coronary heart disease, atrial fibrillation and other cardiac arrhythmias, heart failure, diabetes, thyroid disorders, rheumatoid arthritis, and other inflammatory polyarthropathies and systematic connective disorders, irritable bowel syndrome, treated constipation, diverticular disease of the intestine, inflammatory bowel disease, treated dyspepsia (daily medication), viral hepatitis, and chronic liver disease, chronic kidney disease, peripheral vascular disease, prostate disorders, glaucoma, stroke and transient ischemic attack, epilepsy, migraine, Parkinson disease, multiple sclerosis, dementia, depression, schizophrenia/nonorganic psychosis or bipolar disorder, psoriasis, anxiety and other stress-related and somatoform disorders, painful condition (daily use of analgesic medication), and COPD (Ilmarinen et al. 2016; Barnett et al. 2012). COPD was not considered as a comorbidity in the group of patients with ACO in the studies evaluating ACO.

4.1.9. Assessment of smoking history

The detailed, lifelong smoking history of every patient was assessed based on both the questionnaire on their background information and the respiratory nurse's interviews, at the baseline and at the follow-up visit. Smoked pack-years (20 cigarettes per day for one year) were evaluated. Smoking status (never/ex/current smoker) of the patients was assessed.

4.1.10. Ethical permissions and study registration

The study protocol of Seinäjoki Adult asthma study has been approved by the Ethics committee of Tampere University Hospital, Tampere, Finland (R12122). Institutional permission (Seinäjoki Central Hospital, Seinäjoki, Finland) was obtained. All patients signed an informed consent at the enrolment into the study. Seinäjoki Adult Asthma Study is registered at ClinicalTrials.gov with identifier number NCT02733016.

4.2. Finnish asthma reimbursement registry (III)

Every patient with doctor-diagnosed asthma is entitled to have asthma medication reimbursement by the Social Insurance Institution in Finland. The reimbursement

covers 65% of the medical costs caused by asthma therapy. A statement by a physician on the patient's asthma diagnosis must be presented to receive the reimbursement. The statement must describe the fulfilment of the diagnostic criteria of asthma in objective lung function measurements. The reimbursement registry is administered by the Social Insurance Institution.

Data on the new asthma reimbursements in Finland during the years 2012-2013 were obtained from the registry for the current study. The numbers of new asthma reimbursements were considered to reflect the new asthma diagnoses made annually. Patients with special reimbursement due to other obstructive diseases (e.g., COPD) are also categorized in the original reimbursement data under the title "asthma reimbursements". Thus, the patients with new reimbursement due to any other obstructive disease than asthma were excluded, and only the patients who were entitled to special reimbursement due to asthma were included in the analyses (Kankaanranta et al. 2017).

4.3. Study design and setting of COREA (VI)

The Cohort for Reality and Evolution of Adult Asthma (COREA) is a cohort of 4,846 asthma patients in South Korea (Kim et al. 2009; Kim et al. 2013; Lee et al. 2011; Park et al. 2015; Park et al. 2018; Park et al. 2019; Park et al. 2019). The COREA started in year 2005 and includes patients with doctor-diagnosed (pulmonologist or allergist) asthma from 21 centres of Korea. Informed consent is obtained from every patient and the protocol has been approved by the institutional review board of each centre. The inclusion criteria of COREA were a diagnosis of asthma based on a positive bronchodilator test in spirometry after administration of 200 μg of salbutamol (≥ 12% and ≥ 200 mL), or airway hyperresponsiveness in a methacholine test (PC20 FEV_1 ≤ 25 mg/mL methacholine).

The current study included 785 patients from the COREA cohort. These patients were aged ≥ 15 years, steroid-naïve and had bronchodilator test performed at the time of diagnosis.

4.4. Statistical analyses

Statistical analyses were performed using SPSS software, versions 22-25 (IBM SPSS, Armonk, NY). $p<0.05$ was considered statistically significant. The normality of the data distribution was analyzed with the Kolmogorov-Smirnov's test and by visual

evaluation of the distribution. Group comparisons in normally distributed, continuous data were performed with one-way analysis of variance test (ANOVA) when three or more groups were analyzed, or with t-test when two groups were analyzed. Tukey's post hoc test was used. Categorical data were analyzed with Chi-squared test. Group comparisons on continuous data with skewed distribution were analyzed with Mann-Whitney U test between two groups, or Kruskal-Wallis test between three or more groups. Correlations were analyzed by using Spearman's test or, in COREA data, Pearson's test. Multivariable linear and logistic regression analyses were performed to analyze associations between independent variables and the dependent factor. The correlation matrix was analyzed and explanatory variables not strongly correlated ($R<0.7$) were included in the analyses. Before the regression analyses, the correlation matrix was analyzed, and forward, backward and enter methods were used to select variables in to the final model. In linear regression analysis, outliers were removed to ensure homoscedasticity. R^2 in linear regression analysis and Nagelkerke's R^2 in logistic regression analysis were considered when selecting the best model.

5. SUMMARY OF THE RESULTS

5.1. Description of the study population

The included population varied somewhat in the original communications of the current thesis based on the available information on lung function, bronchodilator response or pack-years (Table 2). The patients included in study I, i.e., the patients who returned for the follow-up visit (n=203), reflect the SAAS study cohort well. Those patients were, on average, 46 years old at the baseline, and 58 years at the follow-up. Female predominance was seen with 42 % of patients being males. The patients' median BMI was shown to be 27 kg/m^2 at the baseline. Weight increase was seen during the follow-up and median BMI of the patients reached 28kg/m^2 by the follow-up. Proportions of never-, ex- and current smoking patients at baseline were shown to be 49%, 33% and 18%, respectively. The proportion of current smokers was shown to decrease during the follow-up, and 15% of the patients reported current smoking at follow-up. Smoked pack-years of ex- and current smoking patients were seen to increase during the follow-up from median of 11 pack-years to 16, respectively. At baseline, 8% of the patients were using daily ICS, whereas 76 % reported daily use of ICS at the follow-up. The median post BD FEV_1 % of predicted was at baseline 88% and increased by the follow-up visit to 90%. The number of patients having post BD $FEV_1/FVC < 0.7$ was 31 (16%) at baseline and 54 (27%) at follow-up, respectively. The proportion of patients having fixed airway obstruction (i.e., post BD $FEV_1/FVC < 0.7$) and ≥ 10 pack-years was found to be 8% at the baseline and 15% at follow-up, respectively (Tuomisto et al. 2016). A total of 37% of the patients were shown to be atopic. Blood eosinophil levels were shown to be median of 0.28 x10^9/l at baseline and 0.17 0.28 x10^9/l at follow-up. The IgE levels were found to be 84 kU/l at baseline and 61 kU/l at follow-up.

The characteristics of the study population included in each different study (II, IV-VI), have been described in detail in the original communications (II, IV-VI).

Patients from the COREA and SAAS cohorts were included to study VI. The patients in both cohorts were mainly similar, adult-onset asthma patients. However, all patients included from the COREA cohort were steroid naïve at the diagnostic time of asthma.

5.2. The effect of smoking on adult-onset asthma

5.2.1. Lung function

Lung function decline was found to be accelerated among adult-onset asthma patients with a smoking history of ≥ 10 pack-years when compared to patients with less than 10 pack-years of smoking. The lung function was found to increase in both groups after the baseline, when the medication for asthma was started (Figure 6). Between the points of $Max_{0\text{-}2.5}$ and follow-up, the median annual decline in FEV_1 was 54 mL among patients with ≥ 10 pack-years smoked, and 36 mL in patients with less than 10 pack-years (p=0.003) (Figure 6). Because lung function decline has previously been suggested to be associated with current smoking, and the accelerated lung function decline might be a feature of COPD, we further excluded the current smoking patients, and those with fixed airway obstruction and ≥ 10 pack-years of smoking from the analyses. The finding remained similar even after the exclusion of current smokers, and also when patients with a possibility of having COPD were excluded. It was further evaluated, whether the increased lung function decline in those patients with ≥ 10 pack-years smoked is associated with active smoking or with the history of smoked pack-years. The patients with ≥ 10 pack-years of smoking were further divided into groups based on whether the number of pack-years increased after the baseline (i.e., patients who continued smoking after asthma diagnosis) or not (i.e., patients who were ex-smokers or did not continue smoking after asthma diagnosis), and the rates of lung function decline were compared. Among the patients with ≥ 10 pack-years of smoking history, the increased rate of lung function decline did not differ between the groups when they were further divided by whether the number of pack-years increased during the 12-year follow-up or not. Factors associated with annual FEV_1 (mL) decline were evaluated by a multivariable linear regression analysis. Smoking history of ≥ 10 pack-years, the baseline value of FEV_1 % of predicted, the change in FEV_1 (mL) from baseline to $Max_{0\text{-}2.5}$, FeNO > 20 ppb at follow up, and the level of blood eosinophils at follow-up were found to be independently associated with FEV_1 decline. In contrast, age, weight gain, use of oral corticosteroid courses during follow-up, sex, or the use of daily inhaled corticosteroids did not become significantly associated with annual FEV_1 decline, although a trend towards negative association was seen for age, weight gain, use of oral steroid courses and female sex. (I)

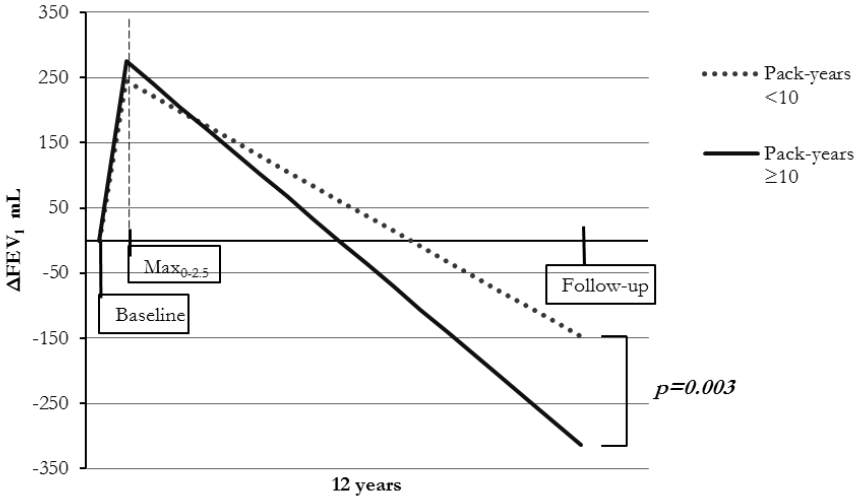


Figure 6. A schematic presentation of the change in FEV_1 (mL) during the 12-year follow-up in the groups with smoking history of <10 or ≥ 10 pack-years. Presentation based on group medians.

5.2.2. Disease burden

To evaluate the effect of smoking history, i.e., pack-years on the disease burden of asthma and to assess the previously suggested dose-dependency effect of smoking, hospitalisations, comorbidities and symptoms were analysed in groups divided according to smoked pack-years. The proportion of hospitalized patients during the 12-year follow-up was found to increase in relation to smoked pack-years, and patients with ≥ 20 pack-years were most frequently hospitalized. The proportion of patients hospitalized for any respiratory reason during the follow-up was up to 41% in the group of patients with ≥ 20 pack-years smoked (Figure 7). Asthma-related hospitalizations were also found to increase in relation to smoked pack-years. To evaluate whether the finding was related to current smoking or to pack-years, current smokers were excluded. Both findings remained after exclusion of current smokers. Furthermore, the number of smoked pack-years correlated with the number of hospital inpatient periods. In addition, a positive correlation between the number of comorbidities and smoked pack-years was found (Figure 8). Patients with ≥ 10 pack-years of smoking history had a higher number, a median of 2 comorbidities, when

compared to patients with less than 10 pack-years of smoking with 0 comorbidities. More severe symptoms, as measured by the Asthma Control Test (ACT) and COPD Assessment Test (CAT), were found to correlate with an increasing number of smoked pack-years (Figure 8). It was further evaluated, which factors were independently associated with hospitalizations. In a multivariable logistic regression analysis smoking history of ≥ 20 pack-years was independent of other factors associated with hospitalization for any respiratory reason (OR 2.5; 95% CI 1.0-6.0; p-value 0.043). Another factor predicting hospitalizations was the number of comorbidities of ≥ 2, whereas age of ≥ 50 years, current smoking, or use of oral corticosteroid courses were not associated with hospitalization for any respiratory reason. (IV)

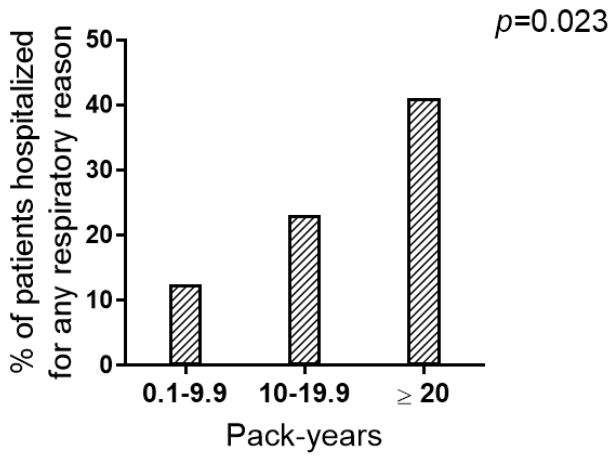


Figure 7. Proportion of patients hospitalized for any respiratory reason during the follow-up in groups with different smoking history in pack-years. P=0.023 for comparison between three groups.

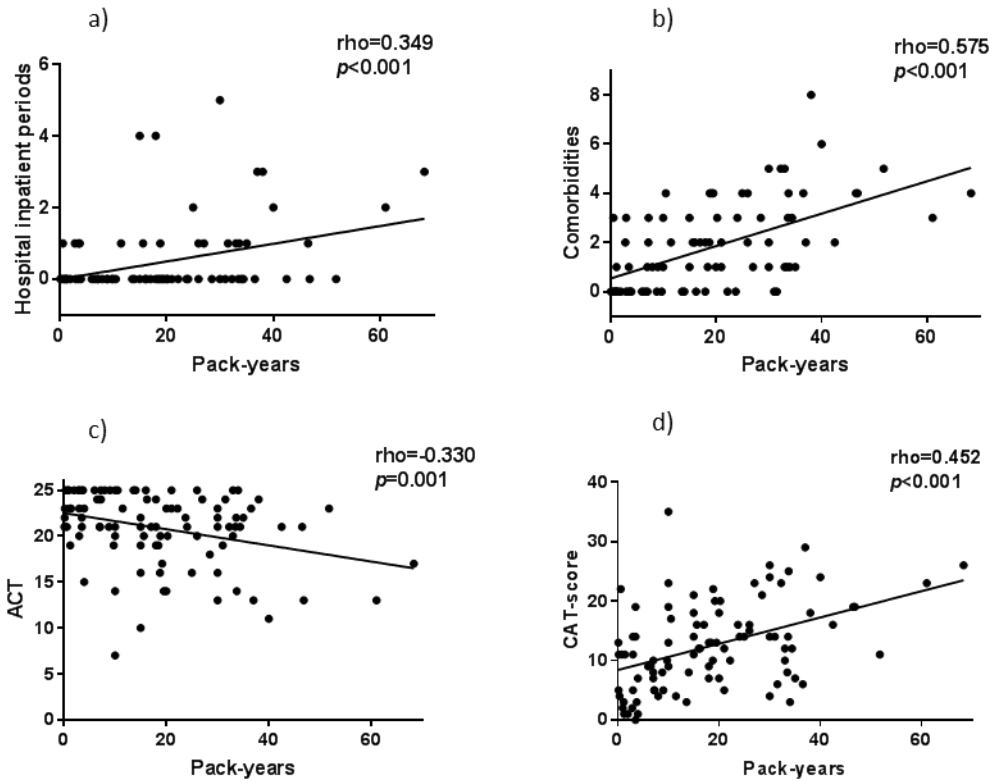


Figure 8. Spearman's correlations (rho) between smoked pack-years and a) hospital inpatient periods (one outlier removed), b) number of comorbidities, c) Asthma Control Test (ACT) -score, d) COPD Assessment Test (CAT) –score

5.3. Asthma-COPD overlap (ACO)

5.3.1. Differences between ACO and asthma

In the current study, the categorization of patients as having ACO was based on objective lung function measurement and smoking history. All patients had been diagnosed with asthma at the baseline. Patients were categorized as having ACO if they had ≥ 10 pack-years of smoking history and post bronchodilator FEV_1/FVC <0.7 at follow-up. The prevalence of asthma-COPD overlap was found to be 18% among adult-onset patients. The differences that could help to identify ACO among

patients with asthma were analyzed. ACO patients were found to have lower diffusing capacity of the lung when compared to patients with asthma alone. ACO patients also had higher levels of blood neutrophils, higher serum IL-6 levels, reduced lung function, higher remaining bronchial reversibility and a higher number of comorbidities compared to patients with asthma alone (Table 3). In contrast, the levels of blood eosinophils, hsCRP, IgE and FeNO did not differ significantly between the groups of ACO, never- and ex-smoking asthma patients with < 10 pack-years of smoking, and the non-obstructive asthma patients with ≥ 10 pack-year smoking history. Patients with ACO had more likely uncontrolled asthma (55.9% of the patients uncontrolled), but no differences were found in the use of oral corticosteroid courses or in the proportion of patients using daily inhaled corticosteroids between the groups of ACO and asthma. However, higher doses of inhaled corticosteroids were used by the non-obstructive asthma patients with ≥ 10 pack-years of smoking, and they also had long-acting beta-agonist medication more often in daily use.

Table 3. Diffusing capacity values, blood biomarkers and number of comorbidities in the groups of ACO, and two groups of asthma with low or heavy smoking history

	Never and ex-smokers with <10 pack-years n=122	**Non-obstructive patients with ≥10 pack-years n=32**	**ACO n=34**	**p-value**
DLco/VA % predicted	98 ±13	96 ±18	86 ± 22 ǂβ	**<0.001**
B-Neutrophils x10*9/L	3.60 (2.70-4.60)	3.85 (2.95-4.98)	4.50 (3.50-5.53) ǂ	**0.008**
IL-6 pg/mL	1.52 (1.12-2.48)	2.10 (1.09-5.69)	2.88 (1.88-4.99) ǂ	**<0.001**
Number of comorbidities	1 (0-2)	1 (0-3)	2 (1-3) ǂ	**0.008**

Data is shown as mean ± SD, or median (interquartile range). ACO= asthma-COPD overlap, DLco = diffusing capacity of the lung for carbon monoxide, VA= Alveolar volume, B=blood, IL-6= Interleukin 6
ǂ: as compared to group 1. (Never and ex-smokers with <10 pack-years) p<0.05
β: as compared to group 2. (Non-obstructive patients with ≥10 pack-years) p<0.05

No consensus exists on what type of patients should be categorized as having ACO. Asthma patients with fixed airway obstruction have been commonly described in previous studies as having ACO even without any smoking history or exposures to other noxious inhaled particles. Therefore, it was evaluated in the current study

whether patients with ACO, the diagnosis based on smoking history and airway obstruction, differed from those with asthma and fixed airway obstruction. When ACO patients were compared with obstructive asthma patients with persistent bronchial obstruction but non- or low smoking history (i.e. $FEV_1/FVC < 0.7$ but less than 10 pack-years), the results revealed ACO patients to have lower diffusing capacity than those with obstructive asthma (Figure 9). In addition, the levels of blood neutrophils and serum IL-6 were found to be higher in the ACO group (Figure 9), whereas the levels of blood eosinophils, IgE, hsCRP or FeNO did not differ between the groups with ACO or obstructive asthma. Moreover, CAT scores were found to be higher in patients with ACO as compared with obstructive asthma patients (Figure 10). (II)

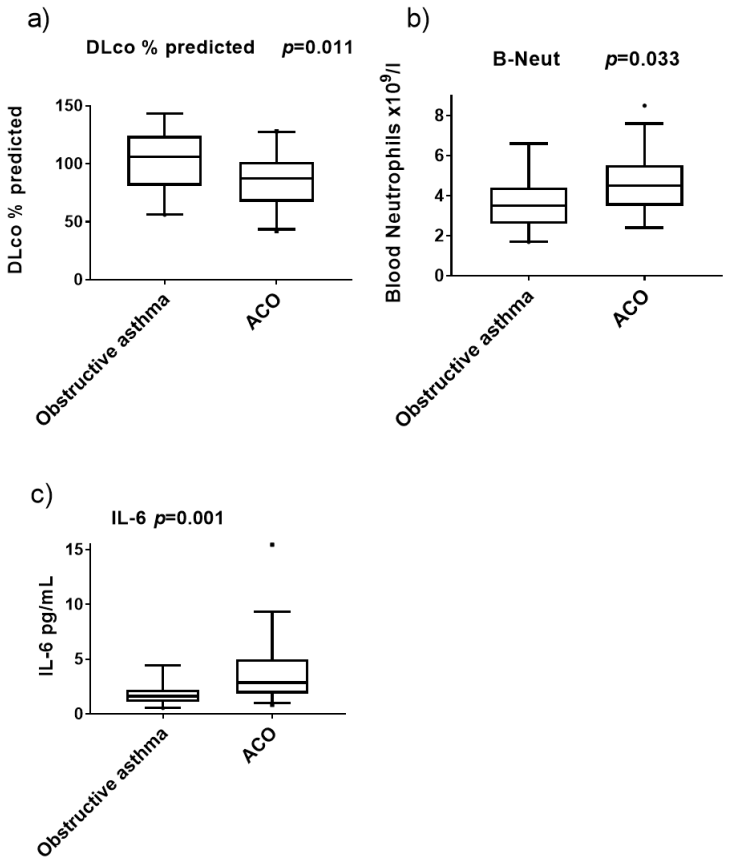


Figure 9. The differences between the groups of patients with obstructive asthma (n=19) or ACO (n=34). Shown are median, 25-75 percentiles and 5-95 percentiles (whiskers) in a) diffusing capacity of the lungs (DLco), b) blood neutrophil counts, c) serum interleukin (IL)-6 levels

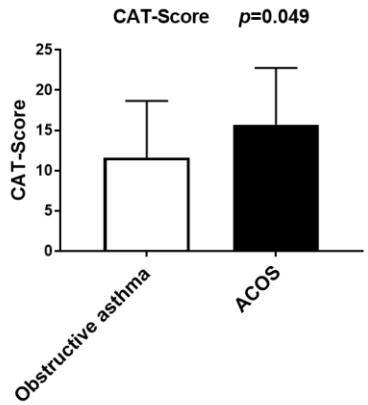


Figure 10. Mean (SD) COPD Assessment Test (CAT) scores in the groups of obstructive asthma and ACO

5.3.2. Occupational exposures in development of ACO

The role of occupational exposures in developing ACO is unknown. The association between occupational exposure to VGDF and the development of ACO among patients with adult-onset asthma was evaluated. The prevalence of ACO was found to be higher in the group of asthma patients with occupational exposure to VGDF as compared to the patients without exposure (Figure 11). The pack-year history was assessed in both groups, those with occupational exposure to VGDF and those without. There were no significant differences in the pack-year history between the groups, and the mean pack-year history was 27.4 years in both groups.

To investigate, whether occupational exposure to VGDF was associated with persistent obstruction, the prevalence of obstructive asthma in groups with different occupational exposure history was evaluated. The prevalence of obstructive asthma (i.e., $FEV_1/FVC < 0.7$ but less than 10 pack-years of smoking) was similar between the groups with or without occupational exposure to VGDF. The association of occupational exposure to VGDF with airway obstruction among all patients, and also among those without any smoking history was further evaluated in a regression model. Multivariable logistic regression analysis showed that the number of smoked pack-years and age were associated with bronchial obstruction among all patients. Obesity was associated with lower risk for obstruction, whereas sex or occupational exposure to VGDF were not associated with airway obstruction among all patients with adult-onset asthma.

A smoking history of ≥ 10 pack-years was considered a major criteria for ACO in the current study; therefore the factors associated with ACO among patients with ≥ 10 pack-years of smoking history were analyzed with another multivariable logistic regression analysis. Results revealed that occupational exposure to VGDF was independent of other factors associated with ACO (OR 4.2; 95% CI 1.1-15.3; *p*-value 0.030). Obesity was again found to be associated with lower risk of ACO, whereas pack-years were no longer associated with ACO among patients with ≥ 10 pack-years of smoking history. Sex, age or smoking status (never/ex-/current smoker) were not associated with ACO in backward, forward or enter method analyses, and therefore, were not included in the final model. (V)

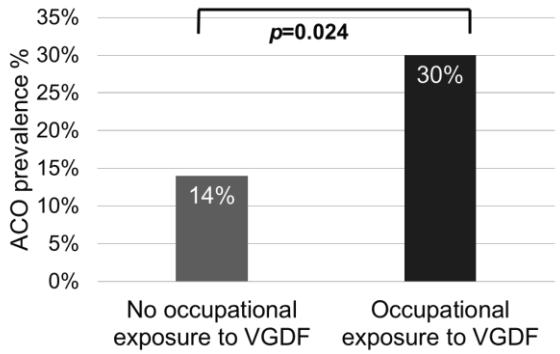


Figure 11. ACO prevalence in the groups of adult-onset asthma patients with or without occupational exposure to vapors, gases, dusts or fumes

5.4. Asthma-COPD overlap diagnostics

5.4.1. Age cut-off of 40-years

It has previously been suggested that one major criterion for ACO diagnosis should be asthma diagnosis before the age of 40 years or a patient should have high bronchial reversibility (Sin et al. 2016). To assess the usability and validity of the proposed criteria, the incidence of new-onset asthma in Finland in age groups of

<40 years and ≥ 40 years was analyzed. Analyses of the asthma special reimbursement data of Social Insurance Institution of Finland showed that 26281 patients were approved for the new special reimbursement for asthma medication in Finland during 2012-2013. The majority of these patients (54%) were aged 40 years or older, whereas only 46% of the patients were 0-39 years of age (Figure 12). Among females, 57.9% of patients receiving the new special reimbursement for asthma medication were aged ≥ 40 years. Among males, 50.5% of the patients were aged less than 40 years when reimbursement was granted. (III)

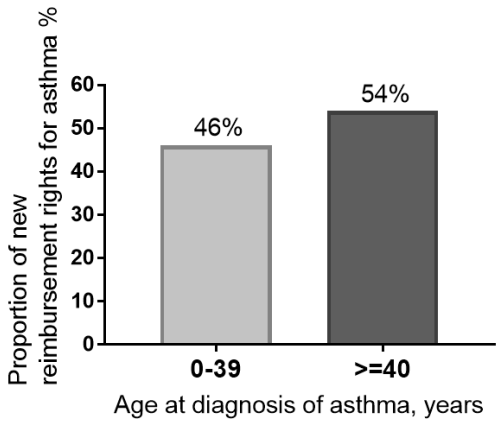


Figure 12. Proportion of all novel asthma reimbursement rights in age groups 0-39 years and ≥ 40 years

5.4.2. Bronchial reversibility

The presence of high bronchial reversibility in FEV_1 (>400mL) has been suggested as a major criterion for the diagnostics of ACO if a patient is diagnosed with asthma later than 40 years of age (Sin et al. 2016). In the current study, the usability and validity of the proposed criteria were analyzed in two, separate, well-defined cohorts of real-life patients with adult-onset asthma. If a higher BDR cut-off would be correct in patients with a higher age of onset of asthma, the hypothesis would be that the BDR is higher in older patients with new-onset asthma, and the 400mL limit is more often reached in older patients with new-onset asthma. In the present study, the bronchial reversibility in FEV_1 % from baseline value did not differ between the

groups of patients with age at asthma diagnosis < 40 years, 40-59.9 years or ≥ 60 years. No differences were found in the SAAS cohort, either in FEV_1 BDR in mL between the groups with different ages at onset. In addition, the proportions of patients having high bronchial reversibility of > 400mL did not differ between the age groups (Figure 13), neither did the proportion of patients achieving reversibility of 200mL, 12% or both. Among the patients in the COREA cohort, FEV_1 BDR in mL was higher in patients with the youngest age at diagnosis, and the percentage of patients fulfilling BDR of > 400mL or ≥ 200 mL in FEV_1 was highest in the group of patients aged < 40 years at diagnosis. However, there were no differences in the proportions of patients with BDR in FEV_1 of ≥12%, or ≥12% and ≥200 mL between the groups of patients with different ages at asthma diagnosis. The main finding of both cohorts remained after excluding patients who could be considered to have ACO.

The proportions of patients who would fulfil the proposed BDR cut-off of 400mL were analyzed. In patients with asthma onset at the age of ≥ 40 years, the BDR limit of 400mL in FEV_1 was reached only among 18 % of the patients in the SAAS cohort and 5% of the COREA cohort patients (Figure 13). Moreover, in correlation analyses, bronchodilator response in FEV_1 (in mL or in percentages from baseline) did not have a clinically relevant correlation with age at onset of asthma. (VI)

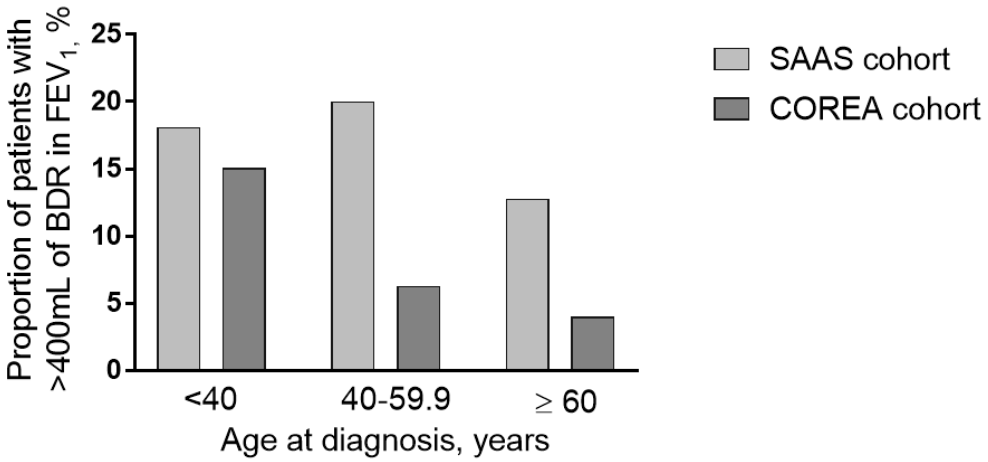


Figure 13. Proportion of patients reaching the 400mL cut-off of BDR in FEV_1 in groups with different age at asthma diagnosis.

6. DISCUSSION

6.1. Methodology

The Seinäjoki Adult Asthma Study is a cohort study consisting of real-life, clinical patients with new-onset adult asthma. Previous asthma studies have commonly excluded smoking patients and patients with smoking history; thus, only a few study cohorts including smoking patients with asthma have previously existed. Therefore also the knowledge of the association between smoking and asthma has remained minimal. The only exclusion criteria for the patients in the Seinäjoki Adult Asthma Study were a previous diagnosis of asthma in childhood or inability to give consent. Thus, the study cohort includes patients well reflecting the real-world patient flow at the clinics, including patients with a heavy smoking history, active smoking, comorbidities and occupational exposures.

Previous asthma studies have commonly focused on asthma starting in childhood, but the disease starting at adult age has remained less studied. In addition to Seinäjoki Adult Asthma Study cohort, only a few other cohorts of adult-onset asthma patients exist (Kauppinen et al. 2019; Rönmark et al. 2007; Westerhof et al. 2014; Westerhof et al. 2018). The importance of adult-onset asthma, however, has already been recognized, and the majority of asthma has been shown to be diagnosed at adult age, especially among females (Honkamäki et al. 2019; Kankaanranta et al. 2017; Sood et al. 2013). A strength of the current study is that the Seinäjoki Adult Asthma Study cohort includes only patients with the adult-onset disease, enabling us to achieve valuable knowledge on this rarely investigated adult-onset phenotype.

Due to the lack of real-world patient cohorts, previous studies on asthma and smoking have mainly been population-based or registry studies, suggesting negative effects of smoking on asthma. Population based-studies have commonly used self-reported asthma or self-reported, doctor-diagnosed asthma as a basis for subject to be categorized as having an asthma diagnosis (Table 1). Therefore, a considerable limitation of these previous population-based studies still exists, because the diagnosis of asthma may be incorrect. Similarly, in previous registry-based studies notable limitations exist in the reliability of the diagnosis.

The major strength of the current clinical cohort study is that the diagnosis of asthma was based on symptoms and objective lung function measurements; thus, the asthma diagnosis can be considered reliable (Kankaanranta et al. 2015). It has been reported even in western countries, that a considerable proportion of patients is left without spirometry measurements at diagnostic time of asthma (Gershon et al. 2012). In the current study, all patients underwent spirometry at diagnosis, follow-up and even several times during the follow-up for most patients. This is a strength of the current study. Another strength is the exceptionally long follow-up period of 12 years that, in addition, started at the diagnostic moment of asthma. After 12 years of follow-up, 79% of the patients returned for the control visit; thus, the high response rate further increases the value and reliability of our findings (Kankaanranta et al. 2015).

Some limitations remain concerning the current study. Pack-years were assessed based on patients' self-reports and research nurse's interviews. Thus, there is a possibility of underestimating the true smoked pack-years. The number of current smokers in our study was somewhat low, leading to a loss of power in the analyses when evaluating the effect of smoking status (i.e., never-/ex-/current smoker) on asthma. The data on medication in the current study was based on patients' self-reporting. Using the data of self-reported medication in the analyses may lead to overestimating the real use of medication. The categorization and the diagnosis of a patient's obstructive airway disease includes always a possibility of bias, because there is no absolutely definite way to categorize a patient as having asthma or COPD. During the past decade, it has been recognized that there may also be some bronchial reversibility present in COPD (Albert et al. 2012; Tashkin et al. 2008). However, the evidence on using any cut-off values of BDR in differentiating asthma from COPD is shown to be weak (Tuomisto et al. 2019). The current study also included patients with a heavy smoking history, and since the differential diagnostic criteria between asthma and COPD are not absolute, a possibility exists of misclassifying some smoking patients as having asthma. However, the asthma diagnosis of every patient in the current study was based on objective lung function measurements and made by following the guidelines. Additionally, the mean diffusing capacity value of the lungs was shown to be normal among patients with ≥ 10 pack-years of smoking, and the number of patients having post BD FEV_1/FVC <0.7 at baseline was low. Therefore possible COPD is not explaining the results in the current study. It also needs to be recognized that a possibility exists of low- or non-smoking patients having some other environmental exposure than smoking that would increase the risk of a patient developing COPD. However, only a minor proportion of the

patients was considered to have a working history linked to occupational exposures. The quantity measurements of the exposure were not performed in the current study's assessment of occupational exposures, which could be considered another limitation of the study.

The age at diagnosis of asthma was considered to be the age at onset. It is possible, however, that a patient may have presented some asthmatic symptoms already years before the diagnosis; thus, the onset of the disease might actually have been during childhood. However, the duration of symptoms before the diagnosis of the patients in the SAAS cohort has been previously assessed by Tuomisto et al. They reported a median of 12-24 months duration of symptoms before the diagnosis (Tuomisto et al. 2016). Considering that the mean age of the patients in the SAAS-cohort at diagnosis of asthma was 46 years, the patients can reliably be considered to have adult-onset asthma.

The bronchial reversibility status of a patient has been previously shown to vary over time (Hanania et al. 2011, Calverley et al. 2013). In the current study, BDR was assessed at one time point. This could be considered as a limitation. However, when evaluating BDR at the time of the asthma diagnosis on therapy naïve patients, BDR evaluation at any additional time points would not have been informative due to the therapy effect caused by asthma medication.

Using the Finnish medical reimbursement registry data in our study has provided both strengths and limitations. All Finnish patients with an asthma diagnosis based on objective lung function measurements showing significant bronchial reversibility or variability are entitled to the special medical reimbursement right. The reimbursement registry includes all special reimbursement rights granted in Finland, and the reimbursement rights numbers directly reflect the number of new asthma diagnoses made. Because of the strict criteria for obtaining the reimbursement right, the registry data about asthma diagnoses can be considered reliable, although some mild asthma cases without significant asthmatic findings in lung function measurements are left outside the registry. It should be noted, however, that in the reimbursement registry, the medication special reimbursement due to COPD and asthma are both documented under the label "asthma". Different ICD-10 codes are used to differentiate COPD from asthma. The criteria for obtaining the special reimbursement right for COPD are strict, and in some cases of COPD patients, an additional diagnosis of asthma may have been set if findings compatible with asthma diagnosis in lung function measurements have been seen. Similar diagnostic challenges due to unclear, guideline-based differential diagnostics between asthma and COPD as just described for the SAAS-cohort, concerns also for the

reimbursement registry data. However, the subjects having COPD as the first marked diagnosis in the reimbursement application were excluded in the current study. The special reimbursement right is obtained 6 months after the asthma diagnosis. Therefore, using the age cut-off of 40-years when dividing the subjects into two groups may lead to a possibility that some subjects actually have asthma onset at the age of 39 years and the reimbursement is obtained after 40 years of age. However, this possibility of bias is minor and does not explain the results.

The Cohort for Reality and Evolution of Adult Asthma (COREA) consists of real-life patients with new-onset asthma. Using the data from two different cohorts of asthma patients (SAAS and COREA) increases the reliability and generalizability of the results. However, due to somewhat different diagnostic procedures, the cohorts may include slightly different patients. The diagnosis of asthma was mainly based on the methacholine challenge test in the COREA study; thus, the patients included from the COREA cohort may be more hyper-reactive than those of the SAAS cohort.

Statistical limitations remain as well. The regression analyses are performed by choosing the independent variables that are considered clinically relevant or shown by previous studies to have relevance in association with the dependent variable. Thus, there is always a possibility of some meaningful variable to be missing from the regression analysis models or for others less important to be included. The explanation rate R^2 may be used to evaluate the goodness of the model. In our study, the R^2 of the linear regression model was moderate a 0.3, although there are no valid reference values.

6.2. The effect of smoking on lung function, morbidity and disease burden

In the present study among patients with adult-onset asthma, cigarette smoking was associated with accelerated loss of lung function. The annual decline in FEV_1 (mL and % predicted), FVC (mL) and FEV_1/FVC ratio was shown to be accelerated when smoking history reaches ≥ 10 pack-years, and a smoking history of ≥ 10 pack-years was also independently associated with a more rapid loss of lung function in FEV_1 (mL). The previous population-based studies have suggested smoking to be associated with more rapid lung function decline among smoking asthma patients when compared to nonsmokers (Aanerud et al. 2015; Apostol et al. 2002; Colak et al. 2015; Hancox et al. 2016; James et al. 2005; Lange et al. 1998). However, there

still has been a lack of clinical, long-term follow-up studies reporting the negative influence of smoking on lung function. Previous published clinical studies have reported cross-sectional findings or short-term effects of smoking, and even some negative studies, showing no relationship between smoking and lung function decline, have been previously published (Boulet et al. 2006; Grol et al. 1999; O'Byrne et al. 2009; Ulrik et al. 1992). Thus, the impact of smoking on lung function in patients with asthma has remained controversial. The current study was the first clinical study in real-world patients with asthma to report the harmful effect of tobacco smoking on long-term lung function in asthma. Furthermore, no previous studies reporting the effect of smoking on lung function among adult-onset asthma patients have been published.

The findings of the present study are in line with the results of the previously published epidemiologic studies suggesting the negative impact of smoking on lung function. In addition, it was found, that accelerated loss of lung function may continue when ≥ 10 pack-years of smoking has been reached, even if smoking has stopped. This is a new finding in the field of asthma research but similar results have already been reported in the field of COPD research. In COPD, the most rapid lung function decline is suggested to take place at the early phase of the disease, and loss of lung function has been reported to continue accelerated even in the patients who have stopped smoking (Csikesz & Gartman 2014; Drummond et al. 2012; Tantucci & Modina 2012).

Previous studies have reported the effects of momentary smoking status (never-/ex-/current smoker) on disease burden of asthma, but the effect of life-long, cumulative smoking history in pack-years has rarely been assessed (Eisner & Iribarren 2007; Kauppi et al. 2014; Polosa et al. 2011; Thomson et al. 2013). In the current study, the cumulative smoking history measured in pack-years, was found to dose-dependently increase the disease burden and multi-morbidity in asthma. The proportion of patients hospitalized for a respiratory reason increased in relation to smoked pack-years. The result remained even after excluding current smokers, indicating that the adverse effects may not be associated to plain smoking status of the patient, but the history of smoking. A smoking history of ≥ 20 pack-years was found to be independently associated with hospitalization. The findings are supported by the previous studies proposing an association between smoking and higher risk for hospitalizations and unscheduled healthcare visits (Eisner & Iribarren 2007; Kauppi et al. 2014; Thomson et al. 2013). Furthermore, the number of comorbidities and symptoms increased in relation to smoked pack-years in the current study. A previous study has similarly suggested more symptoms and lower

asthma control questionnaire (ACQ) scores in smoking asthma patients when compared to never-smokers (Chaudhuri et al. 2008). Our findings further suggest that the CAT-score test may be more sensitive in showing the symptoms of smoking asthma patients than is the Asthma Control Test.

Tobacco smoke contains thousands of compounds, many of them toxic (Talhout et al. 2011). The direct toxic effects of tobacco on the bronchial wall, the ongoing inflammatory process and oxidative stress lead to airway remodeling and may also cause systemic inflammation, which is further suggested to associate with comorbidities in asthma (Ilmarinen et al. 2016; Polosa & Thomson 2013). The results of the current study suggest that the irreversible and adverse effects of smoking on asthma might take place even earlier than is generally thought. The finding of accelerated lung function loss even after smoking has stopped, if ≥ 10 pack-years has been reached, is an important finding that emphasizes the major need for early intervention in asthma patients to stop smoking, and the need for smoking preventive actions. The current study also shows the importance and significance of evaluating the cumulative, life-long smoking history of adult-onset asthma patients.

Future studies should address the effects of smoking in asthma in the early phase of the smoking history. Currently, the early findings in COPD are under active investigation. However, not until recently have smoking patients been included in the studies on asthma; thus the effects of smoking on asthma have remained relatively unknown. Additionally, patients with severe comorbidities have commonly been excluded from the previous studies on asthma. The current study included the patients with any kind of smoking history or comorbidities in the study cohort. Interestingly, the proportion of active smokers (15%) in the current study reflects well the number of smokers in the general population (Honkamäki et al. 2019; Vartiainen 2018). The future asthma research increasingly needs real-world data and real-life cohorts that also include smoking patients with asthma and patients with comorbidities. We also increasingly need information on asthma in the elderly in addition to information on the early effects of smoking on asthma. Thus, even longer follow-up studies are needed on actual patients with asthma.

6.3. Clinical implications of the smoking studies

The current study is the first study to show the negative effect of smoking on lung function in clinical patients with asthma. The impact of patients' life-long smoking history was shown to be important and ≥ 10 pack-years of smoking was found to be a predictor for accelerated lung function decline. Pack-year calculation is an easy and usable tool for clinical work, and should be used routinely in addition to evaluating patients' plain smoking status, as also previously proposed (Polosa et al. 2011). The results of the present study indicate that after 10 pack-years of smoking, the accelerated lung function decline may continue even after smoking cessation. Thus, active intervention to get patients to stop smoking should be carried out as early as possible. The results of the current study also suggest that the symptoms of asthma patient with marked smoking history may be better recognized by using the CAT score questionnaire. The disease burden and multimorbidity were shown to dose-dependently increase with the number of smoked pack-years. To avoid patients' hospitalizations and to increase asthma patients' health and well-being, the accumulation of pack-years should be prevented. Early intervention for smoking cessation is expected to decrease the economic burden because of the decreased morbidity (Ekpu & Brown 2015). This further increases the need not only for early interruption of smoking but also for smoking preventive actions, including legislative restrictions, to be implemented.

6.4. The differences between ACO and adult-onset asthma

Asthma-COPD overlap has been recognized quite recently, and only a little is known about it. Previous studies have reported mostly the differences between ACO and COPD, but the description used for ACO has been variable (De Marco et al. 2013; De Marco et al. 2015; Hardin et al. 2014; Menezes et al. 2014; Miravitlles et al. 2013). Therefore, the reported results have not reached coherence. The previous asthma studies have generally excluded active smokers and patients with ≥ 10 pack-years of smoking history; and thus, ACO among asthma patients has still been far less studied.

The prevalence of ACO among patients with asthma was found to be 18% in the current study, which is in line with the previous studies (Kauppi et al. 2011; Wurst et al. 2016). Higher prevalence numbers have also been suggested in some studies, but the previous studies have not used consistent definitions for ACO, which reflects on the reported prevalence numbers. Nevertheless, a marked proportion of patients with asthma are shown to have ACO, a finding demonstrating the relevance of ACO identification. The current study showed that asthma-COPD overlap differentiated from asthma by having lower diffusing capacity values, higher levels of blood neutrophils and higher IL-6 values. ACO patients also had higher numbers of comorbidities and higher remaining bronchial reversibility after 12 years of therapy. This study was the first to evaluate the differences in clinical characteristics and blood biomarkers between ACO and asthma on real-life patients with adult-onset asthma.

The diffusing capacity of the lungs was found to be lower in patients with ACO when compared to those with asthma alone. In differential diagnostics between obstructive airway diseases, declined diffusing capacity in smoking patients is considered as an indicator for emphysema, a characteristic of COPD (GINA/GOLD 2017). The previous ACO research has rarely evaluated diffusing capacity. One previous study has reported that COPD patients with asthmatic symptoms (defined as ACO) have lower values of diffusing capacity when compared to patients with asthma (Kitaguchi et al. 2016). Thus, the finding in the current study, of lower diffusing capacity values among ACO patients with objectively confirmed diagnosis of asthma is a novel result. Declined diffusing capacity may contribute to the increased disease burden of ACO patients, suggested by the previous studies (Andersén et al. 2013; Kauppi et al. 2011; Menezes et al. 2014; Milanese et al. 2014). Furthermore, based on the results of the present study, the diffusing capacity measurements could be used as a tool for ACO recognition among patients with asthma. This study also showed that ACO patients had higher remaining bronchial reversibility after 12 years of asthma therapy when compared to patients with asthma, while the medication between these two groups did not differ. This finding further suggests steroid resistance playing a role in ACO (Barnes 2013). Based on these results, ACO cannot be distinguished from asthma by using the clinically validated symptom questionnaires ACT, CAT or AQ20, although previously higher CAT scores among ACO patients had been reported (Kurashima et al. 2016). Asthma control among ACO patients was found to be poorer than that of asthma patients. Poor control in the present study might, however, partially be explained by lower

lung function among ACO patients, because control was evaluated according to the GINA 2010 report (GINA 2010).

Previous studies have reported higher sputum neutrophils among patients with ACO when compared to asthma patients (Fu et al. 2014; Gao et al. 2016). Thus, the current study reported finding of higher blood neutrophil levels among patients with ACO when compared to asthma patients is in line with the previous studies. It should be noted that in the current study the use of inhaled corticosteroids was similar between ACO and asthma patients, and thus, possible iatrogenic neutrophilia caused by inhaled corticosteroids does not explain the results (Zhang et al. 2001; Zhang et al. 2002). The levels of IL-6 were found to be higher among ACO patients when compared to asthma patients. This finding is in line with previous studies reporting higher serum or sputum IL-6 levels among patients with ACO (Fu et al. 2014; Gao et al. 2016). IL-6 has been considered as a marker for systemic inflammation and is suggested to promote the neutrophilic inflammation in asthma (Ilmarinen et al. 2016). CRP is another commonly studied biomarker for systemic inflammation (Ilmarinen et al. 2016; Paone et al. 2016). Previously, it has been suggested that the systemic inflammation in ACO might resemble that of COPD with elevated levels of CRP (Gibson & McDonald 2015). Surprisingly, the levels of hsCRP in the current study did not differ between the patients with ACO or asthma, a finding also supported by a previous study (Fu et al. 2014).

Interestingly, when comparing asthma patients with fixed airway obstruction but a low smoking history with ACO patients, ACO differed most clearly by having a lower diffusing capacity, a higher number of comorbidities, higher blood neutrophil levels and higher IL-6 levels. This finding suggests that smoking-induced airway obstruction indeed differs from the obstructive disease caused by ongoing asthma inflammation. Therefore, non- or low smokers' asthma with fixed obstruction may not be routinely categorized as ACO as has commonly been done in the field of research. However, this finding also further demonstrates the heterogeneity of ACO. Previous studies have reported lower FeNO levels and lower blood eosinophil counts among patients with COPD when compared to patients with asthma-induced fixed airway obstruction (Contoli et al. 2010; Fabbri et al. 2003; Rogliani et al. 2016). The current study showed that blood eosinophil levels, FeNO or hsCRP cannot separate ACO patients from asthma patients, because no differences between ACO and asthma were found concerning these biomarkers.

The current study also evaluated the association between ACO and occupational exposures. There was a higher ACO prevalence among patients with occupational exposures to VGDF when compared to patients without exposures. The similar

smoking history of the exposed patients and those without exposures indicate that heavier smoking does not explain the result. Furthermore, the prevalence of airway obstruction was similar between the groups of different occupational exposures, showing that occupational exposure alone does not explain the increased ACO prevalence. Moreover, a regression model showed occupational exposure to VGDF to be associated with ACO. These findings suggest an interplay and additive effect between smoking and occupational exposure to VGDF in development of ACO. The role of occupational exposures in ACO have been reported previously by only a few studies (DeMarco et al. 2015; Ojanguren et al. 2018; Singh et al. 2018). However, considering the small number of previous studies, and the limitations of the few previously published studies, the current study is the first clinical study in patients with asthma to report the interplay between occupational exposures and smoking in development of ACO.

The future research on ACO will be interesting. The need still exists for tools to identify ACO among patients with any obstructive airway disease. We need larger real-world cohorts with objectively diagnosed obstructive airway disease to better characterize ACO. It would also be interesting to assess the differences between ACO and COPD with reversibility; how do those two entities separate? Better identification on ACO will increase the use of correct, individually chosen therapy and thus, directly affect the well-being of the patients.

6.5. What is the validity of the proposed criteria for ACO diagnostics

The findings of the present study have been part of the active scientific discussion on the proposed diagnostic criteria for ACO. Since ACO is no longer considered a separate disease or a syndrome, at least by some authors, but rather two diseases overlapping, it is extremely important that the diagnostics of asthma and COPD among patients suitable for having ACO are valid and based on strong scientific proof. It has been suggested that patients with a smoking history of ≥ 10 pack-years and fixed airway obstruction who do not have high bronchial reversibility in FEV_1 (>400mL), asthma or atopy should be diagnosed before the age of 40 years to fulfill the ACO diagnosis (Kankaanranta et al. 2015; Martin et al. 2019; Miravitlles et al. 2016; Sin et al. 2016; Soler-Cataluña et al. 2012). That is, if a patient, as just described, has significant, asthma like BDR but still less than 400mL and is aged ≥ 40 years at the time of diagnosis, the asthma diagnosis would be excluded if the proposed

criteria were to be used. This would lead to the further exclusion of several therapeutic options, including leukotriene antagonists and biologic medication for asthma.

The perception of asthma starting in childhood, or, at the least, earlier than 40 years of age, has been a dogma in the scientific research on obstructive airway diseases until the very recent understanding of different phenotypes, including adult-onset asthma. Previous population-based research from the 1980-90 decades have proposed that the prevalence of self-reported asthma start decreasing after 40 years of age, and that of COPD start increasing, making COPD the most prevalent obstructive airway disease among adults (van Schayck et al. 2004). This concept has led to the persistent impression that obstructive airway disease among patients ≥ 40 years of age should be primarily evaluated as COPD unless asthmatic symptoms are reported before the age of 40 years. The current study showed, by analyzing the medication reimbursement data, that 54% of all new asthma cases are actually diagnosed later than 40 years of age in Finland. This finding was even more outstanding among females, because 57.9% of all women obtained their reimbursement right for asthma medication after 40 years of age. These findings are consistent with the previous modern studies reporting adult-onset asthma as the most prevalent phenotype of asthma, especially among women (Kankaanranta et al. 2017; Sood et al. 2013). Furthermore, the results of the present study are in line with even the most recent studies, which have reported adult-onset asthma becoming the dominant phenotype of asthma by the age of 38 years in women and 50 years in men, with a particularly high asthma incidence among middle-aged females (Honkamäki et al. 2019). Thus, the previous perceptions of asthma starting usually before 40 years of age, and of middle-aged onset obstructive lung disease being primarily COPD can already be considered old-fashioned. Moreover, as the scientific basis is weak for using an age limit of 40 years in differential diagnostics of asthma, further studies are needed to create valid diagnostic criteria for ACO. Currently, the evidence indicates that no age limit what-so-ever can be used reliably for ACO diagnostics.

Another question is the suggested criterion for bronchial reversibility; is the cut-off of 400mL BDR in FEV_1 a acceptable tool in differential diagnostics between asthma, COPD and ACO? A BDR of 200mL and 12 % in a patient with asthmatic symptoms is considered diagnostic for asthma (GINA 2019). A higher cut-off of 400mL has been suggested to increase the reliability of the asthma diagnosis (GINA 2019). The question, however is, whether it is legitimate or even necessary to use more strict criteria for ACO among middle-aged and older patients? What is the

basis for the higher BDR criteria, and what are the consequences of using it? COPD prevalence has previously been suggested to increase after 40 years of age (van Schayck et al. 2004), and the differential diagnostics of obstructive lung diseases among adult patients plays a highly significant role. Using a higher cut-off of BDR among obstructive patients with smoking history and aged 40 years when diagnosed with obstructive lung disease would probably lead to less use of inhaled corticosteroids. In other words, patients would be categorized as having COPD, not ACO. This is to say, overuse of ICS would be avoided but at the cost of underdiagnosing asthma among patients with smoking history. This would especially concern women, as the majority of female asthma has been reported to be diagnosed at adult age (Honkamäki et al. 2019; Kankaanranta et al. 2017; Sood et al. 2013). Furthermore, in addition to an underuse of ICS, following this diagnostic protocol would lead to overuse of (long-acting) bronchodilators, which have already been shown to be deleterious in patients with asthma (Nelson et al. 2006; Patel et al 2013; Suissa et al. 2000; Suissa et al. 2002)

The current study showed that only a minor proportion of therapy-naïve patients with novel asthma actually fulfill the suggested BDR cut-off of 400mL in FEV_1 at diagnosis. The proportion of patients fulfilling the criterion was found to decline with increasing age at asthma diagnosis, and after 40 years of age, only 5-18% of the patients reached the limit. This is to say, 82-95% of adult-onset asthma patients do not reach the BDR limit of 400mL in FEV_1 at the diagnosis. There was a lack of correlation between age and BDR in both cohorts of SAAS and COREA, a finding that further questions the usability of the proposed criterion of a higher BDR in older patients. The possibility of biased results in the present study, caused by some patients with ACO hypothetically having lower BDR, was further eliminated by excluding all patients with possible ACO (i.e., $FEV_1/FVC < 0.7$ and pack-years ≥ 10 at diagnosis). The findings remained similar. Thus, patients with adult-onset asthma are commonly shown not to reach the 400mL BDR limit, which has been suggested as a major criterion in the differential diagnostics for asthma, COPD and ACO. This further indicates that the usability is also poor of the suggested BDR limit among patients compatible with ACO.

The future research should focus on identifying the differences between ACO and asthma. The results of the current study suggest that the proposed diagnostic criteria for ACO (patient having 400mL BDR in FEV_1 if asthma is diagnosed later than 40 years of age) should be abandoned. Additionally, the developed criteria should be based on strong scientific evidence when creating new diagnostic

guidelines. Current evidence does not support any use of higher BDR limits in ACO diagnostics.

6.6. What is the future of ACO?

Most recently, the scientific discussion about the need for ACO has divided along two different paths. The GINA report recognizes the importance and need for identification of ACO patients in order to choose targeted therapy for those patients (GINA 2019), whereas the current GOLD report does not discuss ACO (GOLD 2019). It has even been suggested that all labels such as "asthma" and "COPD" should be abandoned in the future, instead, all obstructive diseases should be addressed only by treatable traits (Agusti et al. 2016; Cazzola & Rogliani 2016; Gibson & McDonald 2015). Based on current knowledge, asthma-COPD overlap does not represent a single disease or a syndrome but two currently acknowledged diseases overlapping in the same patient (GINA 2019; GINA/GOLD 2017). The identification of these two diseases is nevertheless important. Ignoring the coexistence of these two diseases would probably lead to under-treatment of patients and further to increased morbidity and mortality. Considering the modern options for personalized therapy in asthma, especially biologic medications, the understanding of the overlapping of asthma in a patient with previous COPD is crucially important.

The most recent discussion of treatable traits among obstructive airway diseases is extremely important, especially in a scientific way. The treatable factors and the characteristics that play a significant role in the course of patients' asthma or the prognosis are highly valuable to recognize (Agusti et al. 2016; McDonald et al. 2019). Identification of biomarkers also generates an opportunity to develop new targeted medications. Phenotyping adult-onset asthma, however, has managed to point out that despite all the modern molecules in the pharmacological field of therapy, the basic factors such as smoking and obesity still remain the most important factors causing adverse effects on an asthma patient's health (Ilmarinen et al. 2017). Therefore, if we would only try to recognize blood biomarkers or measurable molecules instead of a full clinical presentation, some fundamental knowledge would be missed.

Furthermore, good communication between physicians and patients provides a basis for good results in asthma therapy and adherence (GINA 2019); therefore, a need exists for understandable and easy-to-remember terminology. Communication

that use scientifically valid and informative terms, may not be as successfully implemented in the real-world clinical practice. Therefore, even though increasing recognition of treatable traits is highly valuable in the field of obstructive diseases research, practicing physicians will still need informative, clear definitions of patients' conditions and diseases. Even though asthma and COPD are heterogeneous diseases with various overlapping characteristics, the prevalence of the asthma-COPD overlap is suggested to be high. One fourth of asthmatics and nearly one third of COPD patients are evaluated to have ACO (Hosseini et al. 2019). Thus, ACO is a highly relevant condition among clinical patients, and various therapy options require adequate identification of the disease. In addition, switching the current labels of asthma and COPD to address the conditions merely as obstructive lung diseases with various treatable traits would especially make future population-based research impossible to carry out. For these reasons, the current asthma, COPD and ACO labels should not be abandoned, although the increasing research on biomarkers and treatable traits is important. Future research should focus on better identification and characterization of ACO in well-described patient cohorts. It would also be interesting to evaluate the differences between eosinophilic COPD and ACO and the differences between COPD with bronchial reversibility and ACO. The clinical findings in a study setting, particularly as just described, would bring significant knowledge of therapy and prognosis of these patients.

6.7. Clinical implications of the ACO studies

The present study has demonstrated differences between asthma and ACO. Diffusing capacity measurements may be used as a tool to better identify ACO among patients with asthma. ACO patients are shown to have increased levels of blood neutrophils and IL-6, indicating the presence of non-T2 type inflammation, which usually is not as steroid-sensitive disease as the T2-type disease (Sze et al. 2019). This finding may lead to better selection of personalized therapy for the patients with ACO. Furthermore, the current study introduced differences between ACO and asthma with fixed airway obstruction but a low smoking history. This result indicates that fixed airway obstruction caused by smoking or ongoing asthma inflammation are not similar and should not be categorized as the same disease. Furthermore, the inflammatory profile of ACO was found to differ from the previously reported inflammatory profile of COPD (Barnes 2016; Gibson & McDonald 2015). The hsCRP levels did not differ between ACO and asthma, further

increasing knowledge of the differences between ACO and COPD. Occupational exposures to VGDF were reported to have an association with ACO. The clinical implication of that finding is that active interventions for smoking cessation should take place at early phase in the patient's smoking history. This is especially important in primary care and occupational health care. Additionally, attention should be paid to protections against occupational exposures. Strong preventive actions towards tobacco smoking should also be carried out at the population level.

The previously proposed diagnostic criteria of ACO should be re-evaluated. The suggested age limit of 40 years for an asthma diagnosis to be diagnosed with ACO has been shown by the current study to be invalid, because the majority of asthma is diagnosed after 40 years of age. This finding is also supported by previous, recent studies (Honkamäki et al. 2019; Kankaanranta et al. 2017; Sood et al. 2013). The previously proposed higher reversibility in bronchodilator test as a criterion for ACO should be abandoned. The current study showed only a minority of adult-onset asthma patients to have high reversibility at diagnosis of asthma, and the BDR did not correlate with age at diagnosis. The finding indicates that a higher BDR cut-off in the differential diagnostics between obstructive airway diseases would lead to underdiagnosing asthma and ACO. This is further supported by very recent research (Tuomisto et al. 2019).

7. CONCLUSIONS

Smoking patients and those with smoking history have generally been excluded in the previous studies on asthma. Therefore, the effect of smoking on asthma has remained relatively unknown, although some previous population-based and registry studies have suggested negative effects. Asthma-COPD overlap has recently been recognized and included in guidelines, but remained rarely studied. Identification of ACO has nevertheless, been considered important, because better recognition increases the use of more personalized, modern therapy options. The diagnostic criteria for ACO are currently not confirmed, but some criteria have been suggested. The aim of the present study was to evaluate the effect of smoking on asthma and to investigate the differences between asthma and ACO. Additional aims were to evaluate the usability and validity of the proposed criteria for ACO, and to investigate the role of occupational exposures in developing of ACO.

The major findings and conclusions were:

1. Smoking was found to accelerate lung function decline in patients with adult-onset asthma. The loss of lung function was shown to be accelerated after ≥ 10 pack-years of smoking and the decline in FEV_1 remained accelerated even after smoking had stopped if 10 pack-years had been reached. A smoking history of ≥ 10 pack-years was independent of other factors associated with accelerated lung function decline. Pack-year history was found to dose-dependently increase hospitalizations, symptoms and comorbidities among patients with adult-onset asthma.
2. Asthma-COPD overlap was found to differ from asthma by having lower diffusing capacity values, higher levels of blood neutrophils, higher IL-6 values and higher remaining bronchial reversibility despite therapy. ACO was also found to differ from asthma with fixed airway obstruction but no- or low smoking history. Diffusing capacity measurement may be used to identify ACO in clinical work.
3. Occupational exposures to VGDF were found to be associated with ACO among patients with adult-onset asthma. The results indicated that

occupational exposure alone may not result in ACO but the combination of occupational exposures and smoking. The results thus suggested smoking and occupational exposure to VGDF may have an additive effect in development of ACO.

4. Bronchodilator response was found to be stable despite the age at diagnosis of adult-onset asthma and BDR did not correlate with age. The result indicates that using different BDR at different ages when diagnosing obstructive airway diseases is not reasonable. The majority of asthma was found to be diagnosed after 40 years of age in Finland. The validity of the previously suggested criteria for ACO was found to be poor, and to lead to underdiagnosing asthma and ACO among smokers, especially in women. The previously suggested ACO criteria should be re-evaluated.

The results of the present study emphasize the importance of smoking history as a cause of adverse outcome in asthma. Smoking history of every patient should be assessed in pack-years, and active interventions towards smoking cessation should be undertaken. Future research should be executed more often in clinical settings among real-life patients with asthma, and longer follow-ups are needed. The early effects of smoking on asthma need to be studied in the future, and the differences between ACO, asthma and COPD should be further investigated.

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REFERENCES

Aanerud M, Carsin AE, Sunyer J, Dratva J, Gislason T, Jarvis D, deMarco R, Raherison C, Wjst M, Dharmage SC, Svanes C. Interaction between asthma and smoking increases the risk of adult airway obstruction. Eur Respir J. 2015;45(3):635-43.

Aaron SD, Boulet LP, Reddel HK, Gershon AS. Underdiagnosis and Overdiagnosis of Asthma. Am J Respir Crit Care Med. 2018;198(8):1012-1020.

Agusti A, Bel E, Thomas M, Vogelmeier C, Brusselle G, Holgate S, Humbert M, Jones P, Gibson PG, Vestbo J, Beasley R, Pavord ID.Treatable traits: toward precision medicine of chronic airway diseases. Eur Respir J. 2016;47(2):410-9.

Albert P, Agusti A, Edwards L, Tal-Singer R, Yates J, Bakke P, Celli BR, Coxson HO, Crim C, Lomas DA, Macnee W, Miller B, Rennard S, Silverman EK, Vestbo J, Wouters E, Calverley P. Bronchodilator responsiveness as a phenotypic characteristic of established chronic obstructive pulmonary disease. Thorax. 2012;67(8):701-8.

Al-Muhsen S, Johnson JR, Hamid Q. Remodeling in asthma. J Allergy Clin Immunol. 2011;128(3):451-62.

Amelink M, de Nijs SB, de Groot JC, van Tilburg PM, van Spiegel PI, Krouwels FH, Lutter R, Zwinderman AH, Weersink EJ, ten Brinke A, Sterk PJ, Bel EH. Three phenotypes of adult-onset asthma. Allergy 2013;68:674-80.

American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 2005; 171: 912–930.

Andersén H, Lampela P, Nevanlinna A, Säynäjäkangas O, Keistinen T. High hospital burden in overlap syndrome of asthma and COPD. Clin Respir J. 2013;7(4):342-6.

Apostol GG, Jacobs DR Jr, Tsai AW, Crow RS, Williams OD, Townsend MC, Beckett WS. Early life factors contribute to the decrease in lung function between ages 18 and 40: the Coronary Artery Risk Development in Young Adults study. Am J Respir Crit Care Med 2002;166: 166–172.

Arnson Y, Shoenfeld Y, Amital H. Effects of tobacco smoke on immunity, inflammation and autoimmunity. J Autoimmun 2010; 34:J258-265

Asthma. Current Care Guidelines. Working group set up by the Finnish Medical Society Duodecim, the Finnish Respiratory Society, the Finnish Peadiatric Society, and the Finnish society of Clinical Physiology. Helsinki: The Finnish Medical Society Duodecim, 2012 (referred November 25th, 2019). Available online at: www.kaypahoito.fi

Astman diagnostiikka ja hoito. Current Care Guidelines. Working group set up by the Finnish Medical Society Duodecim, the Finnish Respiratory Society and the Finnish Peadiatric Society. The Finnish Medical Society Duodecim 2000; 116: 2568-84.

Bai TR, Vonk JM, Postma DS, Boezen HM. Severe exacerbations predict excess lung function decline in asthma. Eur Respir J. 2007;30(3):452-6.

Balmes J, Becklake M, Blanc P, Henneberger P, Kreiss K, Mapp C, Milton D, Schwartz D, Toren K, Viegi G; Environmental and Occupational Health Assembly, American Thoracic Society. American Thoracic Society Statement: Occupational contribution to the burden of airway disease. Am J Respir Crit Care Med. 2003;167(5):787-97.

Barley EA, Quirk FH, Jones PW. Asthma health status measurement in clinical practice: validity of a new short and simple instrument. Respir Med 1998; 92: 1207–1214.

Barnes PJ. Corticosteroid resistance in patients with asthma and chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2013;131(3):636-45.

Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2016;138(1):16-27.

Barnett K, Mercer SW, Norbury M, Watt G, Wyke S, Guthrie B. Epidemiology of multimorbidity and implications for health care, research, and medical education: a cross-sectional study. Lancet. 2012;380(9836):37-43.

Barrecheguren M, Esquinas C, Miravitlles M. The asthma-chronic obstructive pulmonary disease overlap syndrome (ACOS): opportunities and challenges. Curr Opin Pulm Med 2015; 21: 74–79.

Benowitz NL. Nicotine addiction. N Engl J Med. 2010;362(24):2295-303.

Bisgaard H, Bønnelykke K. Long-term studies of the natural history of asthma in childhood. J Allergy Clin Immunol. 2010;126:187-97.

Bjermer L, Alving K, Diamant Z, Magnussen H, Pavord I, Piacentini G, Price D, Roche N, Sastre J, Thomas M, Usmani O. Current evidence and future research needs for FeNO measurement in respiratory diseases. Respir Med. 2014;108(6):830-41.

Blanc PD, Iribarren C, Trupin L, Earnest G, Katz PP, Balmes J, Sidney S, Eisner MD. Occupational exposures and the risk of COPD: dusty trades revisited. Thorax 2009;64:6-12.

Boulet LP, Lemière C, Archambault F, Carrier G, Descary MC, Deschesnes F. Smoking and asthma: clinical and radiologic features, lung function, and airway inflammation. Chest. 2006;129(3):661-8.

Brightling C, Greening N. Airway inflammation in COPD: progress to precision medicine. Eur Respir J 2019; 54: 1900651 [https://doi.org/10.1183/13993003.00651-2019].

Burgess JA, Matheson MC, Gurrin LC, Byrnes GB, Adams KS, Wharton CL, Giles GG, Jenkins MA, Hopper JL, Abramson MJ, Walters EH, Dharmage SC. Factors influencing asthma remission: a longitudinal study from childhood to middle age. Thorax. 2011;66:508-13.

Calverley PM, Albert P, Walker PP. Bronchodilator reversibility in chronic obstructive pulmonary disease: use and limitations. Lancet Respir Med 2013;1: 564–73

Cavaillès A, Brinchault-Rabin G, Dixmier A, Goupil F, Gut-Gobert C, Marchand-Adam S, Meurice JC, Morel H, Person-Tacnet C, Leroyer C, Diot P. Comorbidities of COPD. Eur Respir Rev. 2013;22:454-75.

Cazzola M, Rogliani P. Do we really need asthma-chronic obstructive pulmonary disease overlap syndrome? J Allergy Clin Immunol. 2016;138(4):977-983.

Cerveri I, Cazzoletti L, Corsico AG, Marcon A, Niniano R, Grosso A, Ronzoni V, Accordini S, Janson C, Pin I, Siroux V, de Marco R. The impact of cigarette smoking on asthma: a population-based international cohort study. Int Arch Allergy Immunol 2012; 158: 175–183.

Chalmers GW, Macleod KJ, Little SA, Thomson LJ, McSharry CP, Thomson NC. Influence of cigarette smoking on inhaled corticosteroid treatment in mild asthma. Thorax 2002; 57: 226–230.

Chalmers GW, MacLeod KJ, Thomson L, Little SA, McSharry C, Thomson NC. Smoking and airway inflammation in patients with mild asthma. Chest. 2001;120(6):1917-22.

Chaudhuri R, McSharry C, McCoard A, Livingston E, Hothersall E, Spears M, Lafferty J, Thomson NC. Role of symptoms and lung function in determining asthma control in smokers with asthma. Allergy 2008;63:132-5

Çolak Y, Afzal S, Nordestgaard BG, Lange P. Characteristics and prognosis of never-smokers and smokers with asthma in the Copenhagen General Population

Study. A prospective cohort study. Am J Respir Crit Care Med 2015; 192: 172–181.

Contoli M, Baraldo S, Marku B, Casolari P, Marwick JA, Turato G, Romagnoli M, Caramori G, Saetta M, Fabbri LM, Papi A. Fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease: 5-year follow-up. J Allergy Clin Immunol. 2010;125(4):830-7.

Cosio BG, Soriano JB, López-Campos JL, Calle-Rubio M, Soler-Cataluna JJ, de-Torres JP, Marín JM, Martínez-Gonzalez C, de Lucas P, Mir I, Peces-Barba G, Feu-Collado N, Solanes I, Alfageme I, Casanova C; CHAIN Study. Defining the Asthma-COPD Overlap Syndrome in a COPD Cohort. Chest. 2016;149(1):45-52.

Csikesz NG, Gartman EJ. New developments in the assessment of COPD: early diagnosis is key. Int J Chron Obstruct Pulmon Dis. 2014;9:277-86.

De Marco R, Marcon A, Rossi A, Antó JM, Cerveri I, Gislason T, Heinrich J, Janson C, Jarvis D, Kuenzli N, Leynaert B, Probst-Hensch N, Svanes C, Wjst M, Burney P. Asthma, COPD and overlap syndrome: a longitudinal study in young European adults. Eur Respir J. 2015;46(3):671-9.

De Marco R, Pesce G, Marcon A, Accordini S, Antonicelli L, Bugiani M, Casali L, Ferrari M, Nicolini G, Panico MG, Pirina P, Zanolin ME, Cerveri I, Verlato G. The coexistence of asthma and chronic obstructive pulmonary disease (COPD): prevalence and risk factors in young, middle-aged and elderly people from the general population. PLoS One 2013; 8: e62985.

de Nijs SB, Venekamp LN, Bel EH. Adult-onset asthma: is it really different? Eur Respir Rev. 2013;22:44-52.

Dijkstra A, Vonk JM, Jongepier H, Koppelman GH, Schouten JP, ten Hacken NH, Timens W, Postma DS. Lung function decline in asthma: association with inhaled corticosteroids, smoking and sex. Thorax. 2006;61(2):105-10.

Drummond MB, Hansel NN, Connett JE, Scanlon PD, Tashkin DP, Wise RA. Spirometric predictors of lung function decline and mortality in early chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2012;185(12):1301-6.

Eisner MD, Iribarren C. The influence of cigarette smoking on adult asthma outcomes. Nicotine Tob Res 2007; 9:53–56.

Ekpu VU, Brown AK. The Economic Impact of Smoking and of Reducing Smoking Prevalence: Review of Evidence. Tob Use Insights. 2015;8:1-35.

Engelkes M, Janssens HM, de Jongste JC, Sturkenboom MC, Verhamme KM. Medication adherence and the risk of severe asthma exacerbations: a systematic review. Eur Respir J. 2015;45(2):396-407.

European Commission. Attitudes of Europeans Towards Tobacco and Electronic Cigarettes. Report 2015. http://ec.europa.eu/public_opinion/archives/ebs/ebs_429_en.pdf. Accessed September 6th, 2019

Fabbri LM, Romagnoli M, Corbetta L, Casoni G, Busljetic K, Turato G, Ligabue G, Ciaccia A, Saetta M, Papi A. Differences in airway inflammation in patients with fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2003;167(3):418-24.

Fu JJ, McDonald VM, Gibson PG, Simpson JL. Systemic Inflammation in Older Adults With Asthma-COPD Overlap Syndrome. Allergy Asthma Immunol Res. 2014;6(4):316-24.

Gao J, Iwamoto H, Koskela J, Alenius H, Hattori N, Kohno N, Laitinen T, Mazur W, Pulkkinen V. Characterization of sputum biomarkers for asthma-COPD overlap syndrome. Int J Chron Obstruct Pulmon Dis. 2016;11:2457-2465.

George L, Taylor AR, Esteve-Codina A, Soler Artigas M, Thun GA, Bates S, Pavlidis S, Wagers S, Boland A, Prasse A, Boschetto P, Parr DG, Nowinski A, Barta I, Hohlfeld J, Greulich T, van den Berge M, Hiemstra PS, Timens W, Hinks T, Wenzel S, Siddiqui S, Richardson M, Venge P, Heath S, Gut I, Tobin MD, Edwards L, Riley JH, Djukanovic R, Auffray C, De-Meulder B, Erik-Dahlen S, Adcock IM, Chung KF, Ziegler-Heitbrock L, Sterk PJ, Singh D, Brightling CE; U-BIOPRED and the EvA study teams. Blood eosinophil count and airway epithelial transcriptome relationships in COPD versus asthma. Allergy. 2019 Sep 10. doi: 10.1111/all.14016.

Gershon AS, Victor JC, Guan J, Aaron SD, To T. Pulmonary function testing in the diagnosis of asthma: a population study. Chest. 2012;141(5):1190-1196.

Gibson PG, McDonald VM. Asthma-COPD overlap 2015: now we are six. Thorax. 2015;70(7):683-91.

Global Initiative for Asthma and Global Initiative for Chronic Obstructive Lung Disease. Diagnosis and Initial Treatment of Asthma, COPD and Asthma-COPD Overlap 2017. www.ginasthma.org/ Date last accessed: August 17th 2018

Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention. 2010. www.ginasthma.org. Date last accessed: March 29, 2011.

Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention (GINA). Updated 2019. http://www.ginasthma.org/. Date last accessed: October 8th, 2019.

Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease. 2019 Report. http://goldcopd.org/ Date last accessed: November 9th 2019

Grol MH, Gerritsen J, Vonk JM, Schouten JP, Koëter GH, Rijcken B, Postma DS. Risk factors for growth and decline of lung function in asthmatic individuals up to age 42 years. A 30-year follow-up study. Am J Respir Crit Care Med 1999; 160: 1830–1837.

Haahtela T, Klaukka T, Koskela K, Erhola M, Laitinen LA; Working Group of the Asthma Programme in Finland 1994-2004. Asthma programme in Finland: a community problem needs community solutions. Thorax 2001; 56: 806–814.

Hallstrand TS, Leuppi JD, Joos G, Hall GL, Carlsen KH, Kaminsky DA, Coates AL, Cockcroft DW, Culver BH, Diamant Z, Gauvreau GM, Horvath I, de Jongh FHC, Laube BL, Sterk PJ, Wanger J; American Thoracic Society (ATS)/European Respiratory Society (ERS) Bronchoprovocation Testing Task Force. ERS technical standard on bronchial challenge testing: pathophysiology and methodology of indirect airway challenge testing. Eur Respir J. 2018;52(5). pii: 1801033. doi: 10.1183/13993003.01033-2018.

Hanania NA, Celli BR, Donohue JF, Martin UJ. Bronchodilator reversibility in COPD. Chest 2011;140:1055-1063

Hancox RJ, Gray AR, Poulton R, Sears MR. The effect of cigarette smoking on lung function in young adults with asthma. Am J Respir Crit Care Med 2016; 194: 276–284.

Hardin M, Cho M, McDonald ML, Beaty T, Ramsdell J, Bhatt S, van Beek EJ, Make BJ, Crapo JD, Silverman EK, Hersh CP. The clinical and genetic features of the COPD asthma overlap syndrome. Eur Respir J 2014; 44: 341–350.

Hekking PP, Wener RR, Amelink M, Zwinderman AH, Bouvy ML, Bel EH. The prevalence of severe refractory asthma. J Allergy Clin Immunol. 2015;135(4):896-902.

Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. Review. Lancet. 2004;364:709-21.

Hogg JC, Timens W. The pathology of chronic obstructive pulmonary disease. Annu Rev Pathol. 2009;4:435-59.

Honkamäki J, Hisinger-Mölkänen H, Ilmarinen P, Piirilä P, Tuomisto LE, Andersén H, Huhtala H, Sovijärvi A, Backman H, Lundbäck B, Rönmark E, Lehtimäki L, Kankaanranta H. Age- and gender-specific incidence of new asthma diagnosis from childhood to late adulthood. Respir Med. 2019;154:56-62.

Hosseini M, Almasi-Hashiani A, Sepidarkish M, Maroufizadeh S. Global prevalence of asthma-COPD overlap (ACO) in the general population: a systematic review and meta-analysis. Respir Res. 2019;20(1):229.

Ilmarinen P, Tuomisto LE, Kankaanranta H. Phenotypes, risk factors and mechanisms of adult-onset asthma. Mediators Inflamm 2015; 2015: 514868.

Ilmarinen P, Tuomisto LE, Niemelä O, Danielsson J, Haanpää J, Kankaanranta T, Kankaanranta H. Comorbidities and elevated IL-6 associate with negative outcome in adult-onset asthma. Eur Respir J 2016;48:1052-1062.

Ilmarinen P, Tuomisto LE, Niemelä O, Kankaanranta H. Prevalence of Patients Eligible for Anti-IL-5 Treatment in a Cohort of Adult-Onset Asthma. J Allergy Clin Immunol Pract. 2019;7(1):165-174.

Ilmarinen P, Tuomisto LE, Niemelä O, Tommola M, Haanpää J, Kankaanranta H. Cluster Analysis on Longitudinal Data of Patients with Adult-Onset Asthma. J Allergy Clin Immunol Pract. 2017;5:967-978.

James AL, Palmer LJ, Kicic E, Maxwell PS, Lagan SE, Ryan GF, Musk AW. Decline in lung function in the Busselton Health Study: the effects of asthma and cigarette smoking. Am J Respir Crit Care Med. 2005;171(2):109-14.

Janson C, Malinovschi A, Amaral AFS, Accordini S, Bousquet J, Buist AS, Canonica GW, Dahlén B, Garcia-Aymerich J, Gnatiuc L, Kowalski ML, Patel J, Tan W, Torén K, Zuberbier T, Burney P, Jarvis D. Bronchodilator reversibility in asthma and COPD: findings from three large population studies. Eur Respir J. 2019;54(3). pii: 1900561. doi: 10.1183/13993003.00561-2019.

Jayes L, Haslam PL, Gratziou CG3, Powell P, Britton J, Vardavas C, Jimenez-Ruiz C, Leonardi-Bee J; Tobacco Control Committee of the European Respiratory Society. SmokeHaz: Systematic Reviews and Meta-analyses of the Effects of Smoking on Respiratory Health. Chest. 2016;150(1):164-79.

Jones PW, Harding G, Berry P, Wiklund I, Chen WH, Kline Leidy N. Development and first validation of the COPD Assessment Test. Eur Respir J. 2009;34(3):648-54

Kankaanranta H, Harju T, Kilpeläinen M, Mazur W, Lehto JT, Katajisto M, Peisa T, Meinander T, Lehtimäki L. Diagnosis and pharmacotherapy of stable chronic obstructive pulmonary disease: the finnish guidelines. Basic Clin Pharmacol Toxicol. 2015;116:291-307.

Kankaanranta H, Ilmarinen P, Kankaanranta T, Tuomisto LE. Seinäjoki Adult Asthma Study (SAAS): a protocol for a 12-year real-life follow-up study of new-onset asthma diagnosed at adult age and treated in primary and specialised care. NPJ Prim Care Respir Med 2015; 25: 15042.

Kankaanranta H, Tuomisto LE, Ilmarinen P. Age-specific incidence of new asthma diagnoses in Finland. J Allergy Clin Immunol Pract 2017;5:189–191.

Kauppi P, Kupiainen H, Lindqvist A, Haahtela T, Laitinen T. Long-term smoking increases the need for acute care among asthma patients: a case control study. BMC Pulmonary Medicine 2014;14:119

Kauppi P, Kupiainen H, Lindqvist A, Tammilehto L, Kilpeläinen M, Kinnula VL, Haahtela T, Laitinen T. Overlap syndrome of asthma and COPD predicts low quality of life. J Asthma. 2011;48(3):279-85.

Kauppinen R, Vilkka V, Sintonen H, Hedman J. The first year of treatment predicts the prognosis of asthma over 25 y-A prospective study. Allergy. 2019 Jul 15. doi: 10.1111/all.13983.

Kim TB, Jang AS, Kwon HS, Park JS, Chang YS, Cho SH, Choi BW, Park JW, Nam DH, Yoon HJ, Cho YJ, Moon HB, Cho YS, Park CS; COREA Study Group. Identification of asthma clusters in two independent Korean adult asthma cohorts. Eur Respir J. 2013;41:1308-14.

Kim TB, Park CS, Bae YJ, Cho YS, Moon HB; COREA Study Group. Factors associated with severity and exacerbation of asthma: a baseline analysis of the cohort for reality and evolution of adult asthma in Korea (COREA). Ann Allergy Asthma Immunol. 2009;103:311-7.

Kitaguchi Y, Komatsu Y, Fujimoto K, Hanaoka M, Kubo K. Sputum eosinophilia can predict responsiveness to inhaled corticosteroid treatment in patients with overlap syndrome of COPD and asthma. Int J Chron Obstruct Pulmon Dis. 2012;7:283-9.

Kitaguchi Y, Yasuo M, Hanaoka M. Comparison of pulmonary function in patients with COPD, asthma-COPD overlap syndrome, and asthma with airflow limitation. Int J Chron Obstruct Pulmon Dis. 2016 May 9;11:991-7.

Kobayashi S, Hanagama M, Yamanda S, Ishida M, Yanai M. Inflammatory biomarkers in asthma-COPD overlap syndrome. Int J Chron Obstruct Pulmon Dis. 2016;11:2117-2123.

Koskela HO, Hyvärinen L, Brannan JD, Chan HK, Anderson SD. Responsiveness to three bronchial provocation tests in patients with asthma. Chest. 2003;124:2171-7.

Koskela HO, Hyvärinen L, Brannan JD, Chan HK, Anderson SD. Coughing during mannitol challenge is associated with asthma. Chest. 2004;125:1985-92.

Kostikas K, Clemens A, Patalano F. The asthma-COPD overlap syndrome: do we really need another syndrome in the already complex matrix of airway disease? Int J Chron Obstruct Pulmon Dis 2016; 11: 1297–1306.

Kurashima K, Takaku Y, Ohta C, Takayanagi N, Yanagisawa T, Sugita Y. COPD assessment test and severity of airflow limitation in patients with asthma, COPD, and asthma-COPD overlap syndrome. Int J Chron Obstruct Pulmon Dis. 2016;11:479-87.

Lange P, Celli B, Agustí A, Boje Jensen G, Divo M, Faner R, Guerra S, Marott JL, Martinez FD, Martinez-Camblor P, Meek P, Owen CA, Petersen H, Pinto-Plata V, Schnohr P, Sood A, Soriano JB, Tesfaigzi Y, Vestbo J. Lung-Function Trajectories Leading to Chronic Obstructive Pulmonary Disease. N Engl J Med. 2015;373(2):111-22.

Lange P, Çolak Y, Ingebrigtsen TS, Vestbo J, Marott JL. Long-term prognosis of asthma, chronic obstructive pulmonary disease, and asthma-chronic obstructive pulmonary disease overlap in the Copenhagen City Heart study: a prospective population-based analysis. Lancet Respir Med. 2016;4(6):454-62.

Lange P, Parner J, Vestbo J, Schnohr P, Jensen G. A 15-year follow-up study of ventilatory function in adults with asthma. N Engl J Med. 1998;339(17):1194-200.

Lazarus SC, Chinchilli VM, Rollings NJ, Boushey HA, Cherniack R, Craig TJ, Deykin A, DiMango E, Fish JE, Ford JG, Israel E, Kiley J, Kraft M, Lemanske RF Jr, Leone FT, Martin RJ, Pesola GR, Peters SP, Sorkness CA, Szefler SJ, Wechsler ME, Fahy JV; National Heart Lung and Blood Institute's Asthma Clinical Research Network.. Smoking affects response to inhaled corticosteroids or leukotriene receptor antagonists in asthma. Am J Respir Crit Care Med 2007; 175: 783–790.

Lee T, Lee YS, Bae YJ, Kim TB, Kim SO, Cho SH, Moon HB; Cohort for Reality and Evolution of adult Asthma in Korea study group (COREA study group), Cho YS. Smoking, longer disease duration and absence of rhinosinusitis are related to fixed airway obstruction in Koreans with severe asthma: findings from the COREA study. Respir Res. 2011;12:1.

Martin RJ, Bel EH, Pavord ID, Price D, Reddel HK. Defining severe obstructive lung disease in the biologic era: an endotype-based approach. Review. Eur Respir J 2019; 54: 1900108 [https://doi.org/10.1183/13993003.00108-2019].

McCracken JL, Veeranki SP, Ameredes BT, Calhoun WJ. Diagnosis and Management of Asthma in Adults: A Review. JAMA. 2017;318(3):279-290.

McDonald VM, Fingleton J, Agusti A, Hiles SA, Clark VL, Holland AE, Marks GB, Bardin PP, Beasley R, Pavord ID, Wark PAB, Gibson PG; participants of the Treatable Traits Down Under International Workshop; Treatable Traits Down Under International Workshop participants. Treatable traits: a new paradigm for 21st century management of chronic airway diseases: Treatable Traits Down Under International Workshop report. Eur Respir J. 2019;53(5). pii: 1802058. doi: 10.1183/13993003.02058-2018.

McGeachie MJ, Yates KP, Zhou X, Guo F, Sternberg AL, Van Natta ML, Wise RA, Szefler SJ, Sharma S, Kho AT, Cho MH, Croteau-Chonka DC, Castaldi PJ, Jain G, Sanyal A, Zhan Y, Lajoie BR, Dekker J, Stamatoyannopoulos J, Covar RA, Zeiger RS, Adkinson NF, Williams PV, Kelly HW, Grasemann H, Vonk JM, Koppelman GH, Postma DS, Raby BA, Houston I, Lu Q, Fuhlbrigge AL, Tantisira KG, Silverman EK, Tonascia J, Weiss ST, Strunk RC. Patterns of Growth and Decline in Lung Function in Persistent Childhood Asthma.N Engl J Med. 2016;374(19):1842-1852.

Milanese M, Di Marco F, Corsico AG, Rolla G, Sposato B, Chieco-Bianchi F, Costantino MT, Crivellaro MA, Guarnieri G, Scichilone N; ELSA Study Group. Asthma control in elderly asthmatics. An Italian observational study. Respir Med. 2014;108(8):1091-9.

Miller J, Edwards LD, Agustí A, Bakke P, Calverley PM, Celli B, Coxson HO, Crim C, Lomas DA, Miller BE, Rennard S, Silverman EK, Tal-Singer R, Vestbo J, Wouters E, Yates JC, Macnee W; Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) Investigators. Comorbidity, systemic inflammation and outcomes in the ECLIPSE cohort. Respir Med. 2013;107(9):1376-84.

Miranda C, Busacker A, Balzar S, Trudeau J, Wenzel SE. Distinguishing severe asthma phenotypes: role of age at onset and eosinophilic inflammation. J Allergy Clin Immunol 2004;113:101-8.

Miravitlles M, Soler-Cataluña JJ, Calle M, Molina J, Almagro P, Quintano JA, Riesco JA, Trigueros JA, Piñera P, Simón A, López-Campos JL, Soriano JB, Ancochea J. Guía Española de la EPOC (GesEPOC). Tratamiento farmacológico de la EPOC estable. Arch Bronconeumol. 2012;48:247–257

Miravitlles M, Soler-Cataluña JJ, Calle M, Molina J, Almagro P, Quintano JA, Trigueros JA, Piñera P, Simón A, Riesco JA, Ancochea J, Soriano JB. A new approach to grading and treating COPD based on clinical phenotypes: summary of the Spanish COPD guidelines (GesEPOC). Prim Care Respir J 2013; 22: 117–121.

Miravitlles M, Soriano JB, Ancochea J, Muñoz L, Duran-Tauleria E, Sánchez G, Sobradillo V, García-Río F. Characterisation of the overlap COPD-asthma phenotype. Focus on physical activity and health status. Respir Med. 2013;107(7):1053-60.

Miravitlles M, Vogelmeier C, Roche N, Halpin D, Cardoso J, Chuchalin AG, Kankaanranta H, Sandström T, Śliwiński P, Zatloukal J, Blasi F. A review of national guidelines for management of COPD in Europe. Eur Respir J 2016; 47: 625–637.

Miravitlles M, Worth H, Soler Cataluña JJ, Price D, De Benedetto F, Roche N, Godtfredsen NS, van der Molen T, Löfdahl CG, Padullés L, Ribera A. Observational study to characterise 24-hour COPD symptoms and their relationship with patient-reported outcomes: results from the ASSESS study. Respir Res. 2014;15:122.

Menezes AMB, Montes de Oca M, Pérez-Padilla R, Nadeau G, Wehrmeister FC, Lopez-Varela MV, Muiño A, Jardim JRB, Valdivia G, Tálamo C; PLATINO Team. Increased risk of exacerbation and hospitalization in subjects with an overlap phenotype: COPD-asthma. Chest. 2014;145(2):297-304.

Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, D'Agostino R Jr, Castro M, Curran-Everett D, Fitzpatrick AM, Gaston B, Jarjour NN, Sorkness R, Calhoun WJ, Chung KF, Comhair SA, Dweik RA, Israel E, Peters SP, Busse WW, Erzurum SC, Bleecker ER; National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med. 2010;181(4):315-23.

Nakamura K, Nagata C, Fujii K, Kawachi T, Takatsuka N, Oba S, Shimizu H. Cigarette smoking and the adult onset of bronchial asthma in Japanese men and women. Ann Allergy Asthma Immunol. 2009;102(4):288-93

Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, Murray JJ, Pendergraft TB. Development of the asthma control test: a survey for assessing asthma control. J Allergy Clin Immunol. 2004;113(1):59-65.

Nelson HS, Weiss ST, Bleecker ER, Yancey SW, Dorinsky PM; SMART Study Group. The Salmeterol Multicenter Asthma Research Trial: a comparison of usual pharmacotherapy for asthma or usual pharmacotherapy plus salmeterol. Chest. 2006;129(1):15-26

O'Byrne PM, Lamm CJ, Busse WW, Tan WC, Pedersen S; START Investigators Group. The effects of inhaled budesonide on lung function in smokers and nonsmokers with mild persistent asthma. Chest. 2009;136(6):1514-1520.

Ojanguren I, Moullec G, Hobeika J, Miravitlles M, Lemiere C. Clinical and inflammatory characteristics of Asthma-COPD overlap in workers with occupational asthma. PLoS One. 2018;13(3):e0193144. doi: 10.1371/journal.pone.0193144.

Ozkaya S, Dirican A, Tuna T. The objective evaluation of obstructive pulmonary diseases with spirometry. Int J Chron Obstruct Pulmon Dis. 2016;11:2009-15.

Paone G, Leone V, Conti V, De Marchis L, Ialleni E, Graziani C, Salducci M, Ramaccia M, Munafò G. Blood and sputum biomarkers in COPD and asthma: a review. Eur Rev Med Pharmacol Sci. 2016;20(4):698-708.

Park SY, Jung H, Kim JH, Seo B, Kwon OY, Choi S, Oh B, Kwon HS, Cho YS, Moon HB, Won S, Park T, Kim TB. Longitudinal analysis to better characterize Asthma-COPD overlap syndrome: Findings from an adult asthma cohort in Korea (COREA). Clin Exp Allergy. 2019 doi: 10.1111/cea.13339.

Park SY, Jung HW, Lee JM, Shin B, Kim HJ, Kim MH, Song WJ, Kwon HS, Jung JW, Kim SH, Park HW, Jang AS, Chang YS, Cho YS, Cho YJ, Cho SH, Choi BW, Lee SJ, Jee SH, Choi SK, Won S, Park T, Kim C, Moon HB, Kim TB; COREA investigators. Novel trajectories for identifying asthma phenotypes: a longitudinal study in Korean asthma cohort, COREA. J Allergy Clin Immunol Pract. 2019 pii: S2213-2198(19)30176-X. doi:10.1016/j.jaip.2019.02.011.

Park SY, Kim JH, Kim HJ, Seo B, Kwon OY, Chang HS, Kwon HS, Kim TB, Kim H, Park CS, Moon HB, Cho YS. High Prevalence of Asthma in Elderly Women: Findings From a Korean National Health Database and Adult Asthma Cohort. Allergy Asthma Immunol Res. 2018;10:387-396.

Park HW, Song WJ, Kim SH, Park HK, Kim SH, Kwon YE, Kwon HS, Kim TB, Chang YS, Cho YS, Lee BJ, Jee YK, Jang AS, Nahm DH, Park JW, Yoon HJ, Cho YJ, Choi BW, Moon HB, Cho SH. Classification and implementation of asthma phenotypes in elderly patients. Ann Allergy Asthma Immunol. 2015;114:18-22.

Patel M, Pilcher J, Reddel HK, Pritchard A, Corin A, Helm C, Tofield C, Shaw D, Black P, Weatherall M, Beasley R; SMART Study Group. Metrics of salbutamol use as predictors of future adverse outcomes in asthma. Clin Exp Allergy. 2013;43(10):1144-51.

Piipari R, Jaakkola JJ, Jaakkola N, Jaakkola MS. Smoking and asthma in adults. Eur Respir J. 2004;24(5):734-9.

Polosa R, Knoke JD, Russo C, Piccillo G, Caponnetto P, Sarvà M, Proietti L, Al-Delaimy WK. Cigarette smoking is associated with a greater risk of incident asthma in allergic rhinitis. J Allergy Clin Immunol. 2008;121(6):1428-34.

Polosa R, Russo C, Caponnetto P, Bertino G, Sarvà M, Antic T, Mancuso S, Al-Delaimy WK. Greater severity of new onset asthma in allergic subjects who smoke: a 10-year longitudinal study. Respiratory Research 2011;12:16.

Polosa R, Thomson NC. Smoking and asthma: dangerous liaisons. Eur Respir J. 2013;41(3):716-26.

Postma DS, Rabe KF. The asthma-COPD overlap syndrome. N Engl J Med 2015; 373: 1241–1249.

Purokivi M, Koskela HO, Koistinen T, Magga J, Peuhkurinen K, Kiviniemi V, Kontra KM. Utility of hypertonic histamine challenge in distinguishing difficult-to-diagnose asthma. Clin Respir J. 2007;1:91-8.

Purokivi M, Koskela HO, Koistinen T, Magga J, Peuhkurinen K, Kiviniemi V, Kontra KM. Utility of cough response during hypertonic histamine challenge in diagnosing asthma. Respir Med. 2008;102:1379-84.

Quirt J, Hildebrand KJ, Mazza J, Noya F, Kim H. Asthma. Review. Allergy Asthma Clin Immunol. 2018;14(Suppl 2):50.

Reddel HK. Treatment of overlapping asthma-chronic obstructive pulmonary disease: Can guidelines contribute in an evidence-free zone? J Allergy Clin Immunol. 2015;136(3):546-52.

Riley CM, Sciurba FC. Diagnosis and Outpatient Management of Chronic Obstructive Pulmonary Disease: A Review. JAMA. 2019;321:786-797.

Rogliani P, Ora J, Puxeddu E, Cazzola M. Airflow obstruction: is it asthma or is it COPD? Int J Chron Obstruct Pulmon Dis. 2016;11:3007-3013.

Rossi A, Butorac-Petanjek B, Chilosi M, Cosío BG, Flezar M, Koulouris N, Marin J, Miculinic N, Polese G, Samaržija M, Skrgat S, Vassilakopoulos T, Vukić-Dugac A, Zakynthinos S, Miravitlles M. Chronic obstructive pulmonary disease with mild airflow limitation: current knowledge and proposal for future research - a consensus document from six scientific societies. Int J Chron Obstruct Pulmon Dis. 2017;12:2593-2610.

Rönmark E, Lindberg A, Watson L, Lundbäck B. Outcome and severity of adult onset asthma--report from the obstructive lung disease in northern Sweden studies (OLIN). Respir Med. 2007;101(11):2370-7.

Sadhra S, Kurmi OP, Sadhra SS, Lam KB, Ayres JG. Occupational COPD and job exposure matrices: a systematic review and meta-analysis. Review. Int J Chron Obstruct Pulmon Dis. 2017;12:725-734.

Schleich FN, Seidel L, Sele J, Manise M, Quaedvlieg V, Michils A, Louis R. Exhaled nitric oxide thresholds associated with a sputum eosinophil count ≥3% in a cohort of unselected patients with asthma. Thorax. 2010;65(12):1039-44.

Sin DD, Miravitlles M, Mannino DM, Soriano JB, Price D, Celli BR, Leung JM, Nakano Y, Park HY, Wark PA, Wechsler ME. What is asthma-COPD overlap syndrome? Towards a consensus definition from a round table discussion. Eur Respir J 2016; 48: 664–673.

Singh A, Liu C, Putman B, Zeig-Owens R, Hall CB, Schwartz T, Webber MP, Cohen HW, Berger KI, Nolan A, Prezant DJ, Weiden MD. Predictors of Asthma/COPD Overlap in FDNY Firefighters With World Trade Center Dust Exposure: A Longitudinal Study. Chest. 2018;154(6):1301-1310.

Soler-Cataluña JJ, Cosío B, Izquierdo JL, López-Campos JL, Marín JM, Agüero R, Baloira A, Carrizo S, Esteban C, Galdiz JB, González MC, Miravitlles M, Monsó E, Montemayor T, Morera J, Ortega F, Peces-Barba G, Puente L, Rodríguez JM, Sala E, Sauleda J, Soriano JB, Viejo JL. Consensus document on the overlap phenotype COPD-asthma in COPD. Arch Bronconeumol. 2012;48(9):331-7.

Sood A, Qualls C, Schuyler M, Arynchyn A, Alvarado JH, Smith LJ, Jacobs DR Jr. Adult-onset asthma becomes the dominant phenotype among women by age 40 years. Ann Am Thorac Soc 2013; 10: 188–197.

Sorino C, Pedone C, Scichilone N. Fifteen-year mortality of patients with asthma-COPD overlap syndrome. Eur J Intern Med. 2016;34:72-77

Suissa S, Ernst P, Benayoun S, Baltzan M, Cai B. Low-dose inhaled corticosteroids and the prevention of death from asthma. N Engl J Med. 2000;343(5):332-6.

Suissa S, Ernst P, Kezouh A. Regular use of inhaled corticosteroids and the long term prevention of hospitalisation for asthma. Thorax. 2002;57(10):880-4.

Svanes C, Sunyer J, Plana E, Dharmage S, Heinrich J, Jarvis D, de Marco R, Norbäck D, Raherison C, Villani S, Wjst M, Svanes K, Antó JM. Early life origins of chronic obstructive pulmonary disease. Thorax. 2010;65(1):14-20.

Sze E, Bhalla A, Nair P. Mechanisms and therapeutic strategies for non-T2 asthma. Allergy. 2019 Jul 16. doi: 10.1111/all.13985.

Talhout R, Schulz T, Florek E, van Benthem J, Wester P, Opperhuizen A. Hazardous compounds in tobacco smoke. Int J Environ Res Public Health. 2011;8(2):613-28.

Tantucci C, Modina D. Lung function decline in COPD. Int J Chron Obstruct Pulmon Dis. 2012;7:95-9.

Tashkin DP, Celli B, Decramer M, Liu D, Burkhart D, Cassino C, Kesten S. Bronchodilator responsiveness in patients with COPD. Eur Respir J. 2008;31(4):742-50.

Tho NV, Park HY, Nakano Y. Asthma-COPD overlap syndrome (ACOS): A diagnostic challenge. Respirology 2016; 21: 410–418.

Thomson NC. Asthma and smoking-induced airway disease without spirometric COPD. Eur Respir J 2017; 49: 1602061 [https://doi.org/10.1183/13993003.02061-2016].

Thomson NC, Chaudhuri R, Heaney LG, Bucknall C, Niven RM, Brightling CE, Menzies-Gow AN, Mansur AH, McSharry C. Clinical outcomes and inflammatory biomarkers in current smokers and exsmokers with severe asthma. J Allergy Clin Immunol 2013;131:1008-1016

Tomlinson JE, McMahon AD, Chaudhuri R, Thompson JM, Wood SF, Thomson NC. Efficacy of low and high dose inhaled corticosteroid in smokers versus non-smokers with mild asthma. Thorax 2005; 60: 282–287.

Tuomisto LE, Ilmarinen P, Lehtimäki L, Tommola M, Kankaanranta H. Immediate bronchodilator response in FEV1 as a diagnostic criterion for adult asthma. Eur Respir J 2019; 53: 1800904 [https://doi.org/10.1183/13993003.00904-2018].

Tuomisto LE, Ilmarinen P, Niemelä O, Haanpää J, Kankaanranta T, Kankaanranta H. A 12-year prognosis of adult-onset asthma: Seinäjoki Adult Asthma Study. Respir Med. 2016;117:223-9.

Ulrik CS, Backer V, Dirksen A. A 10 year follow up of 180 adults with bronchial asthma: factors important for the decline in lung function. Thorax 1992; 47: 14–18.

Van Schayck CP, Levy ML, Chen JC, Isonaka S, Halbert RJ. Coordinated diagnostic approach for adult obstructive lung disease in primary care. Prim Care Respir J. 2004;13(4):218-21.

Vartiainen E. The North Karelia Project: Cardiovascular disease prevention in Finland. Glob Cardiol Sci Pract. 2018;2018(2):13.

Viljanen AA, Halttunen PK, Kreus KE, Viljanen BC. Spirometric studies in non-smoking, healthy adults. Scand J Clin Lab Invest Suppl 1982; 159: 5–20.

Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med 2012;18:716-725

Westerhof GA, Coumou H, de Nijs SB, Weersink EJ, Bel EH. Clinical predictors of remission and persistence of adult-onset asthma. J Allergy Clin Immunol. 2018;141(1):104-109.

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

WHO Global Report on Trends in Prevalence of Tobacco Smoking. WHO; Geneva, Switzerland: 2015. https://apps.who.int/iris/bitstream/handle/10665/156262/9789241564922_eng.pdf;jsessionid=B72BBF56E24242DE8E9E09C09F0DCC0C?sequence=1 Date last accessed: September 9th 2019

Wurst KE, Kelly-Reif K, Bushnell GA, Pascoe S, Barnes N. Understanding asthma-chronic obstructive pulmonary disease overlap syndrome. Respir Med. 2016;110:1-11.

Yun JH, Lamb A, Chase R, Singh D, Parker MM, Saferali A, Vestbo J, Tal-Singer R, Castaldi PJ, Silverman EK, Hersh CP; COPDGene and ECLIPSE Investigators. Blood eosinophil count thresholds and exacerbations in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2018;141(6):2037-2047.

Zhang X, Moilanen E, Adcock IM, Lindsay MA, Kankaanranta H. Divergent effect of mometasone on human eosinophil and neutrophil apoptosis. Life Sci. 2002;71(13):1523-34.

Zhang X, Moilanen E, Kankaanranta H. Beclomethasone, budesonide and fluticasone propionate inhibit human neutrophil apoptosis. Eur J Pharmacol 2001; 431: 365–371.

PUBLICATIONS

PUBLICATION I

The effect of smoking on lung function: a clinical study of adult-onset asthma

Tommola M, Ilmarinen P, Tuomisto LE, Haanpää J, Kankaanranta T, Niemelä O, Kankaanranta H

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The effect of smoking on lung function: a clinical study on adult-onset asthma

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Take home message: In adult-onset asthma, smoking history ≥10 pack-years is associated with accelerated loss of lung function.

Total word count: 2979

This study is registered at ClinicalTrials.gov with identifier number NCT02733016.

ABSTRACT

The aim of this study was to evaluate the effect of smoking on lung function decline in adult-onset asthma in a clinical, 12-year follow-up study.

In Seinäjoki Adult Asthma Study (SAAS), 203 patients were followed for 12 years (during 1999-2013) after diagnosis of new-onset adult asthma. Patients were divided into 2 groups based on smoking history: pack-years < 10 or ≥10. Spirometry evaluation points were: 1. baseline, 2. the maximum lung function ($Max_{0-2.5}$) during the first 2.5 years after diagnosis, and 3. after 12 years of follow-up.

Between $Max_{0-2.5}$ and follow-up, the median annual decline in FEV_1 was 36mL in the group of patients with < 10 pack-years of smoking, and 54 mL in those with smoking history ≥10 pack-years (p=0.003). The annual decline in FEV_1% predicted (p=0.006), FVC mL (p=0.035), and FEV_1/FVC (p=0.045) were also accelerated in the group of patients with ≥10 pack-years smoked. In multivariate regression analysis smoking history ≥ 10 pack-years became a significant predictor of accelerated decline in FEV_1.

Among patients with clinically-defined adult-onset asthma, smoking history ≥ 10 pack-years is associated with accelerated loss of lung function.

183 words

INTRODUCTION

Asthma is a heterogenic disease that has recently been shown to consist of multiple different phenotypes [1,2], which have been identified by cluster analyses based on different clinical features. Age at onset of asthma has been found to be a key factor in distinguishing asthma phenotypes [1]. Early-onset disease is associated with more atopy and allergies than late- or adult-onset asthma. Suggested adult-onset asthma phenotypes are exercise-induced, obesity-related, late-onset eosinophilic (often severe) and smoking-related neutrophilic asthma [1-3]. Most previous studies on asthma have mainly focused on allergic early-onset asthma starting in childhood, but the long term prognosis of adult-onset asthma is yet unknown. However, the limited data suggests that the prognosis of adult-onset asthma is not good, only 3-4.8% of patients being in remission after 5 years of diagnosis [4]. Smokers have generally been excluded from studies of asthma, because of the concern of possible COPD influencing the results. Therefore relatively little is still known about the relationship between asthma and smoking.

Smoking among patients with asthma is almost as frequent as in general population, and 26% of patients with asthma are active smokers [5]. Smoking is associated with increased severity of asthma [6,7], worse asthma-specific quality of life [7,8], and a greater risk of unscheduled health care visits [8] and hospitalization for asthma [7, 9]. Smoking changes the type of asthmatic inflammation towards more neutrophilic [8,10,11], and the response to corticosteroids is attenuated in smokers with asthma [12-15]. Smoking increases the risk of developing asthma [16], especially in allergic patients [17]. While adverse effects of smoking on asthma control and severity are established, less is known on the association between the duration of smoking and dose-effect relationships between smoking and lung function [6, 18].

The effect of smoking on lung function in clinical asthma is still mainly unknown. In population-based studies active cigarette smoking is suggested to have a negative effect on lung function in patients with asthma [19-24]. However, in these studies with self-reported asthma [19, 21-24], or self-reported doctor-diagnosed asthma [20], the follow-up was not started when asthma was diagnosed. In addition, the baseline of the most pioneering studies reaches back to years before widespread use of inhaled corticosteroids [19,20]. Furthermore the use of asthma diagnosis made by self-reporting questionnaires by patients may lead to misclassification of asthma. There exist, however, negative studies in which no relationship between smoking and lung function decline was reported [25,26]. Therefore the effect of smoking on lung function decline in clinically defined patients with asthma still remains controversial, despite the pioneering population-based studies. Especially, the long-term effect of smoking on adult-onset asthma remains unknown. This study addresses the gap in the literature and increases our knowledge by evaluating the effect of tobacco smoking on lung function decline in a well-defined, clinical cohort of patients with new-onset asthma diagnosed at adult age.

METHODS

Study population and design

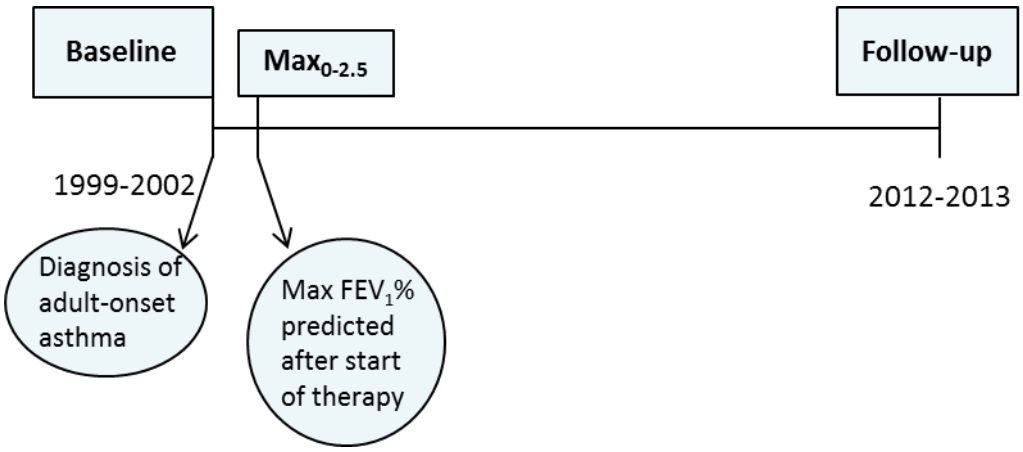
Seinäjoki Adult Asthma Study (SAAS) is a single-center (Department of Respiratory Medicine, Seinäjoki Central Hospital, Seinäjoki, Finland) 12-year follow-up study, in which 257 patients were diagnosed to have new-onset adult asthma during the years 1999-2002. The study protocol and the inclusion and exclusion criteria have been previously published [27]. Patients were recruited from the diagnostic visit, and the diagnosis of new-onset asthma was made by a respiratory physician. Diagnosis was based on typical symptoms and confirmed by objective lung function

measurements [27]. Smokers (current or ex-) were not excluded. Most patients were therapy-naïve (92 % not on inhaled steroids at the time of diagnosis), and the anti-inflammatory therapy was started immediately after the baseline visit. After a mean follow-up of 12.2 years (range 10.8-13.9 years) a total of 203 patients (79%) returned to a control visit. Blood samples were collected (to determine neutrophil and eosinophil counts), and fraction of exhaled nitric oxide (FENO) was measured at the follow-up visit. During the follow-up patients were actively treated for their asthma according to Finnish Asthma Program guidelines [28]. A written informed consent was obtained to a study protocol approved by the Ethics committee of Tampere University Hospital, Tampere, Finland (R12122).

Lung function evaluation points

Lung function measurements were performed with a spirometer (Vmax Encore 22, Viasys Healthcare, Palm Springs, CA) that was calibrated daily. Finnish reference values were used [29]. After the initiation of asthma therapy, only pre-bronchodilator spirometry was measured on most of the patients, and therefore we chose the changes in pre-bronchodilator spirometry values for evaluation throughout the study. Lung function measurement points were: 1.) baseline (i.e. time of asthma diagnosis), 2.) the maximum lung function ($Max_{0\text{-}2.5}$) during the first 2.5 years after diagnosis (i.e. after start of anti-inflammatory therapy) based on the highest pre-bronchodilator FEV_1 % predicted, and 3.) after 12 years of follow-up (Figure 1). Lung function measurements after the diagnosis of asthma were taken while patients were on medication, without pauses or withholding on the therapy.

Figure 1. Measurement points of spirometry



Assessment of smoking

Smoked pack-years (20 cigarettes per day for 1 year) were evaluated both at the baseline and follow-up visits, and patients were divided into two groups based on smoked pack-years: < 10 and ≥10 pack-years. The group of patients that had smoked ≥10 pack-years by the follow-up visit, was further divided into two groups based on whether pack-years increased during the follow up or not, indicating that patients had either continued smoking or not, respectively. The number of currently smoking subjects in this study was too low to statistically evaluate the differences in lung function decline between the groups of never-, ex-, and current smokers.

Statistical analyses

Continuous data is expressed as mean ± SD or median and interquartile range. Groups were compared by using Student's t-test, Mann-Whitney rank sum test or χ^2–test. Comparisons

between three groups were done by one-way ANOVA with Tukey's post hoc test, Kruskal-Wallis test or χ^2–test.

Multiple linear regression analysis was performed to analyze factors associated with FEV_1 decline from point of $Max_{0\text{-}2.5}$ to the follow-up visit. The correlation matrix was analyzed and explanatory variables not strongly correlated (R<0.7) were included in the analysis. Simple linear regression analysis and forward, backward and enter methods were used for selection of variables to the final model. Outliers were removed to ensure homoscedasticity. Statistical analyses were performed using SPSS software, version 22 (IBM SPSS, Chicago, Ill). A *p*-value < 0.05 was regarded as statistically significant.

RESULTS

Baseline characteristics

The baseline characteristics of the study population (n=203) are shown in table 1. The median time from the baseline to the point of maximum spirometry ($Max_{0\text{-}2.5}$) was 0.6 years (range 0.0-2.4 years), and the median increase in FEV_1 between baseline and $Max_{0\text{-}2.5}$ was 260 mL (interquartile range 70-575). Baseline characteristics of the whole cohort (n=257), and those who were lost to follow-up are shown in Table E1.

Table 1. Baseline (years 1999-2002), $Max_{0\text{-}2.5}^{\Omega}$ and follow-up (years 2012-2013) characteristics of the cohort (n=203)

	Baseline	**$Max_{0\text{-}2.5}^{\Omega}$**	**Follow-up**

Age	46.0 ± 13.7	46.7 ± 13.7	58.2 ± 13.6
Males	85 (41.9)	85 (41.9)	85 (41.9)
BMI kg·m^{-2}	27.1 (24.1-29.7)	26.9 (23.9-29.6)	28.1 (24.4-31.2)
Smoking status			
Never-smokers	100 (49.3)	-	96 (47.3)
Ex-smokers	67 (33.0)	-	77 (37.9)
Current smokers	36 (17.7)	-	30 (14.8)
Pack-years[#]	11 (5-20)	-	16 (7-30)
DL % predicted	96.9 ± 18.9	-	93.4 ± 17.7
DLVA % predicted	100.4± 18.4	-	95.2 ± 16.3
Daily use of inhaled corticosteroid	16 (8.0)	188 (96.4)	155 (76.4)
Pre-bronchodilator lung function			
FEV_1 L	2.85 (2.33-3.32)	3.19 (2.60-3.87)	2.64 (2.17-3.16)
FEV_1 % predicted	82.8 (71.0-92.2)	91.0 (83.0-102.0)	86.0 (76.0-96.0)
FEV_1/FVC	0.75 (0.69-0.80)	0.79 (0.73-0.83)	0.73 (0.66-0.79)
FVC L	3.73 (3.18-4.44)	4.07 (3.40-4.91)	3.66 (3.12-4.38)
FVC % predicted	90.3 (79.8-100.4)	97.3 (87.8-105.9)	96.0 (87.0-106.0)
Post-bronchodilator lung function			
FEV_1 L	3.02 (2.51-3.55)	-	2.75 (2.27-3.31)
FEV_1 % predicted	88.0 (76.6-98.9)	-	90.0 (80.0-98.0)
FEV_1/FVC	0.79 (0.74-0.83)	-	0.75 (0.69-0.80)
FVC L	3.85 (3.28-4.52)	-	3.77 (3.16-4.43)
FVC % predicted	94.0 (82.0-102.1)	-	98.5 (88.0-107.3)
FEV_1/FVC ratio<0,7	31 (16.3)	-	54 (26.6)
Atopy[¶]	68 (37.2)	-	-

Data are presented as n (%), mean ± SD or median (interquartile range). [#] of ex- and current smokers. [¶] as defined by positive skin-prick test towards common aeroallergen. [Ω] the point of highest lung function (FEV_1% predicted) during the first 2.5 years after the baseline (i.e. from diagnosis of asthma).

To evaluate the effect of smoked pack-years on lung function, patients were divided to groups based on the amount of smoked pack-years < 10 or ≥10 at follow-up. The baseline characteristics and detailed smoking characteristics of the groups divided by smoked pack-years are shown in Table E2. At the baseline, patients who had smoked ≥10 pack-years were older and more obese.

The number of patients who had post-bronchodilator FEV_1/FVC ratio <0.7 at baseline was higher in the group of patients who had smoked ≥10 pack-years (Table E2).

Effect of smoked pack-years on lung function

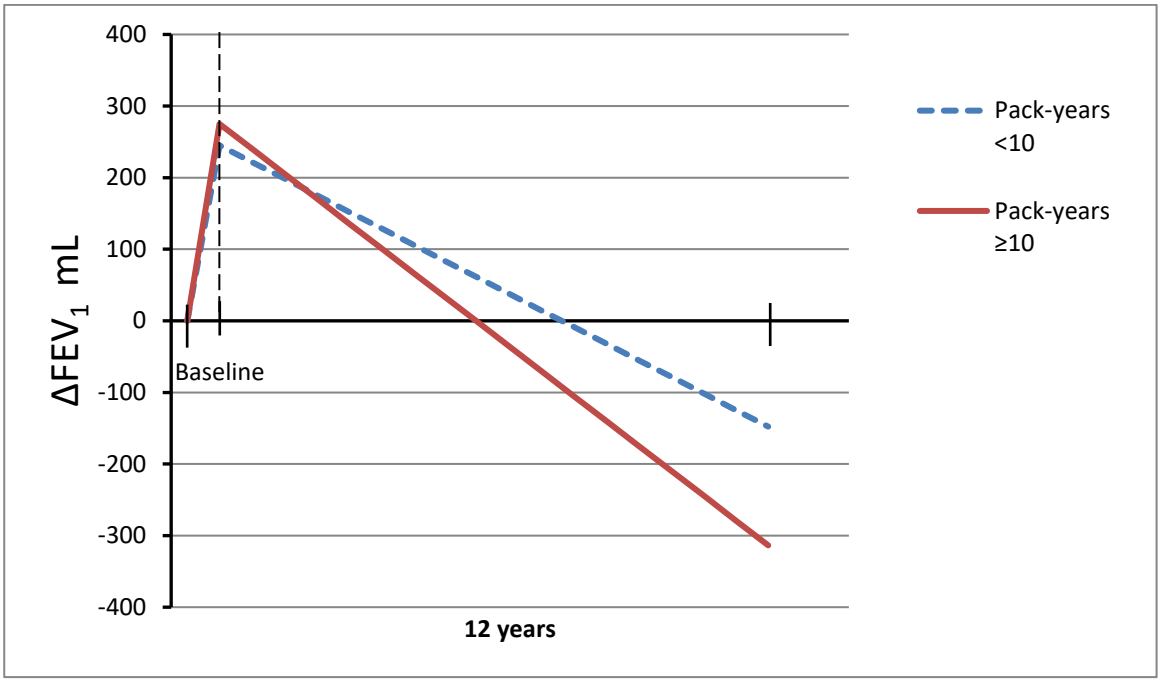
Most of the patients were therapy-naïve at the baseline visit. Evaluating the effect of smoking on lung function in patients with asthma by comparing the values between baseline and follow-up visits would be complicated by the effect of asthma therapy started at the baseline visit. Thus, we decided to evaluate the effect of smoking on lung function decline, by measuring the change between the highest lung function measurement available (as judged by the highest pre-bronchodilator FEV_1 % predicted) during first 2.5 years after the diagnosis ($Max_{0\text{-}2.5}$), and follow up. The annual decline in lung function as measured by FEV_1 (mL/year or % predicted/year), or FVC (mL/year) between $Max_{0\text{-}2.5}$ and follow-up, was significantly more rapid in the group of patients who had smoked ≥10 pack-years as compared to those who had smoked < 10 pack-years. In addition, the decline in FEV_1/FVC ratio was accelerated in the group of patients with ≥10 pack-years smoked. (Table 2, Figure 2)

Table 2. The annual change (Δ) in lung function between $Max_{0\text{-}2.5}$ and follow-up visit

	Pack-years < 10 n=124	**Pack-years ≥ 10 n=65**	***P*-value**
ΔFEV_1 mL/year	-36.1 (-60.7 to -21.6)	-54.1 (-73.2 to -32.6)	**0.003**
ΔFEV_1 % predicted/year	-0.34 (-1.04 - 0.34)	-0.75 (-1.25 to -0.23)	**0.006**
ΔFEV_1/FVC/year	-0.004 (-0.007 to -0.002)	-0.006 (-0.009 to -0.003)	**0.045**
ΔFVC mL/year	-27.6 (-54.9 to -6.8)	-41.7 (-63.2 to -20.8)	**0.035**
ΔFVC % predicted/year	0.05 (-0.68 – 0.78)	-0.12 (-0.62 – 0.44)	0.411

Shown are median (interquartile range). Data presented in bold type are statistically significant

Figure 2. Schematic presentation of the changes in FEV_1 (mL) during 12 years of follow-up in the groups of < 10 or ≥10 pack-years. Model based on group medians.



Definition of abbreviations: ΔFEV_1 mL = the change in FEV_1 mL, $Max_{0\text{-}2.5}$ = the point of highest lung function (FEV_1% predicted) during the first 2.5 years after the baseline. ‡ : p-value for the annual change in FEV_1 between $Max_{0\text{-}2.5}$ and follow-up visit

To exclude the possibility that smoking cessation leads to a further increase in lung function between baseline and $Max_{0\text{-}2.5,}$ which could explain accelerated decrease in lung function in ex-smokers with ≥ 10 pack-years, the lung function level (Table E3) and increase in lung function between baseline and $Max_{0\text{-}2.5}$ (Table E4) were evaluated. However there was no evidence of higher levels of lung function at $Max_{0\text{-}2.5,}$ or enhanced response to therapy in ex-smokers as compared to never smokers (Tables E3 and E4). Furthermore, there is a possibility that inclusion of current smokers in the analysis may lead to a bias. However, when current smokers were excluded from the analysis, the results remained similar, i.e. ex-smokers with ≥ 10 pack-years of smoking showed accelerated decline in lung function (Table E5). To exclude the possibility that patients having COPD with reversibility of the airways could affect the results, patients with DLco ≤ 90%

predicted [30], FEV_1/FVC <0.7 and smoking history ≥ 10 pack-years were excluded. However, after this exclusion, the decline in lung function remained significantly more rapid in those patients with ≥ 10 pack-years of smoking (Table E6).

The effect on lung function when smoking continues

In COPD, it has been proposed that accelerated lung function decline may continue even after smoking cessation [31]. To evaluate whether the accelerated loss of lung function in patients with asthma having smoked ≥ 10 pack-years was related to active smoking, or to history of smoked pack-years, we compared the rate of lung function decline in patients who continued smoking during the follow-up (i.e. pack-years increased), and patients who did not continue smoking after diagnosis. Surprisingly, there were no differences in the rate of lung function decline between these groups (Table 3). This suggests that having ever smoked ≥10 pack-years is associated with accelerated loss of lung function, despite whether patient has stopped smoking or not.

Table 3. The annual change (Δ) in lung function between $Max_{0\text{-}2.5}$ and follow-up visit in group of patients with ≥ 10 pack-years of smoking, divided further by whether smoking continued after baseline or not.

	Pack-years ≥ 10, smoking cessation at baseline n=28	**Pack-years ≥ 10, smoking continued after baseline n=37**	***P*-value**
ΔFEV_1 mL/year	-51.6 (-70.3 to -27.4)	-54.1 (-79.9 to -38.9)	0.643
ΔFEV_1 % predicted/year	-0.64 (-1.26 to -0.12)	-0.77 (-1.20 to -0.43)	0.740
ΔFEV_1/FVC /year	-0.007 (-0.009 to -0.004)	-0.005 (-0.010 to -0.002)	0.286
ΔFVC mL/year	-43.2 (-59.6 to -5.0)	-41.7 (-66.1 to -25.4)	0.434
ΔFVC % predicted/year	-0.04 (-0.40-0.90)	-0.40 (-0.75-0.25)	0.077

Shown are median (interquartile range)

Facing a new adult-onset asthma patient at the time of diagnosis

To evaluate the combined effect of asthma therapy and smoking on lung function changes we compared the lung function data between the baseline (i.e. point of diagnosis) and the 12-year follow-up. This comparison closely reflects the situation in which a clinician is facing an adult patient with novel diagnosis of asthma, and wondering what will be the future of the patient if he/she is a smoker or not. During the whole 12 years of follow-up from the diagnosis of adult-onset asthma the annual decline in FEV_1 % predicted was significantly more rapid in the group of patients who had smoked ≥ 10 pack-years, as compared with the group of patients who had smoked < 10 pack-years (Table 4). The difference in the annual decline in FEV_1 mL was of a borderline significance towards a more rapid loss of lung function among patients with ≥ 10 pack-years of smoking history. In contrast, there were no statistically significant differences in ΔFVC, or in $\Delta FEV_1/FVC$ (Table 4). When using post bronchodilator values, the changes in lung function did not become statistically significant (Table E7). To evaluate whether the accelerated decline in lung function between baseline and follow-up in patients having smoked ≥ 10 pack-years was related to active smoking or to smoked pack-years, we compared the rate of lung function decline in patients who continued smoking during the follow-up (i.e. pack-years increased) with those, in whom the number of pack-years did not increase (i.e. ex-smokers and patients who did not continue smoking after diagnosis). However, there were no significant differences between these groups (Table E8). This suggests that it is ever having smoked ≥ 10 pack-years that is associated with accelerated decline in lung function.

Table 4. The annual change (Δ) in lung function between the baseline and follow-up visit

	Pack-years < 10 n=128	**Pack-years ≥ 10 n=65**	***P*-value**
ΔFEV$_1$ mL/year	-16.6 (-31.4-3.3)	-25.0 (-41.7-0.9)	0.052
ΔFEV$_1$ % predicted/year	0.40 (-0.16-1.06)	0.08 (-0.48-0.78)	**0.022**
ΔFEV$_1$/FVC/year	-0.003 (-0.006-0.002)	-0.003 (-0.007-0.001)	0.452
ΔFVC mL/year	-7.7 (-32.7-17.2)	-16.6 (-44.3-15.7)	0.143
ΔFVC % predicted/year	0.59 (-0.09-1.28)	0.33 (-0.08-1.15)	0.227

Shown are median (interquartile range). Data presented in bold type are statistically significant

Determinants of lung function decline

Multiple linear regression analysis revealed that significant predictors of FEV$_1$ (mL) decline (from Max$_{0\text{-}2.5}$ to follow-up) were pack-years ≥ 10, FEV$_1$ % predicted at baseline, ΔFEV$_1$ mL (baseline to Max$_{0\text{-}2.5}$), FENO > 20 ppb at follow-up, and blood eosinophils at follow-up. A trend towards being predictors of FEV$_1$ decline was shown for age, weight gain, use of oral steroid courses during follow-up, and female gender. Instead, daily ICS use at follow-up did not predict annual FEV$_1$ decline (Table 5).

Table 5. Predictors of annual FEV$_1$ decline (mL) from Max$_{0\text{-}2.5}$ to follow-up in multiple linear regression analysis. n=154

Variable	**Estimate (Δml)**	**95% CI**	***P*-value**
ΔBMI (Max$_{0\text{-}2.5}$ - follow-up) kg/m^2	-1.37	-2.87 to 0.13	0.072
Age at follow-up	-0.30	-0.61 to -0.00	0.052
Female gender	7.64	-1.45 to 16.72	0.099
≥10 pack-years at follow-up	-12.08	-21.36 to -2.80	**0.011**
LOG B-eosinophils at follow-up	-18.23	-32.23 to -4.23	**0.011**
ΔFEV$_1$ mL $^{\Omega}$	-0.04	-0.05 to -0.03	**<0.001**

(baseline - $Max_{0-2.5}$)			
FEV_1 % predicted Ω at baseline	-0.54	-0.84 to -0.24	**<0.001**
Not daily ICS user at follow-up	-2.98	-13.36 to 7.39	0.849
FeNO at follow-up > 20 ppb	-10.70	-20.54 to -0.86	**0.033**
Oral steroids during follow-up	-7.79	-16.60 to 1.02	0.083

Ω: pre bronchodilator values. Data presented in bold type are statistically significant

DISCUSSION

We present here the effect of smoking on lung function during a 12-year follow-up of new-onset asthma in adult patients. Cigarette smoking is significantly associated with the accelerated decline in lung function in patients with adult-onset asthma. When smoking history is ≥ 10 pack-years, the annual decline in FEV_1 (mL and % predicted), FVC (mL), and FEV_1/FVC is significantly accelerated as compared to those who have smoked < 10 pack-years. Smoking history ≥ 10 pack-years is also associated with more rapid loss of lung function, despite whether the patient has stopped smoking or not.

Smokers have generally been excluded from studies of asthma, and therefore little is still known about the relationship of asthma and smoking. Several population-based studies have previously suggested a more rapid lung function decline among smoking patients with asthma as compared with non-smokers [19-24]. Nevertheless, there are no previous clinical, long-term follow-up studies published, showing the negative impact of smoking on lung function in asthma. Previous clinical studies have mostly been cross-sectional evaluations of lung function between smokers and non-smokers without any follow-up [10], or the follow-up period has been short (2-3 years) [18,32]. Studies with longer follow-up have not reported the effect of smoking on lung function [6,13]. In contrast, some negative studies have been published, showing no relationship between

smoking and lung function decline [25,26], so eventually, the effect of smoking on lung function decline in patients with asthma has been controversial. To the best of our knowledge, this is the first clinical study to show the significant negative impact of smoking on long-term lung function decline in a cohort of patients with clinically defined asthma or adult-onset asthma.

Our findings are in line with the results of epidemiologic studies of asthma and smoking. Two of the most recently published population-based studies have reported accelerated loss of lung function in asthmatic individuals who smoke. In the Copenhagen General Population Study, more rapid decline in FEV_1 was reported in smoking asthmatics, as compared with never-smokers with asthma, during 4.5 years of follow-up [23]. In a recent epidemiologic study [24], a birth-cohort was followed to the age of 38. Among young adults with asthma smoking was associated with lower FEV_1/FVC ratio and lower FEV_1 values. The results on the decline in FVC vary between studies [23,24]. In our study of a clinically defined group of patients with adult-onset asthma, we report here a significant negative impact of smoking on lung function in a long-term follow-up, as measured by FEV_1 (mL and % predicted), FVC (mL) and FEV_1/FVC ratio. Our results thus confirm in patients with clinically defined asthma the relationship between smoking and accelerated lung function decline, an association suggested by the previous epidemiologic studies [23,24].

Our finding of continuously accelerated rate of lung function decline, even after smoking is stopped, is supported by studies on COPD. The traditional view of the effects of smoking on lung function in COPD, is based on the findings of Fletcher and Peto, who propose a reduction of excessive decline in FEV_1 after smoking cessation at any state of COPD [33]. However, recent studies of COPD have reported, that the most rapid lung function decline may occur already in the

early state of the disease, and the accelerated decline in FEV_1 is present even in the groups of those patients who have quitted smoking [31, 34, 35]. Our study suggests that a similar accelerated decline in lung function already at early phase is also present in adult-onset asthma patients who smoke. After 10 pack-years smoked, the rate of lung function decline remains accelerated even if smoking is stopped. This highly emphasizes the importance of early intervention to stop smoking before 10 pack-years is reached.

In a multivariate regression analysis we examined the variables associated with ΔFEV_1 mL between $Max_{0\text{-}2.5}$ and follow-up visit. Smoked pack-years ≥ 10 became a significant variable to explain the decline in FEV_1 mL. Other significant variables to predict the decline in FEV_1 were elevated blood eosinophil count and FeNO >20 ppb at follow-up, which are known to be related to existing inflammation and more severe asthma [36,37]. In addition, results of multivariate analysis suggest that those subjects, who originally responded well to the asthma therapy (i.e. high increase in FEV_1 mL between baseline and $Max_{0\text{-}2.5}$), had also more rapid decline in FEV_1 mL at follow-up. Relevance of this finding still remains unknown. It could indicate bronchial reactivity leading to a tendency of intense reaction both positively to anti-inflammatory therapy but also negatively to irritating agents. Well preserved lung function at the baseline became an unexpected predictor of accelerated loss of lung function. This finding might be explained with lung capacity, as higher FEV_1 values at the baseline enable larger decline later on. Studies on asthma and obesity have previously shown that loss of weight is associated with an increase in lung function [38]. However, increased age and weight gain were only of borderline significance in explaining more rapid loss of lung function in asthma. It has previously been suggested that severe asthma exacerbations may predict excess lung function decline [39]. We included exacerbations to the multiple linear

regression analysis by evaluating if patient had used oral corticosteroid courses during the follow-up or not. In our results the use of oral corticosteroid courses did not become a significant predictor of FEV_1 decline, although there was a trend suggesting that exacerbations may predict loss of lung function.

Our study has several strengths. In our real-life clinical cohort, the diagnosis of new-onset adult asthma was made by a respiratory physician. The diagnosis was based on typical symptoms and objective lung function measurements showing reversibility of airway obstruction [27]. The 12-year follow-up is exceptionally long, giving us a strong view of the prognosis of lung function in these patients. The response rate in our study was good as 79% of patients of the original cohort returned to the follow-up visit. The use of $Max_{0\text{-}2.5}$ as a measurement point enabled us to include the optimal lung function of patients after the functional improvement due to the treatment for asthma was achieved. Period of 2.5 years was chosen to allow time for the therapy to affect, and to eliminate bias from practical delays (due to hospital or the patient), although the maximum lung function was usually achieved in 0.6 years. There remain some limitations in the interpretation of our results. The number of current smokers at follow-up was too low (n=21) to statistically evaluate the differences between groups of never-, ex- and current smokers. However, we further analyzed the data current smokers excluded, to better understand the differences between never smokers and ex-smokers (with ≥10 pack-years). The main results of these analyses remained the same, showing more rapid loss of lung function among ex-smokers with ≥ 10 pack-years. This indicates that inclusion of current smokers does not lead to biased results. Another limitation is lack of post-bronchodilator spirometry values at the point of $Max_{0\text{-}2.5,}$ which led to the use of pre-bronchodilator values throughout the study. We acknowledge that in some patients with asthma, large bronchial reversibility may be observed constantly over years, and furthermore, reversibility of the airways rarely remains constant in particular patient [40].

Therefore inevitably using post BD values of spirometry would have been more suitable, and this is to be considered as a limitation of our study. Other limitations of the study are the lack of control group, and lack of data on exposure to second hand smoke. Our study population was a cohort of real-life clinical asthma patients, including smoking subjects. Therefore, the study cohort includes some patients, who could be classified as having the recently defined asthma/COPD overlap syndrome (ACOS) [41,42]. However, the lung diffusion capacity values of patients were well preserved both at the baseline and follow-up, excluding the possibility of significant bias due to emphysema. In addition, the variable airway obstruction, a hallmark of asthma [43], was objectively established in every patient at the baseline, which leads to exclusion of classic COPD patients with no reversibility of the airways. Furthermore, our main findings remained similar in further analyses, when patients with DLco ≤90%, combined with smoking history ≥10 pack-years and FEV_1/FVC <0.7, were excluded.

In conclusion, in patients with new-onset adult asthma, smoking is significantly associated with the decline in lung function. The loss of lung function is more rapid among patients with ≥10 smoked pack-years as compared to those, who have smoked < 10 pack-years. Furthermore our results suggest, that having ever smoked ≥ 10 pack-years is associated with accelerated decline in lung function, and after 10 smoked pack-years, the rate of lung function decline remains accelerated, despite whether patient has stopped smoking or not. Our results highlight the importance of smoking cessation interventions at early phase in patients with adult-onset asthma.

Contributions: M.T, P.I, L.E.T. and H.K. designed the study and wrote the report with input from the other authors. P.I. performed the statistical analyses with help from T.K., O.N. and J.H. contributed to laboratory and clinical physiology analyses, respectively. All authors contributed to interpretation of the data. All authors made critical revisions of the manuscript and approved the final version of the manuscript.

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REFERENCES

1. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med 2012;18:716-725
2. De Nijs SB, Venekamp LN, Bel EH. Adult-onset asthma: is it really different? Review. Eur Respir Rev 2013;22:127,44-52
3. Ilmarinen P, Tuomisto LE, Kankaanranta H. Phenotypes, risk factors and mechanisms of adult-onset asthma. Mediators Inflamm 2015;2015:514868.
4. Tuomisto LE, Ilmarinen P, Kankaanranta H. Prognosis of new-onset asthma diagnosed at adult age. A systematic review. Respir Med 2015;109:944-954
5. Cerveri I, Cazzoletti L, Corsico A, Marcon A, Niniano R, Grosso A, Ronzoni V, Accordini S, Janson C, Pin I, Siroux V, DeMarco R. The impact of cigarette smoking on asthma: a population-based international cohort study. Int Arch Allergy Immunol 2012;158:175-183
6. Polosa R, Russo C, Caponnetto P, Bertino G, Sarvà M, Antic T, Mancuso S, Al-Delaimy WK. Greater severity of new onset asthma in allergic subjects who smoke: a 10-year longitudinal study. Respiratory Research 2011;12:16
7. Eisner MD, Iribarren C. The influence of cigarette smoking on adult asthma outcomes. Nicotine and Tobacco Research 2007;9:53-56
8. Thomson NC, Chaudhuri R, Heaney LG, Bucknall C, Niven RM, Brightling CE, Menzies-Gow AN, Mansur AH, McSharry C. Clinical outcomes and inflammatory biomarkers in current smokers and exsmokers with severe asthma. J Allergy Clin Immunol 2013;131:1008-1016

9. Kauppi P, Kupiainen H, Lindqvist A, Haahtela T, Laitinen T. Long-term smoking increases the need for acute care among asthma patients: a case control study. BMC Pulmonary Medicine 2014;14:119
10. Boulet L-P, Lemière C, Archambault F, Carrier G, Descary MC, Deschesnes F. Smoking and asthma. Chest 2006;129:661-668
11. Chalmers GW, Macleod KJ, Thomson L, Little SA, McSharry C, Thomson NC. Smoking and airway inflammation in patients with mild asthma. Chest 2001;120:1917-1922
12. Chalmers GW, MacLeod KJ, Little SA, Thomson LJ, McSharry CP, Thomson NC. Influence of cigarette smoking on inhaled corticosteroid treatment in mild asthma. Thorax 2002;57:226-230
13. Dijkstra A, Vonk JM, Jongepier H, Koppelman GH, Schouten JP, ten Hacken NHT, Timens W, Postma DS. Lung function decline in asthma: association with inhaled corticosteroids, smoking and sex. Thorax 2006;61:105-110
14. Lazarus SC, Chinchilli VM, Rollings NJ, Boushey HA, Cherniack R, Craig TJ, Deykin A, DiMango E, Fish JE, Ford JG, Israel E, Kiley J, Kraft M, Lemanske RF, Leone FT, Martin RJ, Pesola GR, Peters SP, Sorkness CA, Szefler SJ, Wechsler ME, Fahy JV. Smoking affects response to inhaled corticosteroids or leukotriene receptor antagonists in asthma. Am J Respir Crit Care Med 2007;175:783-790
15. Tomlinson JE, McMahon AD, Chaudhuri R, Thompson JM, Wood SF, Thomson NC. Efficacy of low and high dose inhaled corticosteroid in smokers versus non-smokers with mild asthma. Thorax 2005;60:282-287
16. Nakamura K, Nagata C, Fujii K, Kawachi T, Takatsuka N, Oba S, Shimizu H. Cigarette smoking and the adult onset of bronchial asthma in Japanese men and women. Ann Allergy Asthma Immunol 2009;102:288-293

17. Polosa R, Knoke JD, Russo C, Piccillo G, Pasquale C, Sarvà M, Proietti L, Al.Delaimy WK. Cigarette smoking is associated with a greater risk of incident asthma in allergic rhinitis. J Allergy Clin Immunol 2008;121:1428-34
18. Westerhof GA, Vollema EM, Weersink EJ, Reinartz SM, de Nijs SB, Bel EH. Predictors for the development of progressive severity in new-onset adult asthma. J Allergy Clin Immunol 2014;134:1051-6
19. Lange P, Parner J, Vestbo J, Schnohr P, Jensen G. A 15-year follow-up study of ventilatory function in adults with asthma. N Engl J Med 1998;339:1194-1200
20. James AL, Palmer LJ, Kicic E, Maxwell PS, Lagan SE, Ryan GF, Musk AW. Decline in lung function in the Busselton health study: the effects of asthma and cigarette smoking. Am J Respir Crit Care Med 2005;171:109-114
21. Aanerud M, Carsin A-E, Sunyer J, Dratva J, Gislason T, Jarvis D, de Marco R, Raherison C, Wjst M, Dharmage SC, Svanes C. Interaction between asthma and smoking increases the risk of adult airway obstruction. Eur Respir J 2015;45:635-643
22. Apostol GG, Jacobs DR, Tsai AW, Crow RS, Williams OD, Townsend MC, Beckett WS. Early life factors contribute to the decrease in lung function between ages 18 and 40. Am J Respir Crit Care Med 2002;166:166-172
23. Colak Y, Afzal S, Nordestgaard BG, Lange P. Characteristics and prognosis of never-smokers and smokers with asthma in the Copenhagen General Population Study. Am J Respir Crit Care Med 2015;192(2):172-181
24. Hancox RJ, Gray AR, Poulton R, Sears MR. The effect of cigarette smoking on lung function in young adults with asthma. Am J Respir Crit Care Med 2016; e-pub ahead of print

25. Grol MH, Gerritsen J, Vonk JM, Schouten JP, Koëter GH, Rijcken B, Postma DJ. Risk factors for growth and decline of lung function in asthmatic individuals up to age 42 years. Am J Respir Crit Care Med 1999;160:1830-1837
26. Ulrik CS, Backer V, Dirksen A. A 10 year follow up of 180 adults with bronchial asthma: factors important for the decline in lung function. Thorax 1992;47:14-18
27. Kankaanranta H, Ilmarinen P, Kankaanranta T, Tuomisto LE. Seinäjoki adult asthma study (SAAS): a protocol for a 12-year real-life follow-up study of new-onset asthma diagnosed at adult age and treated in primary and specialised care. NPJ Prim Care Respir Med 2015;25:15042
28. Haahtela T, Klaukka T, Koskela K, Erhola M, Laitinen LA; Working Group of the Asthma Programme in Finland 1994-2004. Asthma programme in Finland: a community problem needs community solutions. Clinical review. Thorax 2001;56:806-814
29. Viljanen AA, Halttunen PK, Kreus KE, Viljanen BC. Spirometric studies in non-smoking, healthy adults. Scand J Clin Lab Invest Suppl 1982;159:5-20
30. Amelink M, de Groot JC, de Nijs SB, Lutter R, Zwinderman AH, Sterk PJ, ten Brinke A, Bel EH. Severe adult-onset asthma: A distinct phenotype. J Allergy Clin Immunol 2013;132:336-41
31. Drummond MB, Hansel NN, Connett JE, Scanlon PD, Tashkin DP, Wise RA. Spirometric predictors of lung function decline and mortality in early chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2012;185 (12):1301-1306
32. O'Byrne PM, Lamm CJ, Busse WW, Tan WC, Pedersen S. The effects of inhaled budesonide on lung function in smokers and nonsmokers with mild persistent asthma. Chest 2009;136:1514-1520

33. Fletcher C, Peto R. The natural history of chronic airflow obstruction. Br Med J 1977;1(6077):1645-1648

34. Csikesz NG, Gartman EJ. New developments in the assessment of COPD: early diagnosis is key. Review. Int J Chron Obstruct Pulmon Dis 2014;9:277-286

35. Tantucci C, Modina D. Lung function decline in COPD. Review. Int J Chron Obstruct Pulmon Dis 2012;7:95-99

36. Kim HB, Eckel SP, Kim JH, Gilliland FC. Exhaled NO: Determinants and clinical application in children with allergic airway disease. Allergy Asthma Immunol Res 2016;8(1):12-21.

37. Miranda C, Busacker A, Balzar S, Trudeau J, Wenzel SE. Distinguishing severe asthma phenotypes: role of age at onset and eosinophilc inflammation. J Allergy Clin Immunol 2004;113:101-108

38. Kankaanranta H, Kauppi P, Tuomisto LE, Ilmarinen P. Emerging comorbidities in adult asthma: risks, clinical associations, and mechanisms. Review. Mediators Inflamm 2016; Article ID 3690628, 23 pages, 2016. doi:10.1155/2016/3690628.

39. Bai TR, Vonk JM, Postma DS, Boezen HM. Severe exacerbations predict excess lung function decline in asthma. Eur Respir J 2007;30:452-456

40. Sharma S, Litonjua AA, Tantisira KG, Fuhlbrigge AL, Szefler SJ, Strunk RC, Zeiger RS, Murphy AJ, Weiss ST. Clinical predictors and outcomes of consistent bronchodilator response in the childhood asthma management program. J Allergy Clin Immunol 2008;122:921-928

41. Global Initiative for Asthma and Global Initiative for Chronic Obstructive Lung Disease; Diagnosis of Diseases of Chronic Airflow Limitation: Asthma, COPD and Asthma-COPD Overlap Syndrome (ACOS). 2015. http://www.ginasthma.org/

42. Kankaanranta H, Harju T, Kilpeläinen M, Mazur W, Lehto JT, Katajisto M, Peisa T, Meinander T, Lehtimäki L. Diagnosis and pharmacotherapy of stable chronic obstructive pulmonary disease: the Finnish guidelines. Basic Clin Pharmacol Toxicol. 2015;116(4):291-307

43. Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention. Updated 2015. http://www.ginasthma.org/

PUBLICATION II

Differences between asthma-COPD overlap syndrome and adult-onset asthma

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Differences between Asthma-COPD overlap syndrome (ACOS) and adult-onset asthma

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Take home message: ACOS differs from adult-onset asthma by lower diffusing capacity, higher serum IL-6 and higher blood neutrophils

This study is registered at ClinicalTrials.gov with identifier number NCT02733016.

Total word count: 2991

Abstract

Differences between asthma-COPD overlap syndrome (ACOS) and adult-onset asthma are poorly known. The aim of this study was to evaluate these differences in a clinical cohort of patients with adult-onset asthma, as a part of Seinäjoki Adult Asthma Study (SAAS).

188 patients were diagnosed with adult-onset asthma and re-evaluated 12 years after diagnosis. Patients were divided into 3 groups based on smoking history and post bronchodilator spirometry values: 1) Never and ex-smokers with <10 smoked pack-years, 2) Non-obstructive (FEV_1/FVC ≥0.7) patients with ≥10 pack-years, and 3) ACOS patients with ≥10 pack-years and FEV_1/FVC <0.7.

ACOS patients had lower diffusing capacity (DLco/VA %predicted 86 vs. 98 or 96, p<0.001), higher blood neutrophil levels (4.50 vs. 3.60 or 3.85 $x10^9$/L, p=0.008), and higher IL-6 levels (2.88 vs. 1.52 or 2.10 pg/mL, p<0.001) as compared to never and ex-smokers with <10 pack-years, or non-obstructive patients with ≥10 pack-years, respectively. ACOS patients had also lower lung function, higher remaining bronchial reversibility, and higher number of comorbidities.

This study shows distinct differences in diffusing capacity, blood neutrophil and IL-6 levels, bronchial reversibility, lung function, and comorbidities between ACOS and adult-onset asthma. The present findings should be considered in comprehensive assessment of adult asthma patients.

Words 199

INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) have previously been categorized as separate entities of obstructive airway diseases with different clinical features [1, 2]. Recently, however, overlapping of these two diseases has been recognized, and a novel clinical phenotype, asthma-COPD overlap syndrome (ACOS) has been described. ACOS is characterized by persistent airway obstruction accompanied with several features from both asthma and COPD [3-6]. ACOS has recently been recognized by Global Initiative for Asthma (GINA) and Global Initiative for Chronic Obstructive Lung Disease (GOLD), and is included in several national guidelines of COPD [3-5, 7]. ACOS is considered to develop mainly by two different pathways: Either patient with COPD develops asthma-like symptoms and/or characteristics typical for asthma (for example large reversibility of the airways), or patient with asthma continues smoking and eventually develops non-reversible airway obstruction indicating COPD [8, 9]. There is also a third pathway suggested, in which patient with asthma develops non-reversible airway obstruction without smoking history [9]. However, the history of exposure to tobacco smoking (or biomass fuels) has been considered as a requirement for COPD diagnosis [2]. Thus, it has been proposed that smoking is to be regarded as a necessary factor when using the asthma-COPD overlap diagnosis as well [8, 10].

Previous studies on asthma have generally excluded smoking patients, and studies of COPD have mostly excluded patients with asthma history or diagnosis of asthma. Therefore relatively little is still known about the differences between ACOS and asthma [6, 10]. Prevalence of ACOS among patients with COPD or asthma is suggested to be 12-61% depending on the criteria used [9], and it is reported to increase with age [11]. Previous studies on ACOS have mainly been executed in COPD cohorts, and ACOS patients are reported to have more frequent exacerbations [12-14] and

hospitalizations [14, 11], worse quality of life [12], reduced physical activity [12], and more dyspnea and wheezing as compared to patients with COPD alone [11, 12].

ACOS among asthmatic patients remains far less studied. Some, mainly epidemiologic and registry based studies on ACOS among patients with asthma have been previously published, leaving a major need for clinical settings with real-life patients. However, these previous studies have reported more frequent exacerbations [14,15], worse asthma control, more dyspnea symptoms [15], impaired lung function [14-16], and worse quality of life [16] in ACOS patients as compared to patients with asthma alone. Patients with ACOS are also reported to have increased rate [14, 17] and length of hospitalization as compared to patients with asthma [17]. Furthermore, the number of comorbidities [15], especially hypertension [15, 16], has been reported to be higher among patients with ACOS as compared with asthma alone, and mortality of patients with ACOS has been suggested to be higher than that of asthmatics [18, 19].

Diagnostics of ACOS is challenging, because no specific single clinical feature, spirometric finding, or biomarker has been identified to differentiate ACOS from asthma [6, 20, 21]. Differentiating ACOS from COPD has been considered important as it affects the choice of therapy, i.e. use of inhaled glucocorticoids. Differentiating ACOS from asthma, however, has received less attention even though there are options of targeted treatment also for COPD, such as long acting muscarinic antagonists (LAMA) and roflumilast. Furthermore, at the moment ACOS is a phenotype with heterogenic and poorly defined clinical features. For that reason there is an urgent need for recognition of specific characteristics and biomarkers for ACOS [20].

The aim of this study was to evaluate the differences between asthma and ACOS in a real-life, clinical cohort of patients with asthma diagnosed at adult age.

METHODS

Study population and design

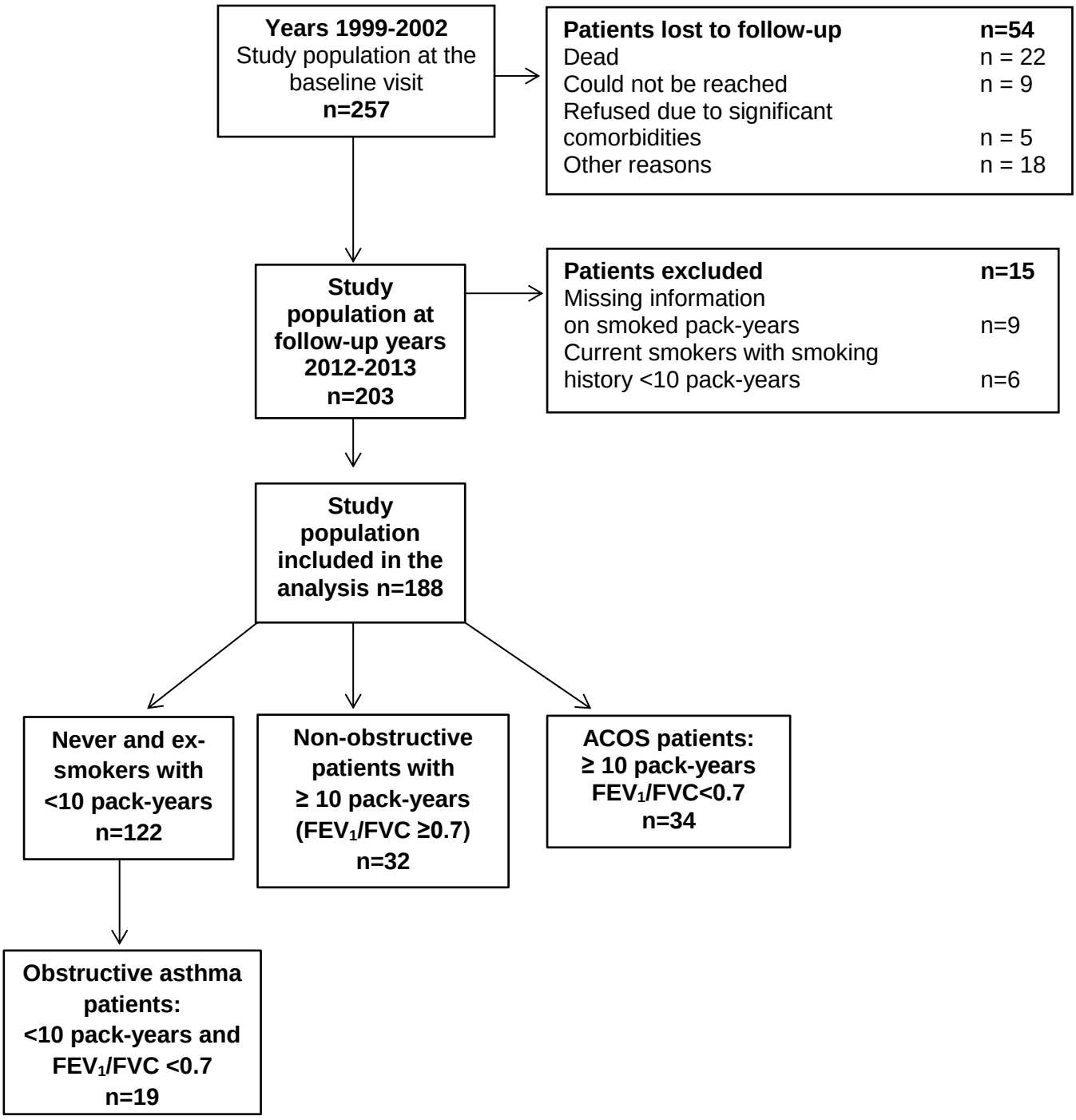
Seinäjoki Adult Asthma Study (SAAS) is a 12-year follow-up study (during the years 1999-2013), in which 257 patients were diagnosed to have new-onset adult asthma (asthma onset at the age of ≥ 15 years) in the Department of Respiratory Medicine of Seinäjoki Central Hospital, Finland. Diagnosis of asthma was made by a respiratory physician, it was based on typical symptoms and confirmed by objective lung function measurements. The study protocol and the inclusion and exclusion criteria have been previously published (Table E1) [22]. Smokers (current or ex-) were included. After a follow-up of 12 years, 203 patients (79%) were re-evaluated (years 2012-2013), and data of 188 patients was included in the analysis (Figure 1). During the follow-up, patients were actively treated for their asthma according to Finnish Asthma Program guidelines [23]. Medication use was collected by using structured questionnaire including self-reported medication patients were taking at the time of follow-up visit. The setting of the present study is cross-sectional, using mostly data from the control visit (years 2012-2013). However, when assessing the use of oral corticosteroid courses, atopy or airway obstruction at baseline, longitudinal data was utilized. A written informed consent was obtained to a study protocol approved by the Ethics committee of Tampere University Hospital, Tampere, Finland (R12122).

Evaluation of smoking and lung function

Lung function measurements were performed with a spirometer (Vmax Encore 22, Viasys Healthcare, Palm Springs, CA) that was calibrated daily. Finnish lung function reference values were used [24]. Lifelong cumulative exposure to tobacco was evaluated by assessing smoked pack-years (20 cigarettes per day for 1 year), and patients were divided into three groups based on smoked pack-years and lung function: 1. Never and ex-smokers with <10 pack-years of smoking

(current smokers excluded), 2. Non-obstructive patients with ≥10 pack-years and post bronchodilator (BD) FEV_1/FVC ≥0.7, and 3. ACOS patients i.e. ≥10 pack-years of smoking and post BD FEV_1/FVC <0.7 (Figure 1). The differences between obstructive asthma (with <10 pack-years of smoking) and ACOS were also analyzed. Patients with obstructive asthma were separated from the group of never- and ex-smokers with <10 pack-years based on post BD FEV_1/FVC: those patients with post BD FEV_1/FVC<0.7 were categorized as obstructive asthma patients (n=19) (Figure 1). Serum interleukin-6 (IL-6), high-sensitivity C-reactive protein (hsCRP), immunoglobulin E (IgE), blood cell counts and fractions of exhaled nitric oxide (FeNO) were measured as previously described [25-27], and patients filled out clinical questionnaires of Asthma Control Test (ACT), COPD Assessment Test (CAT), and Asthma Questionnaire 20 (AQ20) [28] at the visit. Asthma control was evaluated based on the recommendations of the GINA 2010 report [29].

Figure 1. Study profile



Statistical analyses

Continuous data is expressed as mean ± SD or median and interquartile range, as required. Groups were compared by using Student's t-test, Mann-Whitney rank sum test or χ^2–test. Comparisons between three groups were done by one-way ANOVA with Tukey's post hoc test, Kruskal-Wallis test or χ^2–test. Statistical analyses were performed using SPSS software, version 24 (IBM SPSS, Armonk, NY). A *p*-value < 0.05 was regarded as statistically significant.

RESULTS

Out of the 188 patients analyzed, 34 patients (18.1%) were classified as having asthma-COPD overlap syndrome and 32 patients (17.0%) belonged to the group of non-obstructive patients with ≥10 pack-years. The mean (SD) age of asthma onset in total cohort was 46.5 (13.6) years, and in ACOS group it was 53.0 (10.8) years. A majority (122 patients; 64.9%) of patients were never or ex-smokers with smoking history less than 10 pack-years. ACOS patients were older as compared to other groups, and male predominance was seen in the two groups of ≥10 pack-years of smoking history. The duration of asthma was equal in all groups, due to the 12-year follow-up period in each group. Characteristics of the 3 groups are shown in Table 1, and of the excluded patients in Table E2.

In the ACOS group, the number of patients with uncontrolled asthma (55.9%) was higher as compared to other groups. In addition, the percentage of patients with well controlled asthma was lower in the two groups with smoking history ≥ 10 pack-years (Table 1). Surprisingly, the use of oral steroids did not differ between any of the groups, and equal percentages of patients were using daily inhaled glucocorticoids in all groups. However, the group of non-obstructive patients

with smoking history ≥ 10 pack-years used higher doses of inhaled glucocorticoids and more often had long acting beta agonists (LABA) in use. Daily use of LAMA, leukotriene antagonists or theophylline was similar between the groups (Table 1). Prevalence of rhinitis, atopy and allergy did not differ between the groups (Table 1).

We assessed whether ACOS differs from asthma by using questionnaires that are widely validated for clinical work. The results revealed no significant differences in ACT scores or CAT scores between ACOS and non-obstructive patients with history of ≥10 pack-years, although ACT scores were lower, and CAT scores higher in the two groups with smoking history ≥ 10 pack-years as compared to never or ex-smokers with < 10 pack-years of smoking (Table 1). The AQ20 scores did not differ between the groups (Table 1).

Table 1. Characteristics of the study groups

	Never and ex-smokers with <10 pack-years n=122	**Non-obstructive patients with ≥10 pack-years n=32**	**ACOS ≥10 pack-years $FEV_1/FVC<0.7$ n=34**	***p*-value$^{\Phi}$**
Age years	56.7 ± 13.9	59.8 ±12.8	65.0 ±10.7 ǂ	**0.005**
BMI kg·m^{-2}	27.9 (24.3-31.2)	30.6 (25.4-33.8)	28.1 (24.2-30.7)	0.093
Gender male n(%)	36 (29.5)	19 (59.4) ǂ	24 (70.6) ǂ	**<0.001**
Asthma duration years	12.3 ± 0.6	12.4 ± 0.7	12.0 ± 0.7	0.091
Pack-years (of ex/current smokers)	3 (1-5) range 0-9	21 (17-31) ǂ range 10-47	26 (15-34) ǂ range 10-68	**<0.001**
Smoking status n(%)				**<0.001**
Never smoker	96 (78.7)	0 ǂ	0 ǂ	
Ex-smoker	26 (21.3)	17 (53.1) ǂ	25 (73.5) ǂ	
Current smoker	0 $^{\Omega}$	15 (46.9) ǂ	9 (26.5) ǂ	

Asthma control according to GINA n(%)				**<0.001**
Controlled	54 (44.3)	5 (15.6) ǂ	5 (14.7) ǂ	
Partly controlled	41 (33.6)	18 (56.3)	10 (29.4)	
Uncontrolled	27 (22.1)	9 (28.1)	19 (55.9) ǂ	
ICS daily use n(%)	96 (78.7)	26 (81.3)	27 (79.4)	0.950
ICS dose/day bud eq §	800 (400-1000)	1000 (763-1900)	800 (800-1200)	**0.023**
LABA in daily use n(%)	51 (41.8)	21 (65.6) ǂ	21 (61.8)	**0.016**
LAMA, LTRA or theophylline in daily use n(%)	20 (16.5)	7 (21.9)	8 (23.5)	0.575
Use of oral steroid courses ever n(%)	38 (31.7)	14 (43.8)	7 (21.2)	0.149
≥2 oral steroid courses in 2 years n(%)	16 (13.3)	6 (18.8)	4 (12.1)	0.691
ACT score	22 (20-25)	21 (19-24)	21 (16-23) ǂ	**0.025**
CAT score	10 ±7	14±7 ǂ	16±7 ǂ	**<0.001**
AQ20 score	3 (1-7)	4 (2-7)	4 (2-8)	0.291
Post bronchodilator FEV_1/FVC <0.7 at baseline ᵘ n(%)	11 (9.0)	1 (3.1)	16 (47.1) ǂ#	**<0.001**
Skin-prick positive ᵘ n(%)	44 (39.3)	11 (35.5)	5 (20.0)	0.191
Continuous rhinitis n(%)	44 (36.4)	13 (41.9)	8 (23.5)	0.256
Allergic conjunctivitis or rhinitis n(%)	79 (66.9)	20 (62.5)	15 (45.5)	0.079

Data is shown as n (%), mean ± SD, or median (interquartile range) Ω: excluded, §: budesonide equivalent, of daily users, ᵘ: at the moment of asthma diagnosis (1999-2002) [22], BMI= Body mass index, GINA= Global Initiative for Asthma, ICS= inhaled corticosteroids, LABA= long acting beta agonists, LAMA= long acting muscarinic antagonists, LTRA= leukotriene antagonists, ACT= Asthma Control Test, CAT= COPD Assessment Test, AQ20= Asthma Questionnaire 20

Φ: *p*-value across all groups

ǂ: as compared to group 1. (Never and ex-smokers with <10 pack-years) p<0.05

#: as compared to group 2. (Non-obstructive patients with ≥10 pack-years) p<0.05

Diffusing capacity and biomarkers

ACOS patients had significantly lower diffusing capacity of the lungs for carbon monoxide (DLco % and DLco/VA % predicted) as compared to the other groups (p=0.001). Furthermore, blood neutrophil count and serum IL-6 levels were found to be the highest in the ACOS group (Table 2). In contrast, levels of blood eosinophils, hsCRP, IgE, or FeNO did not differ significantly between any of the groups (Table 2).

Table 2. Diffusing capacity and biomarker data in study groups

	Never and ex-smokers with <10 pack-years n=122	Non-obstructive patients with ≥10 pack-years n=32	ACOS ≥10 pack-years $FEV_1/FVC<0.7$ n=34	*p*-value$^{\Phi}$
DLco % predicted	97 ± 16	91 ± 15	85 ± 23 ǂ	**0.001**
DLco/VA % predicted	98 ± 13	96 ± 18	86 ± 22 ǂ#	**<0.001**
B-Neutrophils x10*9/L	3.60 (2.70-4.60)	3.85 (2.95-4.98)	4.50 (3.50-5.53) ǂ	**0.008**
B-Eosinophils x10*9/L	0.16 (0.09-0.28)	0.14 (0.09-0.22)	0.19 (0.10-0.29)	0.409
IgE kU/l	59 (25-167)	95 (26-199)	59 (20-140)	0.516
FeNO ppb	12 (5-21)	8 (5-13)	10 (5-15)	0.063
hsCRP mg/L	1.24 (0.56-2.33)	1.18 (0.74-5.02)	0.93 (0.59-3.04)	0.369
IL-6 pg/mL	1.52 (1.12-2.48)	2.10 (1.09-5.69)	2.88 (1.88-4.99) ǂ	**<0.001**

Data is shown as mean ± SD, or median (interquartile range). DLco = Diffusing capacity of the lung for carbon monoxide, VA= Alveolar volume, B=blood, IgE= Immunoglobulin E, FeNO= Exhaled nitric oxide, hsCRP= high sensitivity C-reactive protein, IL-6= Interleukin 6

Φ: *p*-value across all groups

ǂ: as compared to group 1. (Never and ex-smokers with <10 pack-years) p<0.05

#: as compared to group 2. (Non-obstructive patients with ≥10 pack-years) p<0.05

Lung function

Post BD spirometry values of FEV_1 (p=0.002), FEV_1 % predicted (p<0.001), and FEV_1/FVC ratio (p<0.001) were found to be significantly lower in the group of patients with ACOS as compared to other groups. It needs to be noted, however, that post BD FEV_1/FVC<0.7 was an inclusion criteria for the ACOS group in this study. FVC (liters or % predicted) values did not differ between the groups (Table 3). Pre BD values are presented in Table E3. We evaluated also reversibility (measurements before and after BD) of the airways at this visit, by which patients had been actively treated for their asthma for 12 years. There was significantly higher reversibility of the airways among patients with ACOS as compared with the other groups. This was seen in FEV_1 % predicted, and in FVC mL and % predicted (Table 3).

Table 3. Lung function in study groups

	Never and ex-smokers with <10 pack-years n=122	Non-obstructive patients with ≥10 pack-years n=32	ACOS ≥10 pack-years FEV_1/FVC<0.7 n=34	*p*-value$^{\Phi}$
Post bronchodilator				
FEV_1 L	2.74 (2.30-3.34)	3.08 (2.34-3.60)	2.32 (1.80-2.98) ǂ#	**0.002**
FEV_1 % predicted	93.0 (84.0-102.0)	88.5 (81.0-95.0)	75.0 (57.5-85.5) ǂ#	**<0.001**
FEV_1/FVC	0.77 (0.72-0.81)	0.78 (0.72-0.81)	0.62 (0.54-0.67) ǂ#	**<0.001**
FVC L	3.65 (3.07-4.36)	3.85 (3.04-4.80)	3.92 (3.41-4.47)	0.428
FVC % predicted	99.0 (89.0-110.0)	93.0 (82.5-105.0) ǂ	98.5 (91.0-106.5)	0.080
FEV_1 reversibility$^{\Omega}$				
mL	108.8 ± 137.8	91.6 ± 104.5	135.6 ± 149.9	0.404
%	4.2 ± 5.5	3.2 ± 3.7	6.7 ± 7.7 #	**0.034**
FVC reversibility$^{\Omega}$				
mL	23.9 ± 150.3	52.5 ± 148.2	151.2 ± 239.7 ǂ	**0.001**
%	0.8 ± 4.4	1.6 ± 3.9	4.3 ± 6.6 ǂ	**0.001**

Data is shown as mean ± SD, or median (interquartile range), $^{\Omega}$: change from pre- to postbronchodilator

Φ: *p*-value across all groups

ǂ: as compared to group 1. (Never and ex-smokers with <10 pack-years) p<0.05

#: as compared to group 2. (Non-obstructive patients with ≥10 pack-years) p<0.05

Comorbidities

The overall number of comorbidities was significantly higher in the ACOS group as compared with the other groups (p=0.008). COPD was not considered as a comorbidity in the ACOS group. Similarly, the number of medications used for treatment of comorbidities was highest in the ACOS group. Prevalence of hypertension (p=0.029), coronary heart disease (p=0.012) and hypercholesterolemia (p=0.023) was highest in ACOS group (Table 4). In contrast, there were no differences in prevalence of diabetes, systemic rheumatoid disease, or thyroidal disease. In the group of non-obstructive patients with ≥ 10 pack-years of smoking, the prevalence of obesity was higher as compared to other groups, and obesity among ACOS patients was similar to never or ex smoking patients with < 10 pack-years of smoking (Table 4). In addition, there were no differences in the use of antipsychotic or antidepressant medication, or therapy for dyspepsia or pain between any of the groups (data not shown).

Table 4. Comorbidities in study groups

	Never and ex-smokers with <10 pack-years n=122	**Non-obstructive patients with ≥10 pack-years n=32**	**ACOS ≥10 pack-years $FEV_1/FVC<0.7$ n=34**	***p*-value$^{\Phi}$**
Number of comorbidities	1 (0-2)	1 (0-3)	2 (1-3) ǂ	**0.008**
Obesity §	37 (30.3)	19 (59.4) ǂ	10 (29.4) #	**0.007**
Hypertension	35 (28.7)	10 (31.3)	18 (52.9)ǂ	**0.029**
Coronary heart disease	7 (5.7)	6 (18.8)	7 (20.6) ǂ	**0.012**

Hypercholesterolemia	18 (14.8)	8 (25)	12 (35.3) ǂ	**0.023**
Diabetes	15 (12.3)	5 (15.6)	8 (23.5)	0.264
Systemic rheumatoid disease	4 (3.3)	1 (3.1)	1 (2.9)	0.995
Thyroidal disease	9 (7.4)	3 (9.4)	4 (11.8)	0.707
Number of other medications $^{\Omega}$	1 (0-3)	2 (1-5)	3 (1-7) ǂ	**0.004**

Data is shown as n (%), or median (interquartile range), §: BMI ≥30, $^{\Omega}$: Other than medications for asthma or allergy

Φ: *p*-value across all groups

ǂ: as compared to group 1. (Never and ex-smokers with <10 pack-years) p<0.05

#: as compared to group 2. (Non-obstructive patients with ≥10 pack-years) p<0.05

Differences between obstructive asthma and ACOS

Patients with obstructive asthma (FEV_1/FVC <0.7 but smoking history less than 10 pack-years) had significantly higher diffusing capacity values of the lung as compared to ACOS patients. Blood neutrophil levels and serum IL-6 were found to be higher in ACOS group as compared to patients with obstructive asthma. No differences were found in levels of blood eosinophils, IgE, hsCRP or FeNO (Table 5). CAT-scores were higher in the ACOS group than in the group of obstructive asthma, but no differences were found in AQ20-scores or ACT-scores (Table E4). Furthermore, the use of medication was similar among patients with ACOS or obstructive asthma, and no differences in lung function were found (Tables E4, E5). ACOS patients had higher number of comorbidities, although there were no other significant differences with regards to specific comorbidities (Table E6).

Table 5. Diffusing capacity and biomarkers in the groups of obstructive asthma and ACOS

	Obstructive asthma	**ACOS ≥10 pack-years**	***p*-value**

	<10 pack-years and $FEV_1/FVC<0.7$ n=19	$FEV_1/FVC<0.7$ n=34	
DLco % predicted	103 ± 24	85 ± 23	**0.011**
DLco/VA % predicted	100 ± 17	86 ± 22	**0.018**
B-Neutrophils x10*9/L	3.68 ± 1.33	4.54 ± 1.37	**0.033**
B-Eosinophils x10*9/L	0.16 (0.08-0.20)	0.19 (0.10-0.29)	0.217
IgE kU/l	77 (29-198)	59 (20-140)	0.399
FeNO ppb	11 (5-24)	10 (5-15)	0.524
hsCRP mg/L	0.94 (0.49-1.57)	0.93 (0.59-3.04)	0.475
IL-6 pg/mL	1.64 (1.12-2.21)	2.88 (1.88-4.99)	**0.001**

Data is shown as mean ± SD, or median (interquartile range). DLco = Diffusing capacity of the lung for carbon monoxide, VA= Alveolar volume, B=blood, IgE= Immunoglobulin E, FeNO= Exhaled nitric oxide, hsCRP= high sensitivity C-reactive protein, IL-6= Interleukin 6

DISCUSSION

In this study we evaluated the differences between ACOS and adult-onset asthma. ACOS most clearly separates from asthma by lower pulmonary diffusing capacity and higher levels of blood neutrophils and serum IL-6 levels. ACOS patients have lower lung function and higher reversibility of the airways despite similar medication for asthma, and more comorbidities than asthma patients without COPD. Furthermore, asthma control is significantly worse among ACOS patients as compared to asthma alone. To the best of our knowledge, this is the first study to evaluate both blood biomarkers and clinical characteristics separating ACOS from asthma, in a cohort of clinical asthma patients including also subjects with smoking-related ACOS.

Lower pulmonary diffusing capacity among smokers has been considered as an indicator of emphysema, a characteristic of COPD. However, diffusing capacity among ACOS patients is still poorly known. In the present study, diffusing capacity values of ACOS patients were found to be significantly lower as compared to asthma patients without COPD. This is supported by previous findings of Kitaguchi et al, who reported lower values of DLco and DLco/VA (% predicted) among COPD patients with asthmatic symptoms (defined as ACOS), as compared to asthma with fixed

airflow limitation [30]. Our results further suggest, that diffusing capacity measurement could be considered as a useful tool in the clinical work when trying to differentiate ACOS patients from those with asthma alone. In addition, lower diffusing capacity among ACOS patients may contribute to the increased disease burden and lower quality of life suggested by this and the previous studies [14-17]. ACOS patients had significantly lower lung function as compared to patients with asthma alone, as measured by FEV_1 (mL and % predicted) and FEV_1/FVC ratio. This is well in line with the previous studies [14-16, 31], and reasonable as FEV_1/FVC<0.7 was inclusion criteria for the ACOS group in this study. Furthermore, our study shows that the reversibility of the airways was significantly higher in ACOS group as compared to asthma alone, at the point when patients had been treated for their asthma already for 12 years. This is supported by findings of Kitaguchi et al, who reported higher increase in FEV_1 after bronchodilator test in the group defined as ACOS (i.e. COPD with asthmatic symptoms) as compared to the group of asthma patients with airflow limitation [30]. Our finding of higher remaining reversibility in ACOS patients, who were yet similarly medicated for their asthma, further suggests steroid resistance [32] to be involved in ACOS.

In the present study we found blood neutrophil levels to be significantly higher among ACOS patients as compared with asthma patients. Previous studies have suggested higher levels of sputum neutrophils in patients with ACOS [33,34]. Given the fact that inhaled glucocorticoids are known to inhibit apoptosis of neutrophils [35,36], there might be a possibility of iatrogenic neutrophilia in ACOS. However, in our study, the dosages of daily inhaled glucocorticoids were not any higher in the ACOS group, in which neutrophil levels were the highest, suggesting that blood neutrophilia among ACOS patients may derive from actual inflammatory pathway rather than is purely an iatrogenic result of the use of glucocorticoids. For example, it has previously been

suggested that IL-6, being higher in ACOS patients, may promote neutrophilic inflammation in asthma [25].

Among obstructive airway diseases, systemic inflammation has previously been typically associated with COPD. However, recently similar prevalence of systemic inflammation has been reported also among patients with ACOS [33]. Most widely studied biomarkers of systemic inflammation have been IL-6 and CRP [37], from which elevated IL-6 has been shown to associate with worse outcome of asthma [25]. We evaluated whether assessing blood biomarkers would help to identify ACOS from asthma. Our results revealed significantly higher levels of IL-6 in ACOS patients as compared with asthma patients. This finding is supported by similar results by Fu et al [33], whose definition of ACOS was, however, not based on smoking history. Another recent study showed significantly higher concentrations of sputum IL-6 in ACOS as compared to asthma [34]. Systemic inflammation in ACOS has been proposed to resemble that in COPD, including elevated CRP levels [38]. In our study, the levels of hsCRP did not differ between the groups, which was a surprising finding considering the existence of systemic inflammation in COPD and ACOS. However, Fu et al reported similar findings in their study showing no significant differences in CRP levels between the groups of ACOS and asthma [33]. The results of our study thus suggest that IL-6, but not hsCRP, separates ACOS from asthma.

Prevalence of ACOS among patients with asthma in our study was 18.1%, which is in line with previous studies [9, 16]. Over a half of ACOS patients suffered from uncontrolled asthma, which was significantly higher proportion of patients than in the other groups. However, asthma control was assessed according to GINA 2010 report [29], thus impaired lung function may partly explain the poor control of asthma in this study. Previously it has been suggested that ACOS patients might have higher CAT scores than patients with asthma [39], so we evaluated, whether ACOS can

be separated from asthma by using questionnaires that are validated for clinical use. We found that ACT scores, CAT scores or AQ20 questionnaires do not separate ACOS from asthma although CAT scores were higher and ACT scores lower among patients with heavier smoking history (≥ 10 pack-years). Thus, ACT, CAT or AQ20 questionnaires may not be useful in diagnosing ACOS among asthmatic patients in clinical daily practice.

Moreover our results show higher number of comorbidities among ACOS patients as compared with asthma patients. Especially cardiovascular morbidity was found to be higher, as the prevalence of hypertension and coronary heart disease was higher in the ACOS group. This finding is supported by previous studies [15, 16].

Fixed airflow obstruction due to asthma or COPD has been previously widely studied. Results have suggested lower diffusing capacity, lower FeNO levels, higher levels of neutrophils and lower eosinophil counts among patients with fixed obstruction caused by COPD, as compared to those induced by asthma [40-42]. However, the differences between ACOS and obstructive asthma have been far less known [42]. When comparing obstructive asthma (with <10 pack-years of smoking history) and ACOS in our study, the results revealed that ACOS separates from obstructive asthma most clearly by lower diffusing capacity, higher number of comorbidities, and higher levels of blood neutrophils and IL-6. Levels of eosinophils, IgE, FeNO or hsCRP did not separate obstructive asthma from ACOS.

Our study has several strengths. In our real-life clinical cohort, the diagnosis of new-onset adult asthma was made by a respiratory physician, and the diagnosis was based on typical symptoms and objective lung function measurements showing reversibility of airway obstruction [22]. The diagnosis of ACOS among our patients with asthma was based on significant history of smoking (≥ 10 pack-years) combined with post BD $FEV_1/FVC<0.7$. The duration of asthma was equal in all of

the groups, which gave us a possibility to reliably compare variables without bias from different duration of the disease. There remain some limitations in the interpretation of our results. The numbers of patients in the two groups of ≥ 10 pack-years of smoking were somewhat low (n=32 and 34, respectively), which may lead to loss of power in the analyses. Thus, further clinical studies with larger study cohorts are still needed. We did not have a control group of healthy persons, which could also be considered as a limitation of this study. We acknowledge that recently a consensus definition of ACOS has been published, in which it is suggested that a key feature of ACOS should be diagnosis of asthma or atopy before 40 years of age [10]. However, in another recent study it has been showed, that the majority of adult-onset asthma is actually diagnosed at older age [43], which leads to proposed age limit of 40 being somewhat low. The present study cohort included only adult-onset asthma patients, and the age of onset of asthma was on average 46.5 years, and in ACOS group 53.0 years. The diagnosis of asthma was made by guidelines, and based on typical symptoms and objective lung function measurements showing bronchial variability. Therefore possible bias due to incorrect categorization of ACOS is not likely in our study, despite the higher age of asthma onset. However, since some of the subjects with COPD have significant reversibility of obstruction and some subjects with smoking history and asthma have only partially reversible airway obstruction, there is no exact way in putting the patients into diagnostic categories. Therefore, we acknowledge the possibility of misclassification, although the diagnoses were made by carefully following the existing guidelines.

In conclusion, ACOS separates from adult-onset asthma most clearly by lower pulmonary diffusing capacity, and higher levels of blood neutrophils and serum IL-6. ACOS patients have lower lung function, more reversibility of the airways despite equal medication for asthma, and more comorbidities than asthmatic patients without COPD. Diffusing capacity measurements could be

considered as a useful tool in clinical work to help identify ACOS patients among those with asthma alone.

Contributions: M.T, P.I, L.E.T., L.L. and H.K. designed the study and wrote the report with input from the other authors. M.T. performed the statistical analyses with help from P.I. O.N. and J.H. contributed to laboratory and clinical physiology analyses, respectively. All authors contributed to interpretation of the data. All authors made critical revisions of the manuscript and approved the final version of the manuscript.

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REFERENCES

1. Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention. Updated 2016. http://www.ginasthma.org/. Date last accessed: November 15th 2016.
2. Global Initiative for Chronic Obstructive Lung Disease: Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. Updated 2016. http://goldcopd.org/. Date last accessed: May 10th 2016.
3. Global Initiative for Asthma and Global Initiative for Chronic Obstructive Lung Disease; Diagnosis of Diseases of Chronic Airflow Limitation: Asthma, COPD and Asthma-COPD

Overlap Syndrome (ACOS). 2015. http://www.ginasthma.org/. Date last accessed: May 2nd 2016.

4. Kankaanranta H, Harju T, Kilpeläinen M, Mazur W, Lehto JT, Katajisto M, Peisa T, Meinander T, Lehtimäki L. Diagnosis and pharmacotherapy of stable chronic obstructive pulmonary disease: the Finnish guidelines. Basic Clin Pharmacol Toxicol. 2015;116(4):291-307
5. Miravitlles M, Soler-Cataluña JJ, Calle M, Molina J, Almagro P, Quintano JA, Trigueros JA, Piñera P, Simón A, Riesco JA, Ancochea J, Soriano JB. A new approach to grading and treating COPD based on clinical phenotypes: summary of the Spanish COPD guidelines (GesEPOC). Prim Care Respir J. 2013;22:117-121
6. Postma DS, Rabe KF. The Asthma-COPD Overlap Syndrome. Review. N Engl J Med 2015;373:1241-1249
7. Miravitlles M, Vogelmeier C, Roche N, Halpin D, Cardoso J, Chuchalin AG, Kankaanranta H, Sandström T, Śliwiński P, Zatloukal J, Blasi F. A review of national guidelines for management of COPD in Europe. Eur Respir J 2016;47:625-637
8. Barrecheguren M, Esquinas C, Miravitlles M. The asthma-chronic obstructive pulmonary disease overlap syndrome (ACOS): opportunities and challenges. Review. Curr Opin Pulm Med. 2015;21(1):74-79
9. Wurst KE, Kelly-Reif K, Bushnell GA, Pascoe S, Barnes N. Understanding asthma-chronic obstructive pulmonary disease overlap syndrome. Respir Med 2016;110:1-11
10. Sin DD, Miravitlles M, Mannino DM, Soriano JB, Price D, Celli BR, Leung JM, Nakano Y, Park HY, Wark PA, Wechsler ME. What is asthma-COPD overlap syndrome? Towards a consensus definition from a round table discussion. Eur Respir J 2016;48:664-673

11. De Marco R, Pesce G, Marcon A, Accordini S, Antonicelli L, Bugiani M, Casali L, Ferrari M, Nicolini G, Panico MG, Pirina P, Zanolin ME, Cerveri I, Verlato G. The Coexistence of Asthma and Chronic Obstructive Pulmonary Disease (COPD): Prevalence and Risk Factors in Young, Middle-aged and Elderly People from the General Population. PLoS One 2013;8(5):e62985
12. Miravitlles M, Soriano JB, Ancochea J, Muñoz L, Duran-Tauleria E, Sánchez G, Sobradillo V, García-Río F. Characterisation of the overlap COPD-asthma phenotype. Focus on physical activity and health status. Respir Med 2013;107:1053-1060
13. Hardin M, Cho M, McDonald M-L, Beaty T, Ramsdell J, Bhatt S, van Beek EJR, Make BJ, Crapo JD, Silverman EK, Hersh CP. The clinical and genetic features of the COPD asthma overlap syndrome. Eur Respir J 2014;44(2):341-350
14. Menezes AMB, de Oca MM, Pérez-Padilla R, Nadeau G, Wehrmeister FC, Lopez-Varela MV, Muiño A, Jardim JRB, Valdivia G, Tálamo C. Increased Risk of Exacerbation and Hospitalization in Subjects With an Overlap Phenotype COPD-Asthma. Chest 2014;145:297-304
15. Milanese M, Di Marco F, Corsico AG, Rolla G, Sposato B, Chieco-Bianchi F, Costantino MT, Crivellaro MA, Guarnieri G, Scichilone N. Asthma control in elderly asthmatics. An Italian observational study. Respir Med 2014;108:1091-1099
16. Kauppi P, Kupiainen H, Lindqvist A, Tammilehto L, Kilpeläinen M, Kinnula VL, Haahtela T, Laitinen T. Overlap Syndome of Asthma and COPD Predicts Low Quality of Life. J Asthma 2011;48:279-285
17. Andersén H, Lampela P, Nevanlinna A, Säynäjäkangas O, Keistinen T. High hospital burden in overlap syndrome of asthma and COPD. Clin Respir J 2013;7:342-346
18. Sorino C, Pedone C, Scichilone N. Fifteen-year mortality of patients with asthma-COPD overlap syndrome. Eur J Intern Med 2016;34:72-77.

19. Lange P, Çolak Y, Ingebrigtsen TS, Vestbo J, Marott JL. Long-term prognosis of asthma, chronic obstructive pulmonary disease, and asthma-chronic obstructive pulmonary disease overlap in the Copenhagen City Heart study: a prospective population-based analysis. Lancet Respir Med. 2016;4:454-462
20. Kostikas K, Clemens A, Patalano F. The asthma-COPD overlap syndrome: do we really need another syndrome in the already complex matrix of airway disease? Int J Chron Obstruct Pulmon Dis. 2016;11:1297-1306
21. Tho NV, Park HY, Nakano Y. Asthma-COPD overlap syndrome (ACOS): A diagnostic challenge. Respirology 2016;21:410-418
22. Kankaanranta H, Ilmarinen P, Kankaanranta T, Tuomisto LE. Seinäjoki adult asthma study (SAAS): a protocol for a 12-year real-life follow-up study of new-onset asthma diagnosed at adult age and treated in primary and specialised care. NPJ Prim Care Respir Med 2015;25:15042
23. Haahtela T, Klaukka T, Koskela K, Erhola M, Laitinen LA; Working Group of the Asthma Programme in Finland 1994-2004. Asthma programme in Finland: a community problem needs community solutions. Clinical review. Thorax 2001;56:806-814
24. Viljanen AA, Halttunen PK, Kreus KE, Viljanen BC. Spirometric studies in non-smoking, healthy adults. Scand J Clin Lab Invest Suppl 1982;159:5-20
25. Ilmarinen P, Tuomisto LE, Niemelä O, Danielsson J, Haanpää J, Kankaanranta T, Kankaanranta H. Comorbidities and elevated IL-6 associate with negative outcome in adult-onset asthma. Eur Respir J. 2016;48:1052-1062.
26. Tuomisto LE, Ilmarinen P, Niemelä O, Haanpää J, Kankaanranta T, Kankaanranta H. A 12-year prognosis of adult-onset asthma: Seinäjoki Adult Asthma Study. Respir Med. 2016;117:223-229.

27. Tommola M, Ilmarinen P, Tuomisto LE, Haanpää J, Kankaanranta T, Niemelä O, Kankaanranta H. The effect of smoking on lung function: a clinical study of adult-onset asthma. Eur Respir J 2016;48:1298-1306
28. Barley EA, Quirk FH, Jones PW. Asthma health status measurement in clinical practice: validity of a new short and simple instrument. Respir Med 1998;92:1207-1214.
29. Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention 2010. Date last accessed: Mach 29th 2011.
30. Kitaguchi Y, Yasuo M, Hanaoka M. Comparison of pulmonary function in patients with COPD, asthma-COPD overlap syndrome, and asthma with airflow limitation. Int J Chron Obstruct Pulmon Dis. 2016;11:991-997.
31. DeMarco R, Marcon A, Rossi A, Antó JM, Cerveri I, Gislason T, Heinrich J, Janson C, Jarvis D, Kuenzli N, Leynaert B, Probst-Hensch N, Svanes C, Wjst M, Burney P. Asthma, COPD and overlap syndrome: a longitudinal study in young European adults. Eur Respir J 2015;46:587-590
32. Barnes PJ. Corticosteroid resistance in patients with asthma and chronic obstructive pulmonary disease. Review. J Allergy Clin Immunol 2013;131:636-645
33. Fu JJ, McDonald VM, Gibson PG, Simpson JL. Systemic Inflammation in Older Adults With Asthma-COPD Overlap Syndrome. Allergy Asthma Immunol Res 2014;6:316-324
34. Gao J, Iwamoto H, Koskela J, Alenius H, Hattori N, Kohno N, Laitinen T, Mazur W, Pulkkinen V. Characterization of sputum biomarkers for asthma-COPD overlap syndrome. Int J Chron Obstruct Pulmon Dis. 2016;11:2457-2465.
35. Zhang X, Moilanen E, Kankaanranta H. Beclomethasone, budesonide and fluticasone propionate inhibit humanneutrophil apoptosis. Eur J Pharmacol 2001;431:365-371

36. Zhang X, Moilanen E, Adcock IM, Lindsay MA, Kankaanranta H. Divergent effect of mometasone on human eosinophil and neutrophilapoptosis. Life Sci 2002;71:1523-34
37. Paone G, Leone V, Conti V, De Marchis L, Ialleni E, Graziani C, Salducci M, Ramaccia M, Munafò G. Blood and sputum biomarkers in COPD and asthma: a review. Eur Rev Med Pharmacol Sci 2016;20:698-708
38. Gibson PG, McDonald VM. Asthma-COPD overlap 2015: now we are six. Review. Thorax 2015;70:683-691
39. Kurashima K, Takaku Y, Ohta C, Takayanagi N, Yanagisawa T, Sugita Y. COPD assessment test and severity of airflow limitation in patients with asthma, COPD, and asthma-COPD overlap syndrome. Int J Chron Obstruct Pulmon Dis. 2016;11:479-487
40. Fabbri LM, Romagnoli M, Corbetta L, Casoni G, Busljetic K, Turato G, Ligabue G, Ciaccia A, Saetta M, Papi A. Differences in airway inflammation in patients with fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2003;167:418-24.
41. Contoli M, Baraldo S, Marku B, Casolari P, Marwick JA, Turato G, Romagnoli M, Caramori G, Saetta M, Fabbri LM, Papi A. Fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease: 5-year follow-up. J Allergy Clin Immunol. 2010 Apr;125(4):830-7
42. Rogliani P, Ora J, Puxeddu E, Cazzola M. Airflow obstruction: is it asthma or is it COPD? Review. Int J Chron Obstruct Pulmon Dis. 2016;11:3007-3013.
43. Kankaanranta H, Tuomisto LE, Ilmarinen P. Age-specific incidence of new asthma diagnoses in Finland. J Allergy Clin Immunol Pract. 2017;5:189-191

PUBLICATION III

Concern of underdiagnosing asthma-COPD overlap syndrome if age limit of 40 years for asthma is used

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Concern of underdiagnosing ACOS if age limit of 40 for asthma is used

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Abstract: Using the suggested asthma-onset age limit of 40 as a criteria for ACOS may lead to severe underdiagnosing of ACOS.

To the Editor:

In a recent issue of European Respiratory Journal, Sin et al. presented recommendations on the definition of asthma-COPD overlap syndrome, ACOS [1]. Presented conclusions based on a round table discussion are very important and notable, especially considering the current lack of specific clinical criteria on ACOS. One of the key recommendations is that asthma or atopy should be diagnosed before age of 40 years (or patients should have very large airway reversibility), in order to fulfill criteria of ACOS. However, the scientific basis of using the 40-year cut-off remains debatable. In addition, the proposal raises a major concern of underdiagnosing adult-onset asthma and ACOS among patients, if the suggested age limit is used.

ACOS has been described to develop mainly by two pathways: 1. patient with previous COPD develops asthma-like symptoms and/or asthmatic characteristics e.g. large reversibility of the airways, or 2. patient with previous asthma continues smoking and develops non-reversible bronchial obstruction, which indicates COPD [2, 3]. The prevalence of ACOS is suggested to be 12-55% among patients with COPD and 13-61% among patients with asthma [2]. These numbers reflect the relatively large impact of ACOS, and oblige us to diagnostic accuracy.

Based on cluster analyses, different phenotypes have been recognized in asthma, and the age of onset has been found to be a key factor distinguishing these phenotypes [4, 5]. Early-onset asthma has typically been associated with atopy and allergies, and characterized with good response to inhaled corticosteroids and relatively high remission rate [4, 5]. Adult-onset asthma, on the other hand, has received less attention. Recent studies have suggested adult-onset asthma to have lower remission rates [5, 6], more rapid loss of lung function [5] and poorer prognosis [6, 7]. In a recent study on age-specific incidence of new asthma diagnoses in Finland, it was reported, that most diagnoses of persistent asthma are actually made in adulthood [8]. In a U.S.-based study the

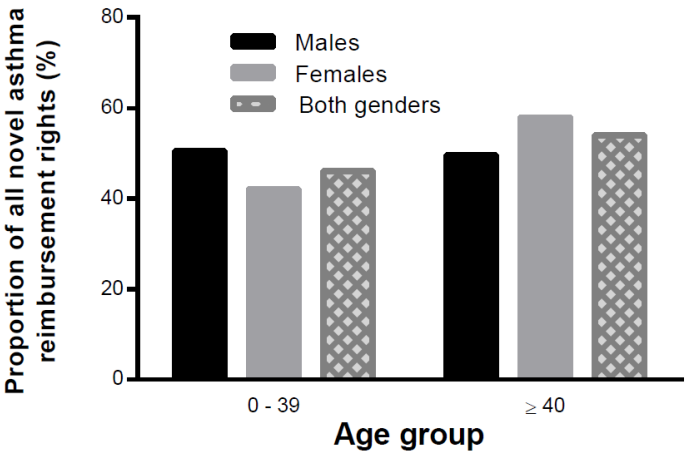
adult-onset phenotype is reported to dominate especially among women [9]. Moreover, in a clinical cohort of consecutive new adult-onset asthma patients (Seinäjoki Adult Asthma Study; SAAS [6, 10]) the mean (SD) age of asthma onset was found to be 46 (13.7) years, which is substantially higher, than the suggested limit [6, 10]. These findings raise a question, whether the age limit of 40 years is actually applicable to the real life and clinical work?

In Finland, every patient with persistent asthma is entitled to asthma reimbursement by the Social Insurance Institution of Finland (SII). The number of new asthma reimbursements reflects the number of novel asthma diagnoses made. We obtained the numbers of patients from the SII, and evaluated the age at which the reimbursement was obtained in Finland during 2012-2013, in order to evaluate whether diagnoses of persistent asthma were made either before, or after 40 years of age. Data acquisition and calculation of novel asthma medication reimbursements were made as previously described [8].

In 2012-2013 in Finland (population 5.4 million), 26,281 new patients were entitled to special reimbursement for their asthma medication (13,941 females and 12,340 males). Of these, only 12,095 persons (46.0 %) belonged to age group 0-39 years, indicating that a majority (54.0 %) of new patients who obtained special asthma medication reimbursement were older than 40 years (Figure 1). More than half (57.9 %) of females were 40 years or older, as in contrast, 50.5 % of men obtained asthma reimbursement before their 40th birthday (Figure 1). As a conclusion, these results further suggest that most asthma is diagnosed after 40 years of age, and thus emphasize the impact and importance of adult-onset asthma. The criteria for asthma reimbursement in Finland is variable airway obstruction demonstrated by using objective lung function measurements [8] and thus

misclassified COPD does not explain the result, even though the current analysis contains patients having smoking history.

Figure 1. Proportion of novel asthma reimbursements in age groups 0-39 or ≥ 40 years



The perception of asthma being merely a childhood-onset disease lives strong among us. However, the adult-onset phenotype of asthma has been identified in several studies and is recognized by guidelines. There is already evidence that majority of persistent asthma may actually start in adulthood [8, 9], making it necessary to take patients with adult-onset asthma into consideration when starting age of asthma is recommended as a diagnostic criteria. In order to obtain a diagnosis and good and increasingly personalized therapy for our ACOS patients, the diagnostics must be accurate and sensitive regardless of gender, age or smoking status. Using the suggested age limit of 40 as a criteria for ACOS may lead to severe underdiagnosing of ACOS, when patients with asthma onset after 40 years of age are not included. This especially concerns women. Thus we propose, that the suggested age limit of asthma onset in the criteria of ACOS should be reconsidered.

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References:

1. Sin DD, Miravitlles M, Mannino DM, Soriano JB, Price D, Celli BR, Leung JM, Nakano Y, Park HY, Wark PA, Wechsler ME. What is asthma-COPD overlap syndrome? Towards a consensus definition from a round table discussion. Eur Respir J 2016;48:664-673
2. Wurst KE, Kelly-Reif K, Bushnell GA, Pascoe S, Barnes N. Understanding asthma-chronic obstructive pulmonary disease overlap syndrome. Respir Med 2016;110:1-11
3. Barrecheguren M, Esquinas C, Miravitlles M. The asthma-chronic obstructive pulmonary disease overlap syndrome (ACOS): opportunities and challenges. Review. Curr Opin Pulm Med. 2015;21(1):74-79
4. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med 2012; 18: 716–725.

5. de Nijs SB. Venekamp LN, Bel EH. Adult-onset asthma: is it really different? Eur Respir Rev 2013; 22: 44-52.
6. Tuomisto LE, Ilmarinen P, Niemelä O, Haanpää J, Kankaanranta T, Kankaanranta H. A 12-year prognosis of adult-onset asthma: Seinäjoki Adult Asthma Study. Respir Med. 2016;117:223-229.
7. Tuomisto LE, Ilmarinen P, Kankaanranta H. Prognosis of new-onset asthma diagnosed at adult age. Respir Med 2015; 109:944-954.
8. Kankaanranta H, Tuomisto LE, Ilmarinen P. Age-specific incidence of new asthma diagnoses in Finland. J Allergy Clin Immunol Pract. 2017;5:189-191
9. Sood A, Qualls C, Schuyler M, Arynchyn A, Alvarado JH, Smith LJ, et al. Adult-onset asthma becomes the dominant phenotype among women by age 40 years. Ann Am Thorac Soc 2013; 10: 188-197.
10. Tommola M, Ilmarinen P, Tuomisto LE, Haanpää J, Kankaanranta T, Niemelä O, Kankaanranta H. The effect of smoking on lung function: a clinical study of adult-onset asthma. Eur Respir J 2016;48:1298-1306

PUBLICATION IV

Cumulative effect of smoking on disease burden and multimorbidity in adult-onset asthma

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Cumulative effect of smoking on disease burden and multimorbidity in adult-onset asthma

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Take home message: Disease burden and multimorbidity in adult-onset asthma increase dose-dependently with smoked pack-years

This study is registered at ClinicalTrials.gov with identifier number NCT02733016.

Words 1197

To the Editor:

Smokers and patients with heavy smoking history have usually been excluded from clinical studies of asthma. Thus, little is known about the impact of lifelong, cumulative tobacco exposure on asthma [1,2]. The effect of smoking status (never-, ex- or current smoker) to disease burden of asthma has been more commonly recognized, but the impact of pack-year history has rarely been evaluated [3-6]. Impact of smoked pack-years has been previously reported only by few studies, showing adverse effects on lung function and asthma control [5, 7-10], whereas no significant differences in healthcare use, asthma related questionnaires or medication use were reported among ex-smokers with severe asthma when patients were categorized based on smoked pack-years [4]. Assessment of pack-years is an easy and usable tool in clinical work, and the intensity of smoking has been proposed to be even more important factor than plain smoking status [5]. Thus our aim was to evaluate the impact of cumulative smoking history i.e. pack-years on hospitalizations, comorbidities and symptoms in adult-onset asthma, as a part of Seinäjoki Adult Asthma Study (SAAS).

Seinäjoki Adult Asthma Study (SAAS) is a prospective, single-center, 12-year follow-up study of patients with adult-onset asthma. At baseline (years 1999-2002) 257 adults (aged ≥15 years) were diagnosed with new-onset asthma by respiratory physician in Seinäjoki Central Hospital, Finland. Diagnosis was made by following the guidelines, and the study protocol has been previously published [11]. Smokers (ex- or current) were included in the study. After a follow-up of 12 years, 203 (79%) patients were re-evaluated at a control visit. During the 12 years of follow-up, patients were actively treated for their asthma, following the Finnish Asthma Program guidelines [11]. Data on hospitalizations was retrospectively collected from the patient records of hospitals, primary care, private clinics and occupational care. Structured questionnaires were used to collect

information on medication and symptoms. Patients' lifelong smoking history was evaluated based on respiratory nurse's interviews, and smoked pack-years (20 cigarettes per day for 1 year) were assessed. Comorbidities were assessed using structured questionnaire, as previously described [12].

To evaluate the dose-dependent effect of smoking, patients with smoking history (never smokers excluded) were divided into 3 groups based on pack-years smoked: 1) pack-years 0.1-9.9, 2) pack-years 10-19.9 and 3) pack-years ≥ 20. In regression analysis of predictors for hospitalization also never smokers were included. Cross-sectional data from the follow-up visit (years 2012-2013) was used, except when evaluating the use of healthcare resources or medication (oral corticosteroids or antibiotics) during follow-up.

Patients with smoking history exceeding 10 pack-years were older, more obese, and more often males (Table 1). Long-acting beta agonist medication (LABA) was more often in daily use among patients with ≥ 10 pack-years, but otherwise the medication was similar between the groups. There were no significant differences in daily use of inhaled corticosteroids (ICS) or long-acting muscarinic antagonists (LAMA), in ICS dose, or surprisingly, in the use of antibiotics or oral corticosteroids (Table 1).

Table 1. Clinical characteristics, use of medication and effect of cumulative smoking history by pack-year groups

	Pack-years [μ] 0.1-9.9 n=32	**Pack-years 10-19.9 n=26**	**Pack-years ≥20 n=39**	**p-value**
Clinical characteristics				
Age at follow-up, years	51.7 ± 13.3	63.2 ± 11.8 [ǂ]	61.9 ± 12.4 [ǂ]	**0.001**

Gender male	12 (37.5%)	15 (57.7%)	28 (71.8%) ǂ	**0.015**
BMI kg·m^{-2}	25.9 (23.3-28.5)	28.1 (25.0-30.8)	28.7 (24.2-33.4)	**0.045**
Use of medication				
ICS in daily use	20 (62.5%)	21 (80.8%)	31 (79.5%)	0.179
ICS dose/day bud eq[§]	800 (400-1000)	800 (650-1800)	1000 (775-1500)	0.282
LABA in daily use	6 (18.8%)	16 (61.5%) ǂ	25 (64.1%) ǂ	**<0.001**
LAMA, LTRA or theophylline in daily use	3 (9.4%)	7 (26.9%)	8 (20.5%)	0.214
≥ 1 antibiotic course during follow-up	22 (68.8%)	22 (84.6%)	30 (76.9%)	0.366
Use of oral steroid courses ever	9 (28.1%)	9 (34.6%)	11 (28.9%)	0.846
Effect of cumulative smoking history				
≥1 hospitalizations during follow-up for any respiratory reason	4 (12.5%)	6 (23.1%)	16 (41.0%) ǂ	**0.023**
≥1 asthma-related hospitalizations during follow-up	2 (6.3%)	5 (19.2%)	12 (30.8%) ǂ	**0.035**
Comorbidities	0 (0-1)	2 (1-3) ǂ	2 (1-4) ǂ	**<0.001**
ACT	23 (21-25)	21 (17-24) ǂ	21 (17-23) ǂ	**0.003**
CAT	8 ± 5	14 ± 7 ǂ	15 ± 7 ǂ	**<0.001**

Data is shown as n (%), mean ± SD, or median (interquartile range). BMI= Body mass index, ICS= inhaled corticosteroids, [§]: budesonide equivalent of daily users, LABA= long acting beta agonists, LAMA= long acting muscarinic antagonists, LTRA= leukotriene antagonists. ACT= Asthma Control Test, CAT= COPD Assessment Test. Statistical analyses used were ANOVA and Tukey's post hoc test, Kruskall-Wallis test, or χ^2–test

ǂ: as compared to group with 0.1-9.9 pack-years $p<0.05$
µ: median (IQ range) of pack-years in the groups: 4 (1-7); 15 (11-18); 32 (26-37), respectively.

Proportion of patients who were hospitalized for any respiratory reason during the 12-year follow-up increased in relation to smoked pack-years, and was the highest in the group with ≥20 pack-years (Table 1). Similarly, asthma-related hospitalizations increased in relation to pack-years, being highest among patients with ≥20 pack-years (Table 1). These findings still remained after exclusion of current smokers (data not shown). In addition, the number of pack-years was found to correlate to the number of hospital inpatient periods (Spearman's rho 0.349, $p<0.001$). Patients with smoking history ≥10 pack-years had higher number of comorbidities as compared to those with

<10 pack-years (Table 1), and a strong correlation between pack-years and number of comorbidities was found (rho 0.575, p<0.001). Patients with light smoking history (0.1-9.9 pack-years) were less symptomatic and had higher Asthma Control Test (ACT) scores and lower COPD Assessment Test (CAT) scores as compared to those with heavier smoking history. CAT scores increased in relation to smoked pack-years (rho 0.452, p<0.001), being highest among patients with ≥20 pack-years (Table 1).

Predictors of hospitalization for any respiratory reason were analyzed by a multivariable logistic regression model among all patients with the information on pack-years (n=193, never smokers included). Of these, 53 patients were hospitalized during the follow up. Results of the analysis showed that smoking history ≥20 pack-years was significantly associated with hospitalization (OR 2.48; 95% CI 1.03-5.97; p-value 0.043). Number of comorbidities ≥2 was also associated with hospitalization (4.29; 1.98-9.29; <0.001). In contrast, current smoking (0.46; 0.16-1.35; 0.158), age >50 years (0.47; 0.19-1.13; 0.091), or use of oral corticosteroid courses (1.48; 0.72-3.05; 0.288) were not significantly associated with hospitalization for respiratory reason. The lack of association between current smoking and hospitalization may be explained by low number of current smokers.

We showed in this clinical study with long follow-up time that in (current- or ex-) smoking asthmatics, higher numbers of pack-years correlate to more frequent hospitalizations, and a history of ≥20 pack-years is a significant predictor for respiratory-related hospitalization. Previous studies in asthma have suggested association between current smoking and greater risk of unscheduled healthcare visits and hospitalization [3, 4, 6], but the effect of pack-years has rarely been evaluated. In our study, the relation between increasing rate of respiratory-related hospitalizations during 12 years and higher number of smoked pack-years remained significant

even after exclusion of current smokers, which indicates, that it may be the cumulative toxic effect of tobacco smoking that increases the risk of adverse health events, not merely the current smoking status. This finding emphasizes the importance of clinical assessment of smoked pack-years in addition to current smoking status in daily clinical practice.

In our study, number of comorbidities was found to have a strong positive correlation to the number of smoked pack-years. In addition, having ≥2 comorbidities was significantly associated with respiratory-related hospitalization. Tobacco smoke may cause systemic inflammation [13, 14], which has been recently shown to associate with comorbidities in asthma [12]. Our result on the dose-dependent increase of asthma-related morbidity with smoked pack-years may result from the interplay of toxic effects of tobacco smoke and years of ongoing systemic inflammation.

Increasing number of smoked pack-years was found to correlate with worse symptoms as measured by both ACT- and CAT-scores. This finding is supported by a previous study reporting more symptoms and worse asthma control questionnaire (ACQ) score in smokers with asthma as compared to never-smokers [15]. A correlation between pack-years and ACQ-score was also reported [15]. To achieve more reliable assessment of symptoms, we chose both ACT and CAT scores for evaluation, although CAT-score test is more commonly used in COPD. Interestingly, the correlation of pack-years to CAT-scores was stronger than to ACT-scores. The result suggests that CAT-score may show the increasing symptoms more sensitively in asthma of smokers, and thus could be considered as a tool for comprehensive evaluation of symptoms.

Taken together, we have shown that in adult-onset asthma smoked pack-years are associated with more frequent hospitalizations, higher number of comorbidities and more symptoms in a dose-dependent manner. Furthermore, smoking history of ≥20 pack-years is significantly associated with a higher risk for respiratory-related hospitalization. The routine assessment of lifelong

smoking history in pack-years should be included in the overall future risk-analysis of asthma patients, and early smoking cessation intervention as well as smoking prevention actions are crucial.

Contributions: M.T, P.I, L.E.T, L.L. and H.K. designed the study and wrote the report with input from the other authors. M.T. performed the statistical analyses with help from P.I. and P.N.. O.N. contributed to the laboratory analyses. All authors contributed to interpretation of the data. All authors made critical revisions of the manuscript and approved the final version of the manuscript.

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REFERENCES

1. Polosa R, Thomson NC. Smoking and asthma: dangerous liaisons. Review. Eur Respir J 2013;41:716-726
2. Postma DS, Rabe KF. The Asthma-COPD Overlap Syndrome. Review. N Engl J Med 2015;373:1241-1249
3. Kauppi P, Kupiainen H, Lindqvist A, Haahtela T, Laitinen T. Long-term smoking increases the need for acute care among asthma patients: a case control study. BMC Pulmonary Medicine 2014;14:119
4. Thomson NC, Chaudhuri R, Heaney LG, Bucknall C, Niven RM, Brightling CE, Menzies-Gow AN, Mansur AH, McSharry C. Clinical outcomes and inflammatory biomarkers in current smokers and exsmokers with severe asthma. J Allergy Clin Immunol 2013;131:1008-1016
5. Polosa R, Russo C, Caponnetto P, Bertino G, Sarvà M, Antic T, Mancuso S, Al-Delaimy WK. Greater severity of new onset asthma in allergic subjects who smoke: a 10-year longitudinal study. Respiratory Research 2011;12:16
6. Eisner MD, Iribarren C. The influence of cigarette smoking on adult asthma outcomes. Nicotine Tob Res 2007; 9:53–56.
7. Tommola M, Ilmarinen P, Tuomisto LE, Haanpää J, Kankaanranta T, Niemelä O, Kankaanranta H. The effect of smoking on lung function: a clinical study of adult-onset asthma. Eur Respir J 2016;48:1298-1306
8. Hancox RJ, Gray AR, Poulton R, Sears MR. The effect of cigarette smoking on lung function in young adults with asthma. Am J Respir Crit Care Med 2016;194:276-84
9. Tuomisto LE, Ilmarinen P, Niemelä O, Haanpää J, Kankaanranta T, Kankaanranta H. A 12-year prognosis of adult-onset asthma: Seinäjoki Adult Asthma Study. Respir Med. 2016;117:223-229.

10. Westerhof GA, Vollema EM, Weersink EJ, Reinartz SM, de Nijs SB, Bel EH. Predictors for the development of progressive severity in new-onset adult asthma. J Allergy Clin Immunol 2014;134:1051-6
11. Kankaanranta H, Ilmarinen P, Kankaanranta T, Tuomisto LE. Seinäjoki adult asthma study (SAAS): a protocol for a 12-year real-life follow-up study of new-onset asthma diagnosed at adult age and treated in primary and specialised care. NPJ Prim Care Respir Med 2015;25:15042
12. Ilmarinen P, Tuomisto LE, Niemelä O, Danielsson J, Haanpää J, Kankaanranta T, Kankaanranta H. Comorbidities and elevated IL-6 associate with negative outcome in adult-onset asthma. Eur Respir J 2016;48:1052-1062.
13. Ilmarinen P, Tuomisto LE, Kankaanranta H. Phenotypes, Risk Factors, and Mechanisms of Adult-Onset Asthma. Mediators Inflamm 2015;2015:514868
14. Arnson Y, Shoenfeld Y, Amital H. Effects of tobacco smoke on immunity, inflammation and autoimmunity. J Autoimmun 2010; 34:J258-265
15. Chaudhuri R, McSharry C, McCoard A, Livingston E, Hothersall E, Spears M, Lafferty J, Thomson NC. Role of symptoms and lung function in determining asthma control in smokers with asthma. Allergy 2008;63:132-5

PUBLICATION V

Occupational exposures and asthma-COPD overlap in a clinical cohort of adult-onset asthma

Tommola M, Ilmarinen P, Tuomisto LE, Lehtimäki L, Kankaanranta H

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ORIGINAL RESEARCH LETTER

Occupational exposures and asthma–COPD overlap in a clinical cohort of adult-onset asthma

To the Editor:

Asthma–COPD overlap (ACO) has recently been recognised as a separate phenotype of obstructive airway diseases and is included in several guidelines of asthma and COPD [1–5]. ACO patients have previously been shown to have lower diffusing capacity of the lung, higher blood neutrophil counts and higher interleukin-6 levels compared with asthma patients [6]. In COPD, fixed airway obstruction is considered to develop in response to chronic exposure to noxious inhaled particles [7]. In western countries, the most common cause of COPD is tobacco smoking, but occupational exposure to dusts and fumes has also been shown to increase the risk for developing COPD [7, 8]. However, the role of occupational exposures in the development of ACO is not known.

We studied the association between ACO and occupational exposures to vapours, gases, dusts or fumes (VGDF) in the cohort of the Seinäjoki Adult Asthma Study (SAAS). In the SAAS, 257 patients were diagnosed with new-onset asthma at adult age and followed for 12 years. Diagnosis was made by a respiratory physician and based on objective lung function measurements and medical history [6, 9–12]. Ex- and current smokers were included, and the smoking history of every patient was carefully assessed. After 12 years (years 2012–2013), patients had a control visit, and the occupational data were retrospectively collected. To evaluate the duration of the patients' occupation, the occupation at the time of asthma diagnosis was confirmed from patient records. Patients with detailed smoking history available (n=194) at the follow-up visit were included in the current study. Patients were considered as ACO patients if they had a ⩾10-pack-year history of smoking and post-bronchodilation forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) ratio <0.7 at the follow-up visit. The subjects were divided into two groups based on whether they had occupational exposure history to VGDF (*i.e.* welders, foundry workers, sheet metal workers, smiths, machine workshop workers, mechanics and farmers).

The prevalence of ACO was higher in the group with occupational VGDF exposure, compared to patients with no exposure. Patients with occupational VGDF exposure were older and more often males. A tendency towards higher body mass index (BMI) in patients with occupational VGDF exposure was seen. No differences in the prevalence of allergic conditions or in the use of daily inhaled corticosteroids were seen between the groups (table 1). No statistically significant differences in the rate of airway obstruction at the time of diagnosis were seen between the groups, although a tendency towards more severe obstruction among patients with occupational VGDF exposure was observed: post-bronchodilation FEV_1/FVC (95% CI) was 0.77 (0.72–0.81) among patients with occupational VGDF exposure, and 0.79 (0.75–0.84) in patients with no VGDF exposure (p=0.060).

The mean±SD smoking history of the ACO patients did not differ between the groups: 27.4±13.0 pack-years in ACO patients with no occupational VGDF exposure, and 27.4±17.4 pack-years in ACO patients with VGDF exposure (p=0.992). The prevalence of obstructive asthma was similar between the groups, *i.e.* asthma patients with low smoking history (<10 pack-years) but fixed airway obstruction (post-bronchodilation FEV_1/FVC <0.7) (table 1).

@ERSpublications
Occupational exposure to vapours, gases, dusts or fumes (VGDF) increases the prevalence of asthma–COPD overlap (ACO) in adult-onset asthma. VGDF exposure is independently associated with ACO and an additive effect with smoking is proposed. http://bit.ly/2LiMiXW

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TABLE 1 Clinical characteristics and prevalence of asthma–COPD overlap in groups of different occupational exposure to vapours, gases, dusts or fumes (VGDF)

	No occupational exposure to VGDF	Occupational exposure to VGDF	p-value
Patients n	150	44	
Asthma–COPD overlap	21 (14)	13 (30)	0.024[¶]
Age years	57.3±13.3	62.3±13.9	0.030[¶]
BMI kg·m^{-2}	27.7 (24.2–30.8)	29.6 (25.4–32.0)	0.057
Males	50 (33)	31 (71)	<0.001[¶]
Allergic rhinitis or conjunctivitis	90 (62)	28 (65)	0.723
ICS in daily use	120 (80)	33 (75)	0.530
Smoking history ⩾10 pack-years	47 (31.3)	19 (43.2)	0.152
Obstructive asthma[#]	15 (10)	5 (11)	0.782

Data are presented as n (%), mean±SD or median (interquartile range), unless otherwise stated. BMI: body mass index; ICS: inhaled corticosteroids. [#]: <10 pack-years and forced expiratory volume in 1 s/forced vital capacity ratio <0.7; [¶]: p<0.05.

We further analysed the factors associated with airway obstruction and ACO with two multivariable logistic regression analyses: 1) with a regression model among all patients (n=194), we analysed the factors associated with airway obstruction (*i.e.* FEV_1/FVC <0.7); and 2) with a regression model among patients with ⩾10 pack-years of smoking history (n=65), we analysed the factors associated with ACO. Among all patients, the number of pack-years (OR 1.05, 95% CI 1.02–1.08; p<0.001) and age (OR 1.04, 95% CI 1.01–1.07; p=0.008) were significantly associated with airway obstruction, whereas BMI ⩾30 kg·m^{-2} was associated with lower risk of obstruction (OR 0.37, 95% CI 0.16–0.85; p=0.019). Sex or occupational VGDF exposure were not associated with obstruction. Among patients with ⩾10 pack-years of smoking, a significant factor associated with ACO was occupational VGDF exposure (OR 4.2, 95% CI 1.1–15.3; p=0.030), whereas BMI ⩾30 kg·m^{-2} was associated with lower risk of ACO (OR 0.18, 95% CI 0.06–0.59; p=0.004). The number of pack-years was not associated with ACO among patients with smoking history ⩾10 pack-years (OR 1.02, 95% CI 0.98–1.07; p=0.281). Sex, age or smoking status (never-/ex-/current smoker) were not significantly associated with ACO as analysed by backward, forward or enter methods and, thus, were not included in the final model.

Our results suggest that adult-onset asthma patients with occupational exposure to VGDF more often develop ACO, compared with patients with no such exposure. The smoking history of ACO patients was similar, regardless of their exposure to VGDF, suggesting that heavier smoking is not the reason for increased ACO prevalence in the group with occupational VGDF exposure in our study. In addition, the proportion of patients with smoking history ⩾10 pack-years did not differ between the groups. The patients with occupational VGDF exposure were older than patients without. However, the rate of airway obstruction at the time of asthma diagnosis was not significantly different between the groups. Thus, the increased ACO rate in the group of occupational VGDF exposure is not merely explained by inferior lung function at the diagnosis. Furthermore, when we evaluated the prevalence of non- or low-smoking patients (<10 pack-years) with fixed airway obstruction (*i.e.* obstructive asthma), we found no differences between the groups. This finding suggests that, in adult-onset asthma, occupational exposure alone is not driving the risk of ACO but rather the combination of occupational exposure and smoking. This is in keeping with the previous suggestions of the additive effect of smoking and other environmental exposures in the development of COPD [13]. Furthermore, the multivariable regression analyses showed that occupational VGDF exposure was independently associated with ACO among patients with ⩾10 pack-years of smoking history. In contrast, obesity seemed to lower the risk of ACO. This finding might be explained by reduction of FVC in obese patients, leading to decreased sensitivity of FEV_1/FVC ratio in detecting obstruction [14]. Although patients in the group with occupational exposures were older and more often males, age and sex were not associated with ACO.

In the current study, 17% (n=33) reported farming as their main profession, and when metal workers were also assessed, up to 23% (n=44) were considered as working in a profession linked to increased risk of developing COPD. This gives us a good view of work-related ACO in real-life asthma patients. Furthermore, the diagnosis of asthma was based on objective lung function measurements, and diagnostic guidelines were carefully followed. The quantity of exposure to occupational particles was not measured, which could be considered as a limitation of the current study. However, the reported occupational

information was confirmed from patient records, showing stability of profession during the 12 years of follow-up on nearly all patients. We also recognise the possibility of occupational exposures in other professions (*e.g.* cleaners, waiters). However, considering the current knowledge of occupational exposures, we assessed the professions that are presumed to have the highest exposure related to development of fixed airway obstruction.

Taken together, our results show increased prevalence of ACO in the group of adult-onset asthma patients with occupational VGDF exposure. This is a new finding and in line with what is known on the additive effect of smoking and occupational exposure in the development of fixed airways obstruction and COPD. We thus support active intervention in primary and occupational healthcare, aiming towards smoking cessation and protection against occupational noxious particles in the air.

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References

1 Global Initiative for Asthma, Global Initiative for Chronic Obstructive Lung Disease. Diagnosis of Diseases of Chronic Airflow Limitation: Asthma, COPD and Asthma–COPD Overlap Syndrome (ACOS). 2015. Available from: www.ginasthma.org Date last accessed: 2 May 2016.
2 Kankaanranta H, Harju T, Kilpeläinen M, *et al.* Diagnosis and pharmacotherapy of stable chronic obstructive pulmonary disease: the Finnish guidelines. *Basic Clin Pharmacol Toxicol* 2015; 116: 291–307.
3 Miravitlles M, Soler-Cataluña JJ, Calle M, *et al.* A new approach to grading and treating COPD based on clinical phenotypes: summary of the Spanish COPD guidelines (GesEPOC). *Prim Care Respir J* 2013; 22: 117–121.
4 Postma DS, Rabe KF. The asthma–COPD overlap syndrome. *N Engl J Med* 2015; 373: 1241–1249.
5 Miravitlles M, Vogelmeier C, Roche N, *et al.* A review of national guidelines for management of COPD in Europe. *Eur Respir J* 2016; 47: 625–637.
6 Tommola M, Ilmarinen P, Tuomisto LE, *et al.* Differences between asthma–COPD overlap syndrome and adult-onset asthma. *Eur Respir J* 2017; 49: 1602383.
7 Global Initiative for Chronic Obstructive Lung Disease. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease. 2019 Report. Available from: http://goldcopd.org Date last accessed: 9 November 2018.

8 Sadhra S, Kurmi OP, Sadhra SS, *et al.* Occupational COPD and job exposure matrices: a systematic review and meta-analysis. *Int J Chron Obstruct Pulmon Dis* 2017; 12: 725–734.

9 Kankaanranta H, Ilmarinen P, Kankaanranta T, *et al.* Seinäjoki Adult Asthma Study (SAAS): a protocol for a 12-year real-life follow-up study of new-onset asthma diagnosed at adult age and treated in primary and specialised care. *NPJ Prim Care Respir Med* 2015; 25: 15042.

10 Ilmarinen P, Tuomisto LE, Niemelä O, *et al.* Comorbidities and elevated IL-6 associate with negative outcome in adult-onset asthma. *Eur Respir J* 2016; 48: 1052–1062.

11 Tuomisto LE, Ilmarinen P, Niemelä O, *et al.* A 12-year prognosis of adult-onset asthma: Seinäjoki Adult Asthma Study. *Respir Med* 2016; 117: 223–229.

12 Tommola M, Ilmarinen P, Tuomisto LE, *et al.* The effect of smoking on lung function: a clinical study of adult-onset asthma. *Eur Respir J* 2016; 48: 1298–1306.

13 Blanc PD, Iribarren C, Trupin L, *et al.* Occupational exposures and the risk of COPD: dusty trades revisited. *Thorax* 2009; 64: 6–12.

14 O'Donnell DE, Ciavaglia CE, Neder JA. When obesity and chronic obstructive pulmonary disease collide. Physiological and clinical consequences. *Ann Am Thorac Soc* 2014; 11: 635–644.

PUBLICATION VI

Relationship between age and bronchodilator response at diagnosis in adult-onset asthma

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Relationship between age and bronchodilator response at diagnosis in adult-onset asthma

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Key words: Adult-onset, asthma, cohort study, bronchodilator response, asthma-COPD overlap, spirometry

Abstract

Background: Possible variation in bronchodilator response (BDR) according to age at the diagnosis of adult-onset asthma is unknown. Our aim was to assess if BDR in FEV_1 is related to age at diagnosis of adult-onset asthma and how many subjects fulfill the 400 mL criterion of BDR, the suggested cut-off for asthma-like reversibility in asthma-COPD overlap (ACO).

Methods: A total of 1030 patients with adult-onset asthma were included; 245 from SAAS (Seinäjoki Adult Asthma Study, Finland) and 785 from COREA (Cohort for Reality and Evolution of Adult Asthma in Korea) cohorts. BDR in FEV_1 at the diagnosis of asthma was assessed. Patients were divided into groups based on age at asthma diagnosis: <40, 40-59.9, and ≥60 years. The cohorts were analyzed separately.

Results: BDR % in FEV_1 did not differ between the groups of different age at asthma diagnosis and no correlation between BDR and age was found. Of patients aged ≥40 years, only 18% (SAAS-cohort) and 5% (COREA-cohort) reached the 400mL BDR in FEV_1. After exclusion of possible ACO patients, the results remained similar.

Conclusion: By using two large cohorts of steroid-naive patients with asthma, we have shown that BDR at diagnosis of asthma is constant over large age span range, and the limit of 400mL in BDR in FEV_1 is rarely reached.

INTRODUCTION

Asthma is a chronic, heterogeneous disease, characterized by airway inflammation and variable bronchial obstruction [1]. Reversibility in FEV_1 of ≥12% and ≥200mL after administration of bronchodilator has been regarded significant, and a key finding when diagnosing asthma [1-3]. However, bronchial reversibility has also been reported in COPD [4, 5], although being usually less than 400 mL in FEV_1 [6]. Asthma-COPD overlap (ACO) is a novel recognized phenotype of airways diseases concerning adult patients, but little is still known about ACO and its diagnostics [6-9]. Symptomatic evaluation has been suggested and spirometric features such as FEV_1/FVC <0.70 and a bronchodilator response of at least 12% or 15% and 400mL have been proposed to be compatible with a diagnosis of ACO in subjects with sufficient smoking history [6, 10-12]. Recently, it has been suggested that a patient with fixed airway obstruction and smoking history compatible with COPD could be considered to have ACO if he/she has either a high reversibility of obstruction (>400mL BDR in FEV_1) or a diagnosis of asthma before the age of 40 years [7]. The revised criteria for ACO have already been criticized since the majority of asthma has been reported to be diagnosed after 40 years of age in women [13-16], and a BDR of ≥400mL in FEV_1 in asthma has been shown to detect predominantly young males [17].

There is, unfortunately, lack of high quality evidence on correct cut-off for BDR to distinguish asthmatics from healthy subjects, and even less is known about the ability of BDR to differentiate between asthma, COPD and ACO [3]. Moreover, smoking among patients with asthma is reported to be nearly as common as among healthy population, up to 26% of asthmatics being smokers [18-20]. This obligates us to pay special attention to the differential diagnostics between asthma, COPD and ACO, especially considering the clinical circumstances where patients have severe symptoms but no previous diagnoses. Previous studies of asthma have usually excluded smoking patients and those with heavy smoking history, and thus, an urgent need for real-life asthma studies including smoking patients has been recognized [6, 20].

In reflection to the proposed criteria of ACO, our aim was to evaluate whether BDR varies with age at diagnosis of adult-onset asthma, and how large proportion of patients fulfil the criterion of 400 mL in BDR, by using data of two, well-described, real-world asthma cohorts.

METHODS

Study population and design

This study presents the results from two different cohorts of adult-onset asthma patients: Seinäjoki Adult Asthma Study (SAAS) –cohort (Finland), and Cohort for Reality and Evolution of Adult Asthma in Korea (COREA, Korea). Results are presented separately, but in a similar way. Patients in both cohorts are divided into three different age groups: 1) <40 years, 2) 40-59.9 years, and 3) ≥ 60 years at asthma diagnosis, respectively.

Seinäjoki Adult Asthma Study (SAAS)

In Seinäjoki Adult Asthma Study (SAAS), 257 patients (≥15 years of age) were diagnosed with new-onset adult asthma during the years 1999-2002 in Seinäjoki Central Hospital, Finland. Diagnosis of asthma was made by respiratory physician, as previously described [9, 21-24]. Majority of the patients were therapy naïve at baseline. Protocol, and the exclusion and inclusion criteria of SAAS have been previously published [21]. A written informed consent was obtained from all patients, and the study protocol was approved by the Ethics committee of Tampere University Hospital, Tampere, Finland (R12122).

In SAAS-cohort, objective lung function measurements were performed on every patient and the diagnosis was based on significant reversibility/variability in obstruction of the airway. BDR of at least 200 mL and 15% from baseline value (after inhalation of 200µg of salbutamol) was considered diagnostic for asthma but diagnosis could also be based on peak expiratory flow (PEF) monitoring, bronchial obstruction in response to challenge with allergen or exercise, or reversibility of obstruction with steroid therapy [21]. In the present study, all patients (n=245) with bronchodilator test performed at the time of diagnosis are included, and cross-sectional data from the diagnostic visit is used. Finnish reference values of spirometry were used [25].

Cohort for Reality and Evolution of Adult Asthma Korea (COREA)

The Cohort for Reality and Evolution of Adult Asthma (COREA) is the first asthma cohort in South Korea since 2005 [16, 26-31]. Patients (aged ≥15 years) diagnosed with asthma by allergists or pulmonologists from 21 centers in diverse areas of Korea were enrolled to the study. In COREA, inclusion criteria were a diagnosis of asthma based on clinical symptoms and either a positive bronchodilator test (200µg of salbutamol) or airway hyperresponsiveness (PC_{20} FEV_1 ≤ 25 mg/ml methacholine). All enrolled participants

signed informed consent. The protocol and design of this cohort were approved by the institutional review board of each center. Of the original 4,846 asthma patients in COREA cohort, our study selected a total of 785 patients who were steroid naïve, and had a bronchodilator test performed at the time of diagnosis. In COREA cohort generally, diagnosis of asthma was based on patients having either BDR of at least 200 mL and 12% in spirometry, or at least moderate bronchial hyperreactivity. Majority of the diagnoses in COREA cohort were based on methacholine challenge test.

In both cohorts, smoking status and history were assessed and smoked pack-years (20 cigarettes per day for 1 year) were evaluated. Levels of blood eosinophils and immunoglobulin E (IgE) were measured, skin prick tests were performed, and the use of steroid medication was recorded by a structured questionnaire.

Statistical analyses

Statistical analyses were performed using SPSS software, version 24 (IBM SPSS, Armonk, NY) or R software, version 3.5.0. Continuous data is expressed as mean ± SD or median and interquartile range, as appropriate. Groups were compared by using one-way ANOVA with Tukey's post hoc test, Kruskal-Wallis test or χ^2–test. Correlation analyses were performed by using Spearman's or Pearson's correlation tests. A *p*-value < 0.05 was regarded as statistically significant.

RESULTS

Clinical characteristics by age in SAAS cohort

There were no differences in gender distribution between the 3 groups with different age at asthma diagnosis, but BMI increased by age (Table 1a). Majority of patients in all age groups were never smokers, and the proportion of current smokers decreased with age. As expected, the number of pack-years (among ex and current smokers) increased by age, being highest in the oldest group. Majority of the patients were therapy naïve at the diagnosis of asthma, with <9 % using steroid medication at that time. Furthermore, there were no differences in the levels of blood eosinophils or immunoglobulin E (IgE) between the groups, although the number of atopic patients was found to be significantly higher in the youngest age group (<40 years) as compared to the older groups (Table 1a).

Table 1a. Baseline clinical characteristics of the 245 patients included from the SAAS cohort

	Age at asthma diagnosis < 40 years n=83	Age at asthma diagnosis 40-59.9 years n=115	Age at asthma diagnosis ≥ 60 years n=47	p-value
Age, years	29.2 ± 7.0	50.6 ± 5.3	68.0 ± 5.3	NA
Gender male	33 (39.8%)	48 (41.7%)	22 (46.8%)	0.733
BMI kg·m^{-2}	25.5 (23.1-30.0)	27.1 (24.3-30.1)	28.7 (26.4-31.6) ǂ	**0.006**
Smoking status				**0.003**
Never smokers	46 (55.4%)	49 (42.6%)	23 (48.9%)	
Ex-smokers	14 (16.9%)	43 (37.4%) ǂ	20 (42.6%) ǂ	
Current smokers	23 (27.7%)	23 (20.0%)	4 (8.5%) ǂ	
Pack-years (of ex/current smokers)	5 (3-18)	15 (7-20) ǂ	24 (10-38) ǂβ	**<0.001**
Steroid medication in use	4 (4.9%)	10 (8.7%)	4 (8.5%)	0.582
B-eosinophils x10*9/L	0.30 (0.19-0.46)	0.22 (0.16-0.40)	0.24 (0.18-0.45)	0.341
IgE kU/L $^{\Omega}$	98 (38-237)	75 (28-145)	71 (21-138)	0.108
Skin prick positive	41 (54.7%)	30 (29.1%) ǂ	6 (14.6%) ǂ	**<0.001**

Data is shown as n (%), mean ± SD, or median (interquartile range). NA= not analyzed, BMI=body mass index, B=blood, IgE= immunoglobulin E

ǂ: as compared to group: Age at asthma diagnosis <40 years p<0.05

β as compared to group: Age at asthma diagnosis 40-59.9 years p<0.05

$^{\Omega}$: data available on 187 patients

Clinical characteristics by age in the COREA cohort

Patients with asthma onset ≥60 years were more often males, and BMI increased with increasing age of asthma diagnosis (Table 1b). Majority of patients in the two groups with asthma diagnosis before 60 years of age were never smokers, but in the oldest group (≥60 years) most patients were ex-smokers. Number of smoked pack-years increased with age at diagnosis, as expected. Blood eosinophil levels and prevalence of atopy were the highest among patients with youngest age at diagnosis of asthma (Table 1b). No differences in IgE levels were found between the groups of different age at diagnosis of asthma (Table 1b). All patients included from the COREA cohort were steroid-naïve at the diagnosis of asthma.

Table 1b. Baseline clinical characteristics of the 785 patients included from the COREA cohort

	Age at asthma diagnosis < 40 years n=245	Age at asthma diagnosis 40-59.9 years n=316	Age at asthma diagnosis ≥ 60 years n=224	p-value
Age, years	36.3 ± 11.6	54.4 ± 7.8	68.9 ± 5.3	NA
Gender male	103 (42.0%)	141 (44.6%)	118 (52.7%)	0.055
BMI kg·m^{-2}	23.3 ± 3.6	24.6 ± 3.5 ǂ	24.6 ± 3.1 ǂ	**<0.001**
Smoking status				
never smokers	116 (48.5%)	171 (55.2%)	94 (42.9%)	**<0.001**
ex-smokers	83 (34.3%)	100 (31.3%)	106 (48.4%)	
current smokers	41 (17.2%)	42 (13.6%)	19 (8.7%)	
Pack-years	4 ± 9	9 ± 16 ǂ	17 ± 24 ǂβ	**<0.001**
B-eosinophils x10*9/L	0.44 ± 0.40	0.33 ± 0.32 ǂ	0.28 ± 0.26 ǂ	**<0.001**
IgE kU/L $^{\Omega}$	422 ± 568	320 ± 576	378 ± 650	0.305
Skin prick positive §	115 (64.6%)	85 (47.0%)	18 (19.2%)	**<0.001**

Data is shown as n (%) and mean ± SD. NA= not analyzed, BMI=body mass index, B=blood, IgE= immunoglobulin E

ǂ: as compared to group: Age at asthma diagnosis <40 years p<0.05

β as compared to group: Age at asthma diagnosis 40-59.9 years p<0.05

$^{\Omega}$: data available on 461 patients. §: data available on 463 patients

Lung function by age in cohorts of SAAS and COREA

In both cohorts, lung function as measured in liters and percentages of predicted value at the time of diagnosis was found to decrease by age (Table 2). In addition, the severity of obstruction, as measured by FEV_1/FVC ratio, increased by age. In contrast, no differences were found between the groups in the diffusing capacity values, which were measured only in the SAAS-cohort (Table 2). Both the cohorts of SAAS and COREA included also smoking patients (ex or current) and therefore some patients could be considered as having ACO. The proportion of possible ACO patients, i.e. subjects with smoking history of ≥10 pack-years and post-bronchodilator FEV_1/FVC <0.7, increased by age. Of the patients in the oldest groups, 22% in the SAAS cohort and 37% in the COREA cohort fulfilled the ACO criteria (Table 2).

Table 2. Lung function and prevalence of ACO in cohorts of SAAS and COREA

	Age at asthma diagnosis < 40 years	Age at asthma diagnosis 40-59.9 years	Age at asthma diagnosis ≥ 60 years	p-value
SAAS cohort				
FEV_1 L post BD	3.34 (2.90-4.17)	2.87 (2.40-3.36) ǂ	2.01 (1.75-2.50) ǂ β	**<0.001**
FEV_1 % pred post BD	90 (84-100)	86 (74-99)	79 (60-89) ǂ β	**<0.001**
FEV_1/FVC post BD	0.81 (0.75-0.87)	0.78 (0.73-0.83) ǂ	0.73 (0.62-0.79) ǂ β	**<0.001**
FVC % pred post BD	95 (88-103)	92 (78-103)	87 (73-98) ǂ	**0.012**
DLco % predicted*	100 ± 20	95 ± 19	92 ± 18	0.093
DL/VA % predicted*	104 ± 19	98 ± 19	97 ± 15	0.106
ACO$^{\mu}$	3 (3.7%)	11 (9.7%)	10 (22.2%) ǂ	**0.004**
COREA cohort				
FEV_1 L post BD	2.74 ± 0.90	2.23 ± 0.69 ǂ	1.70 ± 0.57 ǂβ	**<0.001**
FEV_1% pred post BD	84 ± 21	82 ± 23	75 ± 24 ǂβ	**<0.001**
FEV_1/FVC post BD	0.77 ± 0.13	0.72 ± 0.13 ǂ	0.66 ± 0.15 ǂβ	**<0.001**
FVC% pred post BD	90 ± 16	90 ± 17	84 ± 21 ǂβ	**<0.001**
ACO$^{\mu}$	17 (6.9%)	55 (17.4%)	82 (36.6%)	**<0.001**

Data is shown as n (%), mean ± SD or median (interquartile range). DLco = Diffusing capacity of the lung for carbon monoxide, VA= Alveolar volume

ǂ: as compared to group: Age at asthma diagnosis <40 years p<0.05

β as compared to group: Age at asthma diagnosis 40-59.9 years p<0.05

*Data available from 64 (77.1%), 86 (74.8%) and 33 (70.2%) of patients, respectively

$^{\mu}$ACO: post BD FEV_1/FVC <0.7 and pack-years ≥10

Bronchodilator response by age in SAAS cohort

Bronchodilator reversibility in FEV_1 (absolute change in mL, and change in % from the baseline value) was measured at the time of asthma diagnosis in every patient included in the analysis. No significant differences were found between the age groups in FEV_1 BDR measured either as mL or percentages (Table 3a). In addition, the proportion of patients having high reversibility of obstruction (>400 mL in FEV_1) did not differ between the age groups (Table 3a). The findings remained the same even after exclusion of possible ACO patients (Supplementary Table S1). Furthermore, there were no differences between the age groups in the proportions of patients who fulfilled the reversibility criteria of 200 mL, 12% or both (Table 3a).

Table 3a. Bronchodilator response in FEV_1 at asthma diagnosis by age groups in SAAS cohort.

	Age at asthma diagnosis < 40 years n=83	**Age at asthma diagnosis 40-59.9 years n=115**	**Age at asthma diagnosis ≥ 60 years n=47**	**p-value**
FEV_1 BDR mL	190 (100-330)	130 (60-340)	180 (30-310)	0.266
FEV_1 BDR %	6.1 (3.1-11.2)	5.5 (1.9-12.1)	8.9 (2.1-20.6)	0.293
Patients with >400 mL BDR in FEV_1	15 (18.1%)	23 (20.0%)	6 (12.8%)	0.553
Patients with ≥200 mL BDR in FEV_1	41 (49.4%)	46 (40.0%)	20 (42.6%)	0.415
Patients with ≥12 % BDR in FEV_1	18 (21.7%)	29 (25.2%)	18 (38.3%)	0.109
Patients with ≥200 mL and 12% BDR in FEV_1	18 (21.7%)	29 (25.2%)	17 (36.2%)	0.187

Data is shown as n (%) or median (interquartile range). BDR= bronchodilator response

ǂ: as compared to group: Age at asthma diagnosis <40 years p<0.05

β as compared to group: Age at asthma diagnosis 40-59.9 years p<0.05

Bronchodilator response by age in COREA cohort

Bronchodilator reversibility was higher in patients with younger age at diagnosis when measured as absolute change (mL) in FEV_1, but not when measured as % change from the baseline value (Table 3b). Percentage of patients with absolute (either >400 mL or ≥200 mL) change in FEV_1 was the highest in the youngest group (<40 years). However, no differences were found between the groups in the proportions of patients who fulfilled ≥12%, or ≥12% and ≥200 mL of BDR in FEV_1 (Table 3b). After exclusion of possible ACO patients, BDR in FEV_1 did not differ between the age groups either in mL or in %, and proportion of patients with >400 mL BDR in FEV_1 decreased with age (Supplementary Table S2).

Table 3b. Bronchodilator response in FEV_1 at asthma diagnosis by age groups in COREA cohort.

	Age at asthma diagnosis < 40 years n=245	**Age at asthma diagnosis 40-59.9 years n=316**	**Age at asthma diagnosis ≥ 60 years n=224**	**p-value**
FEV_1 BDR mL	153 ± 268	139 ± 192	101 ± 175 ǂ	**<0.001**
FEV_1 BDR %	7.9 ± 14.1	8.4 ± 12.8	8.6 ± 13.9	0.631

Patients with >400 mL BDR in FEV_1	37 (15.1%)	20 (6.3%) ǂ	9 (4.0%) ǂ	**<0.001**
Patients with ≥200 mL BDR in FEV_1	95 (38.8%)	104 (32.9%)	49 (21.9%) ǂβ	**<0.001**
Patients with ≥12 % BDR in FEV_1	58 (23.7%)	94 (29.8%)	73 (32.6%)	0.088
Patients with ≥200 mL and 12% BDR in FEV_1	55 (22.5%)	79 (25.0%)	46 (20.5%)	0.467

Data is shown as n (%) and mean ± SD

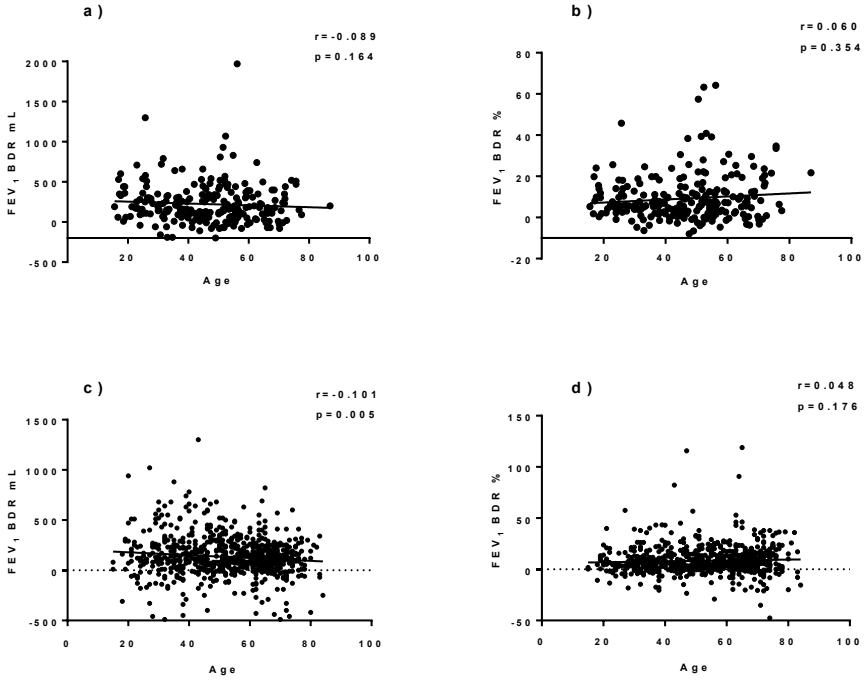
ǂ: as compared to group: Age at asthma diagnosis <40 years $p<0.05$

β as compared to group: Age at asthma diagnosis 40-59.9 years $p<0.05$

Correlation between age at asthma diagnosis and bronchodilator response

To further evaluate the connection between age at asthma diagnosis and bronchial reversibility, correlations were analyzed. No correlation was found between BDR in FEV_1 in % and age at asthma diagnosis in either of the cohorts (Figure 1 b and d). Age at asthma diagnosis and FEV_1 BDR in mL showed statistically, but not clinically, significant negative correlation (i.e. higher reversibility in younger subjects) in COREA cohort (Figure 1c), but not in SAAS cohort (Figure 1a).

Figure 1. Correlations between age at diagnosis of adult-onset asthma and bronchodilator reversibility in FEV_1 a) in mL in SAAS cohort (Spearman's test), b) in percentages in SAAS cohort (Spearman's test), c) in mL in COREA cohort (Pearson's test), d) in percentages in COREA cohort (Pearson's test)



One outlier removed from c) and d). FEV_1=Forced expiratory volume in one second, BDR=bronchodilator response

DISCUSSION

We present here the results on bronchodilator response in patients at the time of diagnosis of adult-onset asthma, as measured in two different, carefully described, clinical cohorts of asthma: the Seinäjoki Adult Asthma Study (SAAS) and the Cohort for Reality and Evolution of Adult Asthma in Korea (COREA). BDR as measured in % in FEV_1 was shown to be similar in different ages of asthma diagnosis. In addition, the majority of patients aged 40 years or older, did not reach the BDR limit of 400mL in FEV_1 at the diagnostic time of asthma.

As previously shown, lung function decreased with age in both cohorts [32-35]. BDR has been previously proposed to decrease with age among general population and healthy persons [32, 33, 36]. A study of

Quanjer et al. evaluated the change in FEV_1 after bronchodilator on obstructive clinical patients (asthma, COPD or ACO), and showed association of BDR with age, height, sex and level of respiratory impairment [36]. The change in FEV_1 was suggested to decline with age, becoming even negative after 50 years of age [36]. Another recent population study on subjects with treated asthma and COPD reported a very limited value of reversibility testing in distinguishing asthma from COPD [37]. In addition, a recent review on BDR in asthma diagnostics stated that the change in FEV_1 after bronchodilator may not be very sensitive tool in asthma diagnostics, and the sensitivity or specificity of any cut-off levels have not been clearly shown [3]. Our study on adult-onset asthma patients showed the relative response to bronchodilator in FEV_1 (% from baseline) to be similar despite the age at diagnosis of asthma, and absolute BDR in FEV_1 (mL) to decrease with increasing age of asthma diagnosis. In keeping with the previous studies, our results thus showed, that BDR in FEV_1 does not increase after age of 40 years. In addition, correlation analyses between age and BDR in FEV_1 did not show clinically meaningful correlation, further indicating that BDR remains stable despite increasing age of asthma onset.

Increasing evidence shows that asthma starting at adult age is very common [14-16]. As compared with child-onset disease, adult-onset asthma patients are less often allergic and have poorer prognosis with low remission rate [24, 38]. At adult age the differential diagnostics between asthma, ACO and COPD becomes essential, because misdiagnosing adult smoking patients' asthma or ACO for COPD may lead to severe morbidity on individual level. However, widely accepted diagnostic criteria for ACO are still missing.

Several COPD guidelines have presented suggestions for ACO criteria [10-12]. Major proposed criteria for ACO among population with COPD have been a significant BDR in FEV_1 (>15 % and >400 mL), sputum eosinophilia and elevated levels of exhaled nitric oxide (FeNO) [10-12]. Further proposal for ACO criteria has included an age cut-off of 40 years: asthma should be diagnosed earlier, or high reversibility in FEV_1 >400 mL should be present [7]. There are, however, different pathways in developing asthma-COPD overlap. The most studied perspective is when a patient has a previous diagnosis of COPD and develops ACO afterwards; a viewpoint widely reflected in the previous guidelines and suggestions for ACO criteria [7, 10-12]. However, ACO may also become diagnosed in patients with previous asthma or, more importantly, in patients without any previous diagnoses. This perspective is only remotely studied, even though the implementation challenges of the previously suggested ACO criteria among general population have already been discussed [13].

Our results showed, that BDR % in FEV_1 does not change with age, and even fewer asthma patients have >400 mL of BDR in FEV_1 at the diagnostic point when age of asthma-onset increases. As partial reversibility of the obstruction is also a feature of COPD, the suggested limit of >400 mL BDR in FEV_1 for asthma-COPD overlap diagnosis after 40 years of age would presumably reduce the overuse of inhaled corticosteroids.

The high BDR cut-off would improve specificity, but on the cost of sensitivity. In practice, this means that a majority of subjects with new onset adult asthma as component of their ACO would have to fulfil this strict criterion of reversibility. In our study, of the patients aged 40 years or older at the time of asthma diagnosis, only 5 % in COREA cohort and 18 % in SAAS cohort fulfilled the limit of BDR >400 mL in FEV_1. That is to say, 82-95 % of the adult-onset asthma patients do not reach the limit of BDR >400 mL in FEV_1. In addition, atopy was shown to decrease with age, in keeping with previous studies [39]. Thus, if using the suggested >400 mL limit in non-atopic patients for asthma-COPD overlap diagnosis, most adult-onset ACO diagnoses would be missed.

In COPD it has been shown that BDR in FEV_1 decreases with increasing severity of COPD [4, 5]. In our study, some patients with smoking history ≥10 pack-years and post BD $FEV_1/FVC<0.7$ could be considered as having asthma-COPD overlap, although the spirometry was measured before the start of the asthma therapy. To avoid bias caused by possible ACO patients having presumably lower response to bronchodilator, we further performed analyses with exclusion of possible ACO patients. The main result remained the same, and thus, our finding is not biased by ACO or COPD.

Major strength of the current study is that we have two large, well defined, real-world cohorts of adult-onset asthma, altogether a study population reflecting clinical reality exceptionally well. The large number of enrolled patients enables us to examine the BDR at the moment of asthma diagnosis in patients over the whole adult-age span, without losing power in analyses. Patients with smoking history are included in the study cohorts, and smoking intensity of the patients is well described. In accordance to the guidelines, the diagnosis of asthma was based on clinical history and objective lung function measurements, and bronchodilator test was measured in every patient. In SAAS and COREA cohorts, however, the diagnostic practices differ slightly from one another. In some patients, the diagnosis of asthma was made based on other objective lung function measurements than positive bronchodilation test, leading to somewhat lower BDR results. This could be considered as a limitation. Despite this, the level of change in FEV_1 after administration of a bronchodilator was similar in these cohorts and the results of both cohorts are in line, increasing the reliability of our results. The reversibility status of individual patients has been shown to vary over time [4, 5]. Thus, another limitation of our study could be that only the BDR at the diagnostic point of asthma was evaluated. However, in our study, most patients were steroid naïve at the diagnostic visit and inhaled corticosteroid medication was started after diagnostic measures. Therefore, evaluating BDR in several time points in our study would not have been informative.

CONCLUSIONS

In conclusion, we have shown that the BDR in FEV_1 at asthma diagnosis is constant over large age span range in adult-onset asthma. In addition, minority of patients with adult-onset asthma have >400 mL BDR in FEV_1 at time of diagnosis. These findings are to be considered when designing diagnostic guidelines concerning asthma starting at adult age, including asthma-COPD overlap.

LIST OF ABBREVIATIONS

FEV_1: forced expiratory volume in one second
FVC: forced vital capacity
COPD: chronic obstructive pulmonary disease
ACO: asthma-COPD overlap
BDR: bronchodilator response
SAAS: Seinäjoki Adult Asthma Study
COREA: Cohort for Reality and Evolution of Adult Asthma in Korea
PEF: peak expiratory flow
$PC_{20}FEV_1$: provocative concentration causing a 20% fall in forced expiratory volume in one second
IgE: immunoglobulin E
BMI: body mass index
DLco: diffusing capacity of the lung for carbon monoxide
DL/VA: diffusing capacity of the lung/ alveolar volume

DECLARATIONS

Ethics approval and consent: All enrolled participants in COREA cohort and in SAAS cohort signed informed consent. The protocol and design of COREA cohort were approved by the institutional review board of each center. The study protocol of SAAS was approved by the Ethics committee of Tampere University Hospital, Tampere, Finland (R12122).

Contributions: M.T, P.I, L.E.T, L.L. H.K.W.,T.-B.K. and H.K. designed the study and wrote the report with input from the other authors. M.T, H.J, and H.K.W. performed the statistical analyses. O.N. contributed to the laboratory analyses. All authors contributed to interpretation of the data. All authors made critical revisions of the manuscript and approved the final version of the manuscript.

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Data availability: All data generated or analyzed during this study are included in this published article (and its Supplementary File). According to ethical permission and patient data-protection laws of Finland, single patient data cannot be made available, but aggregated data is available from authors on reasonable request.

REFERENCES

1. Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention. Updated 2018. http://www.ginasthma.org/. Date last accessed: February 26th, 2019.
2. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, Coates A, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson DC, MacIntyre N, McKay R, Miller MR, Navajas D, Pedersen OF, Wanger J. Interpretative strategies for lung function tests. Eur Respir J. 2005;26:948-68.
3. Tuomisto LE, Ilmarinen P, Lehtimäki L, Tommola M, Kankaanranta H. Immediate bronchodilator response in FEV_1 as a diagnostic criterion for adult asthma. Review. Eur Respir J. 2019;53:pii: 1800904. doi: 10.1183/13993003.00904-2018.
4. Hanania NA, Celli BR, Donohue JF, Martin UJ. Bronchodilator reversibility in COPD. Chest 2011;140:1055-1063
5. Calverley PM, Albert P, Walker PP. Bronchodilator reversibility in chronic obstructive pulmonary disease: use and limitations. Lancet Respir Med 2013;1: 564–73
6. Global Initiative for Asthma and Global Initiative for Chronic Obstructive Lung Disease; Diagnosis of Diseases of Chronic Airflow Limitation: Diagnosis and initial treatment of Asthma, COPD and Asthma-COPD Overlap. Updated 2017. http://www.ginasthma.org/. Date last accessed: August 17th 2018.

7. Sin DD, Miravitlles M, Mannino DM, Soriano JB, Price D, Celli BR, Leung JM, Nakano Y, Park HY, Wark PA, Wechsler ME. What is asthma-COPD overlap syndrome? Towards a consensus definition from a round table discussion. Eur Respir J 2016;48:664-673
8. Postma DS, Rabe KF. The Asthma-COPD Overlap Syndrome. Review. N Engl J Med 2015;373:1241-1249
9. Tommola M, Ilmarinen P, Tuomisto LE, Lehtimäki L, Haanpää J, Niemelä O, Kankaanranta H. Differences between asthma-COPD overlap syndrome and adult-onset asthma. Eur Respir J. 2017;49. pii: 1602383. doi: 10.1183/13993003.02383-2016.
10. Miravitlles M, Vogelmeier C, Roche N, Halpin D, Cardoso J, Chuchalin AG, Kankaanranta H, Sandström T, Śliwiński P, Zatloukal J, Blasi F. A review of national guidelines for management of COPD in Europe. Eur Respir J. 2016;47:625-37
11. Kankaanranta H, Harju T, Kilpeläinen M, Mazur W, Lehto JT, Katajisto M, Peisa T, Meinander T, Lehtimäki L. Diagnosis and pharmacotherapy of stable chronic obstructive pulmonary disease: the finnish guidelines. Basic Clin Pharmacol Toxicol. 2015;116:291-307.
12. Miravitlles M, Soler-Cataluña JJ, Calle M, Molina J, Almagro P, Quintano JA, Riesco JA, Trigueros JA, Piñera P, Simón A, López-Campos JL, Soriano JB, Ancochea J. Guía Española de la EPOC (GesEPOC). Tratamiento farmacológico de la EPOC estable. Arch Bronconeumol. 2012;48:247–257
13. Tommola M, Ilmarinen P, Tuomisto LE, Kankaanranta H. Concern of underdiagnosing asthma-COPD overlap syndrome if age limit of 40 years for asthma is used. Eur Respir J. 2017 Aug 3;50(2). pii: 1700871. doi: 10.1183/13993003.00871-2017.
14. Kankaanranta H, Tuomisto LE, Ilmarinen P. Age-specific incidence of new asthma diagnoses in Finland. J Allergy Clin Immunol Pract. 2017;5:189-191
15. Sood A, Qualls C, Schuyler M, Arynchyn A, Alvarado JH, Smith LJ, et al. Adult-onset asthma becomes the dominant phenotype among women by age 40 years. Ann Am Thorac Soc 2013; 10: 188-197.
16. Park SY, Kim JH, Kim HJ, Seo B, Kwon OY, Chang HS, Kwon HS, Kim TB, Kim H, Park CS, Moon HB, Cho YS. High Prevalence of Asthma in Elderly Women: Findings From a Korean National Health Database and Adult Asthma Cohort. Allergy Asthma Immunol Res. 2018;10:387-396.
17. Appleton SL, Adams RJ, Wilson DH, Taylor AW, Ruffin RE. North West Adelaide Cohort Health Study Team. Spirometric criteria for asthma: adding further evidence to the debate. J Allergy Clin Immunol. 2005;116:976-82.
18. Cerveri I, Cazzoletti L, Corsico A, Marcon A, Niniano R, Grosso A, Ronzoni V, Accordini S, Janson C, Pin I, Siroux V, DeMarco R. The impact of cigarette smoking on asthma: a population-based international cohort study. Int Arch Allergy Immunol 2012;158:175-183

19. To T, Stanojevic S, Moores G, Gershon AS, Bateman ED, Cruz AA, Boulet LP. Global asthma prevalence in adults: findings from the cross-sectional world health survey. BMC Public Health 2012;12:204
20. Thomson NC. Asthma and smoking-induced airway disease without spirometric COPD. Eur Respir J. 2017;49. pii: 1602061. doi: 10.1183/13993003.02061-2016.
21. Kankaanranta H, Ilmarinen P, Kankaanranta T, Tuomisto LE. Seinäjoki adult asthma study (SAAS): a protocol for a 12-year real-life follow-up study of new-onset asthma diagnosed at adult age and treated in primary and specialised care. NPJ Prim Care Respir Med 2015;25:15042
22. Tommola M, Ilmarinen P, Tuomisto LE, Haanpää J, Kankaanranta T, Niemelä O, Kankaanranta H. The effect of smoking on lung function: a clinical study of adult-onset asthma. Eur Respir J 2016;48:1298-1306
23. Ilmarinen P, Tuomisto LE, Niemelä O, Tommola M, Haanpää J, Kankaanranta H. Cluster Analysis on Longitudinal Data of Patients with Adult-Onset Asthma. J Allergy Clin Immunol Pract. 2017;5:967-978.
24. Tuomisto LE, Ilmarinen P, Niemelä O, Haanpää J, Kankaanranta T, Kankaanranta H. A 12-year prognosis of adult-onset asthma: Seinäjoki Adult Asthma Study. Respir Med. 2016;117:223-229.
25. Viljanen AA, Halttunen PK, Kreus KE, Viljanen BC. Spirometric studies in non-smoking, healthy adults. Scand J Clin Lab Invest Suppl 1982;159:5-20
26. Kim TB, Jang AS, Kwon HS, Park JS, Chang YS, Cho SH, Choi BW, Park JW, Nam DH, Yoon HJ, Cho YJ, Moon HB, Cho YS, Park CS; COREA Study Group. Identification of asthma clusters in two independent Korean adult asthma cohorts. Eur Respir J. 2013;41:1308-14.
27. Park SY, Jung HW, Lee JM, Shin B, Kim HJ, Kim MH, Song WJ, Kwon HS, Jung JW, Kim SH, Park HW, Jang AS, Chang YS, Cho YS, Cho YJ, Cho SH, Choi BW, Lee SJ, Jee SH, Choi SK, Won S, Park T, Kim C, Moon HB, Kim TB; COREA investigators. Novel trajectories for identifying asthma phenotypes: a longitudinal study in Korean asthma cohort, COREA. J Allergy Clin Immunol Pract. 2019 pii: S2213-2198(19)30176-X. doi: 10.1016/j.jaip.2019.02.011.
28. Park SY, Jung H, Kim JH, Seo B, Kwon OY, Choi S, Oh B, Kwon HS, Cho YS, Moon HB, Won S, Park T, Kim TB. Longitudinal analysis to better characterize Asthma-COPD overlap syndrome: Findings from an adult asthma cohort in Korea (COREA). Clin Exp Allergy. 2019 doi: 10.1111/cea.13339.
29. Kim TB, Park CS, Bae YJ, Cho YS, Moon HB; COREA Study Group. Factors associated with severity and exacerbation of asthma: a baseline analysis of the cohort for reality and evolution of adult asthma in Korea (COREA). Ann Allergy Asthma Immunol. 2009;103:311-7.
30. Lee T, Lee YS, Bae YJ, Kim TB, Kim SO, Cho SH, Moon HB; Cohort for Reality and Evolution of adult Asthma in Korea study group (COREA study group), Cho YS. Smoking, longer disease duration and

absence of rhinosinusitis are related to fixed airway obstruction in Koreans with severe asthma: findings from the COREA study. Respir Res. 2011;12:1.

31. Park HW, Song WJ, Kim SH, Park HK, Kim SH, Kwon YE, Kwon HS, Kim TB, Chang YS, Cho YS, Lee BJ, Jee YK, Jang AS, Nahm DH, Park JW, Yoon HJ, Cho YJ, Choi BW, Moon HB, Cho SH. Classification and implementation of asthma phenotypes in elderly patients. Ann Allergy Asthma Immunol. 2015;114:18-22.
32. Kainu A, Lindqvist A, Sarna S, Lundbäck B, Sovijärvi A. FEV1 response to bronchodilation in an adult urban population. Chest. 2008;134:387-393
33. Johannessen A, Lehmann S, Omenaas ER, Eide GE, Bakke PS, Gulsvik A. Post-bronchodilator spirometry reference values in adults and implications for disease management. Am J Respir Crit Care Med. 2006;173:1316-25
34. Reed CE. Asthma in the elderly: Diagnosis and management. J Allergy Clin Immunol 2010;126:681-7
35. de Marco R, Marcon A, Rossi A, Antó JM, Cerveri I, Gislason T, Heinrich J, Janson C, Jarvis D, Kuenzli N, Leynaert B, Probst-Hensch N, Svanes C, Wjst M, Burney P. Asthma, COPD and overlap syndrome: a longitudinal study in young European adults. Eur Respir J. 2015;46:671-9
36. Quanjer PH, Ruppel GL, Langhammer A, Krishna A, Mertens F, Johannessen A, Menezes AMB, Wehrmeister FC, Perez-Padilla R, Swanney MP, Tan WC, Bourbeau J. Bronchodilator response in FVC is larger and more relevant than in FEV1 in severe airflow obstruction. Chest 2017;151:1088-1098
37. Janson C, Malinovschi A, Amaral AFS, Accordini S, Bousquet J, Buist AS, Canonica GW, Dahlén B, Garcia-Aymerich J, Gnatiuc L, Kowalski ML, Patel J, Tan W, Torén K, Zuberbier T, Burney P, Jarvis D. Bronchodilator reversibility in asthma and COPD: findings from three large population studies. Eur Respir J. 2019 Sep 5;54(3). pii: 1900561. doi: 10.1183/13993003.00561-2019.
38. de Nijs SB. Venekamp LN, Bel EH. Adult-onset asthma: is it really different? Eur Respir Rev 2013; 22: 44-52.
39. Scichilone N, Callari A, Augugliaro G, Marchese M, Togias A, Bellia V. The impact of age on prevalence of positive skin prick tests and specific IgE tests. Respir Med. 2011;105:651-8