Obstructive lung disease

- Occupational exposures, smoking and airway inflammation

Nicola Murgia

Department of Public Health and
Community Medicine
Institute of Medicine at
Sahlgrenska Academy
University of Gothenburg
Gothenburg, Sweden, 2017

UNIVERSITY OF
GOTHENBURG

Obstructive lung disease
– Occupational Exposures, smoking and airway inflammation

nicola.murgia@unipg.it
ISBN 978-91-629-0055-7 (Print)
ISBN 978-91-629-0056-4 (PDF)
Printed in Gothenburg, Sweden 2017
Ineko AB

Obstructive lung disease

Occupational exposures, smoking and airway inflammation

Nicola Murgia
Department of Public Health and Community Medicine
Institute of Medicine at Sahlgrenska Academy
University of Gothenburg, Sweden

Abstract

Obstructive lung disease is a group of respiratory diseases characterized by airways obstruction. Among them the more frequent are asthma and chronic obstructive pulmonary disease (COPD). Obstructive lung diseases are caused by a complex interaction between environmental exposure (e.g. smoking, occupational, allergens, air pollution) and genetic predisposition. Obstructive lung diseases are usually characterized by airway inflammation. Fractional exhaled nitric oxide (FeNO) is one method to study eosinophilic airway inflammation. Population based survey have been used extensively to study obstructive lung disease; however, some concerns have been raised because their design and methodology. The overall aim of this thesis is to evaluate the effectiveness of a population based survey in Western Sweden to study several aspects of obstructive lung diseases. One aspect is the diagnostic accuracy of questionnaire items in defining airway obstruction by questions regarding diagnosis of COPD and chronic bronchitis symptoms. In this thesis, the sensitivity of these questions in catching airway obstruction was low, while specificity was very high, indicating that participants reporting a medical diagnosis of COPD have a high likelihood of having airway obstruction. Another aspect is the role of subclinical airway inflammation, assessed by FeNO, in predicting obstructive lung diseases. In asymptomatic subjects, high FeNO was associated to new onset asthma and wheezing in a follow-up. Given the importance of FeNO in obstructive lung diseases, this population was also investigated to provide reference values of FeNO, which can be very useful in surveys and clinical practice to discriminate between normal and abnormal findings. Finally, this population was explored to assess potential risk factors for airway obstruction. The results confirmed the role of smoking and atopy as a risk factors, while occupational exposure to vapours, dust, gas and fumes, assessed by a job exposure matrix, seems to play a role especially when coupled to smoking exposure. All the results, despite some limitations, confirm that large population based studies are still useful for exploring different aspects of obstructive lung disease.

Keywords: airway obstruction, COPD, asthma, FeNO, Occupational
ISBN : 978-91-629-0056-4 (PDF); ISBN 978-91-629-0055-7 (Print)

Sammanfattning på svenska

Obstruktiva lungsjukdomar är en grupp lungsjukdomar som kännetecknas av luftvägsobstruktion. Astma och kronisk obstruktiv lungsjukdom (KOL) are de vanligaste bland de obstruktiva lungsjukdomar. Obstruktiva lungsjukdomar orsakas av en komplex interaktion mellan miljö exponeringar (t.ex. cigarettrök, skadliga nämnde i yrkesmiljö, allergener, miljöförstörning) och genetisk ärftlighet. Kronisk inflammation i luftvägarna är en vanlig egenskap i obstruktiva lungsjukdomar. Fraktionerad NO-mättning (fractional exhaled nitric oxide, FeNO) är en metod för att studera eosinofil inflammation i luftvägarna. Populationsbaserad undersökning har använts i stor utsträckning för att studera obstruktiva lungsjukdomar, men det finns flera frågor angående validitet av resultaten på grund av metodologin som använts i såna studier.

Syftet med denna avhandling är att utvärdera effektiviteten av en populationsbaserad undersökning i Västra Sverige för att studera flera aspekter av obstruktiva lungsjukdomar. Ett mål med avhandlingen är att bevisa diagnostisk exakthet av enkätfrågor för att definiera luftvägsobstruktion med ett frågeformulär om diagnos KOL och symptom vid kronisk bronkit. I denna avhandling, frågeformulärets känslighet för att fånga luftvägsobstruktion var låg, medan specificiteten var mycket hög, vilket indikerar att deltagarna som rapporterar en medicinsk diagnos av KOL har en hög sannolikhet för att visa luftvägsobstruktion vid lungfunktion. Ett annat mål med avhandlingen är den potentiella rollen av subklinisk inflammation i luftvägarna, mätt med FeNO, för att förutsäga obstruktiva lungsjukdomar. Hög FeNO kopplades till nydebuterad astma och pip i uppföljningen i försökspersoner utan tidigare symtom. Med tanke på vikten av FeNO i obstruktiva lungsjukdomar, var denna population undersökt också för att definiera referensvärden för FeNO, vilket kan vara mycket användbart i undersökningar och klinisk praxis för att skilja mellan normala och avvikande resultat. Till sist, denna population undersöktes för att bedöma potentiella riskfaktorer för luftvägsobstruktion. Rollen av rökning och atopi som riskfaktorer bekräftades av resultaten, medan yrkesexponering för ånga, damm, gas och rök, bedömda med en yrkesexponeringsmatris, tycks spela en roll, särskilt när den är kopplad till rökning exponering.

Samtliga resultat, trots vissa begränsningar, bekräftar att stora populationsbaserade studier är fortfarande användbara för att utforska olika aspekter av obstruktiva lungsjukdomar.

List of papers

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

I. **Murgia N**, Brisman J, Claesson A, Muzi G, Olin AC, Torén K. Validity of a questionnaire-based diagnosis of chronic obstructive pulmonary disease in a general population study. BMC Pulm Med 2014;14:4.

II. **Murgia N**, Schiöler L, Torén K, Olin AC. Exhaled nitric oxide at different flow rates is a predictor of new-onset wheeze and asthma in a general population. 2016 (submitted)

III. Torén K, **Murgia N**, Schiöler L, Bake B, Olin AC. Reference values of fractional excretion of exhaled nitric oxide among non-smokers and current smokers. 2016 (submitted).

IV. **Murgia N**, Brisman J, Dahlman-Höglund A, Andersson E, Olin AC, Torén K. Occupational risk factors for airway obstruction in a population-based study in Northern Europe (in manuscript).

Content

Abbreviations

ACOS	Asthma-COPD overlap syndrome
ATS	American Thoracic Society
BAL	Bronchoalveolar lavage
BMI	Body Mass Index
COPD	Chronic Obstructive Pulmonary Disease
ERS	European Respiratory Society
FeNO	Fractional exhaled nitric oxide
FEV1	Forced expiratory volume at first second
FVC	Forced vital capacity
GLI	Global lung function initiative
GOLD	Global Initiative for Obstructive Lung Disease
iNOS	Inducible nitric oxide synthase
JEM	Job exposure matrix
LLN	Lower limit of the normal
LR+	Positive likelihood ratio
NHANES	National Health and Nutrition Examination Survey
NO	Nitric Oxide
NPV	Negative predictive value
OR	Odds ratio
PPV	Positive predictive value
VC	Vital Capacity
VGDF	Vapours, gas, dust and fumes
WHO	World Health Organization

1 Introduction

1.1 Definition of obstructive lung disease

Obstructive lung disease is a class of lung diseases characterized by airways obstruction. The more frequent diseases of this category are asthma and chronic obstructive pulmonary disease (COPD); others, less common are bronchiectasis, bronchiolitis and cystic fibrosis. Airways obstruction could also be a feature of other diseases such as sarcoidosis, vasculitis and other autoimmune diseases (1,2).
Obstructive lung disease is one of the leading cause of morbidity and mortality worldwide. Asthma prevalence rates are very high and vary widely in different countries, ranging between 5% to 16% (3). Mortality for asthma has decreased in the last twenty years but, according to WHO, 250000 people still die prematurely for asthma (4). The estimated prevalence of COPD based on the larger epidemiological studies is 11.7% and COPD is the fourth cause of death worldwide, accounting for three million deaths each year (5). Although asthma prevalence seems to have reached a plateau in the western countries and mortality is decreasing (6), it is estimated that, given the trend in risk factors spreading, COPD incidence and mortality will increase greatly in the next future (5).
Cystic fibrosis, even if it is one of the more frequent genetic diseases in western countries, remain a rather rare disease compared to asthma and COPD, however recent improvements in diagnosis and treatment will increase prevalence in the next decades (7). For other obstructive diseases, such bronchiolitis and bronchiectasis, lack of data do not allow to have an accurate estimate of morbidity and mortality, which are usually underestimated (8).
Asthma is defined by the Global Initiative for Asthma as a disease characterized by variable symptoms of wheeze, shortness of breath, chest tightness and/or cough, and by variable expiratory airflow limitation. Both symptoms and airflow limitation characteristically vary over time and in intensity. These variations are often triggered by factors such as exercise, allergen or irritant exposure, change in weather, or viral respiratory infections. Asthma is usually associated with airway hyperresponsiveness to direct or indirect stimuli, and with chronic airway inflammation.
(9). In this definition three key concept are embedded, airway obstruction, typically variable over time, often reversible, exposure to trigger and chronic airway inflammation.

COPD is defined by the Global Initiative for Obstructive Lung Disease (5) as a common, preventable e treatable disease characterized by persistent respiratory symptoms, airflow limitation due to airway and/or alveolar abnormalities usually caused by the exposure to noxious particle and gases (5). As for asthma, also in this definition the three key concept mentioned above are present, making the two diseases closer than usually is thought. As a matter of fact, the so called "Dutch hypothesis", since 1961, proposed that asthma and chronic bronchitis could be seen as different manifestation of the same disease, a chronic non-specific respiratory disease. Despite some criticism, this theory is still debated today, especially in the last years, when the trend is to define different phenotypes of obstructive lung diseases, toward a better prevention and treatment of them. For this reason, having a more comprehensive view of this subject become actual and grey areas and controversial concepts, as the asthma-COPD overlap syndrome (ACOS), become matter of discussion worldwide (10).

1.2 Airway obstruction

One key concept which characterized both diseases (asthma and COPD) is airway obstruction. Airway obstruction is a multifaceted phenomenon which finally produce airflow limitation and consequently reduction of blood oxygen partial pressure and, in some stage of the process, raise in blood carbon dioxide. Airway obstruction is a result of a mixed effect of three moments. One moment is bronchial smooth muscle contraction, usually mediated by the activation of β2 adrenergic receptor and airway muscarinic receptors, as the M3 receptor (11). Another important moment is airway inflammation which leads mainly to mucosal edema and mucus secretion. The type of airway inflammation is often matter of debate in the scientific community, in asthma inflammation is frequently eosinophilic and the response is mainly Th2 type, while in COPD neutrophilic inflammation is usually predominant, with T CD8 lymphocyte infiltrates (12). However, many reports highlighted the presence of a specific eosinophilic phenotype in COPD and a neutrophilic phenotype in asthma, often associated to a more severe and longstanding form of asthma (13). These characteristics, which can be perceived as confusing at a first stage, are the demonstration of the complexity and the interaction of the two diseases. The third moment, commonly present in COPD and Asthma, is airway remodelling, a complex phenomenon characterized by aberrant repair of the epithelium and accumulation of fibroblasts and myofibroblast, which contribute to extracellular matrix deposition, resulting in fixed bronchial obstruction (14).
Since XIX century spirometry is the main tool to measure lung volumes, flows and thus airway obstruction. Even if many techniques have been made available

to study airways physiology and pathology, such as analysis of induced sputum, fraction exhaled nitric oxide, exhaled breath condensate, oscillometry, etc. spirometry remain the most widespread and reliable way to measure airway obstruction. The most common parameters used to study airway obstruction are the forced expiratory volume at the first second (FEV1), the vital capacity (VC) and their ratio (FEV1/VC). In fact, despite the availability of many other parameters, sometimes very useful as the residual volume, or quite unclear as the FEF 25-75, FEV1 and VC are still the most widely used for clinical decision making on obstructive lung disease (15).

To define airway obstruction by these parameters there is a need of normal reference values to compare with. Reference values are usually made by non-smoker healthy subjects form the general population. Historically many attempts have been made to obtain a reference equation which can be used worldwide, the last one was the introduction of the global lung function initiative (GLI) equations in 2012 which consider also older subjects than in the past ERS equations (16), however the proposed adoption of GLI equations worldwide upraise some criticism, especially in Northern Europe (17), where a country-based reference equations were proposed to fit better well known ethnic differences (18).

The adoption of a reference equation will allow to express the FEV1 and the other parameters as a percentage of the predicted value. Moreover, after adopting a reference equation, another issue will raise, the definition of obstruction.

In the past, in epidemiological studies and in clinical practice a fixed percentage of the proposed parameters (FEV1/CV ratio, FEV1) was used to define and grade airway obstruction. The parameter adopted and the percentage varied over time and it was different by region/country. A good example of this classification is the definition given by GOLD od airway obstruction which classified as obstructed every subject, regardless the age, with a FEV1/VC ratio, after pharmacological bronchodilation, under 0.70. This way of classifying obstruction is still in use in research and clinical practice, however it upraises a lot of criticism among lung function experts, because it will not consider the physiological lung aging, yielding to a misclassification of patients with obstructive lung disease, especially COPD (19).

For this reason, the main western respiratory scientific respiratory societies, the American Thoracic Society (ATS) and the European Respiratory Society (ERS) proposed a definition, adopted worldwide, which define a subject obstructed when the FEV1/VC ratio falls under the lower limit of the normal (LLN), which is the fifth percentile of the reference population distribution (20).

1.3 Airway inflammation

Another key concept in obstructive lung disease is airway inflammation, which is not just a contributor of airway obstruction, but also a more complex phenomenon implicated, for example, in systemic effect of obstructive lung disease (21). From the clinical point of view, one of the typical symptom of airway inflammation is chronic cough, which is usually dry in asthma and productive in COPD. In this last case, if it last more than 3 months in a row and 2 years consecutively, it fulfils the criteria of "chronic bronchitis". However, chronic bronchitis could be present without airway obstruction, while it could possible to have airway obstruction without chronic bronchitis symptoms (5). These features could generate some confusion in COPD diagnosis and classification, especially in primary care, and this could explain COPD underdiagnosis by general practitioners (22,23).
In these last years, the mechanism beyond airway inflammation in asthma and COPD have been studied deeply, especially because of the availability of new target therapies for patients with a more severe disease, not responding to common treatments. For asthma, these studies focused mostly on the interaction between innate, now recognized as a pivotal factor in starting /maintaining inflammation, and adaptive immunity with the two know distinct pathways Th2 and Th1, and the involvement of Th17 (24). As a results of these types of inflammatory response it would be possible to assign the subject with the disease to different inflammatory phenotypes, the eosinophilic one, where eosinophils are predominant in airways and retrievable by bronchoalveolar lavage (BAL) or induced sputum and a neutrophilic form, where the predominant cells in respiratory media (BAL or sputum) are neutrophils. Eosinophilic inflammation can be associated with the whole range of disease severity, from mild-to-moderate to severe uncontrolled disease, while neutrophilic inflammation occurs more frequently in more severe asthma (25).

A simplified summary of airway inflammation in severe asthma, but applicable also to mild-moderate disease, is displayed in Figure 1.

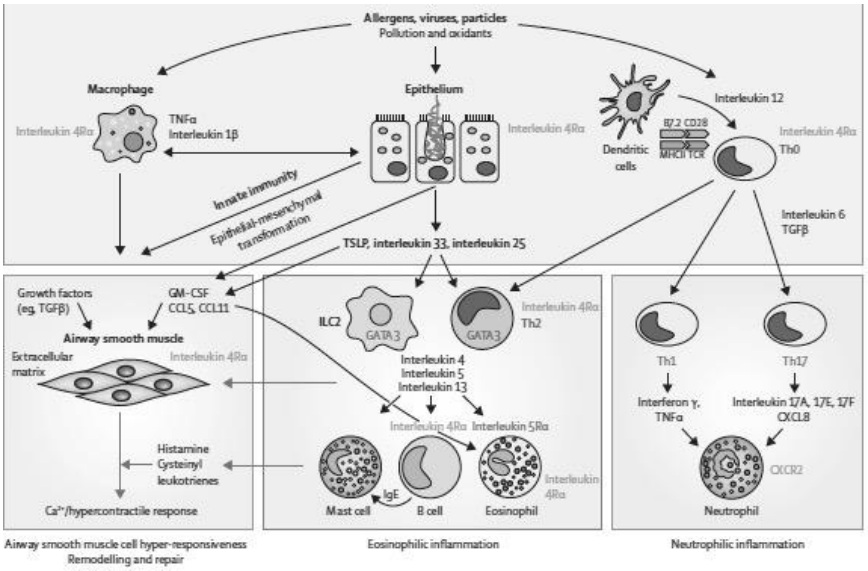

Figure 1. Re-used from Chung KF (24), with the permission of the publisher.

For COPD, mechanisms underlying the pathogenesis of the disease are still more unclear, because the heterogeneity of the disease. While in the past COPD and one of the possible its clinical feature, chronic bronchitis, were seen as an unspecific chronic inflammation triggered by different stimuli (smoking, infections, environmental exposure), in this last decade more attention was paid to ascertain the complex interactions between innate and adaptive immunity in COPD. As a matter of fact, beside the innate inflammatory burst, mediated by reactive oxygen species, proteolytic enzymes and generic pro-inflammatory chemokines, an activation of the adaptive immune system, mediated through the Th1 and Th17 pathways, was demonstrated (26). More details on innate and adaptive response in COPD are provided in Figure 2.

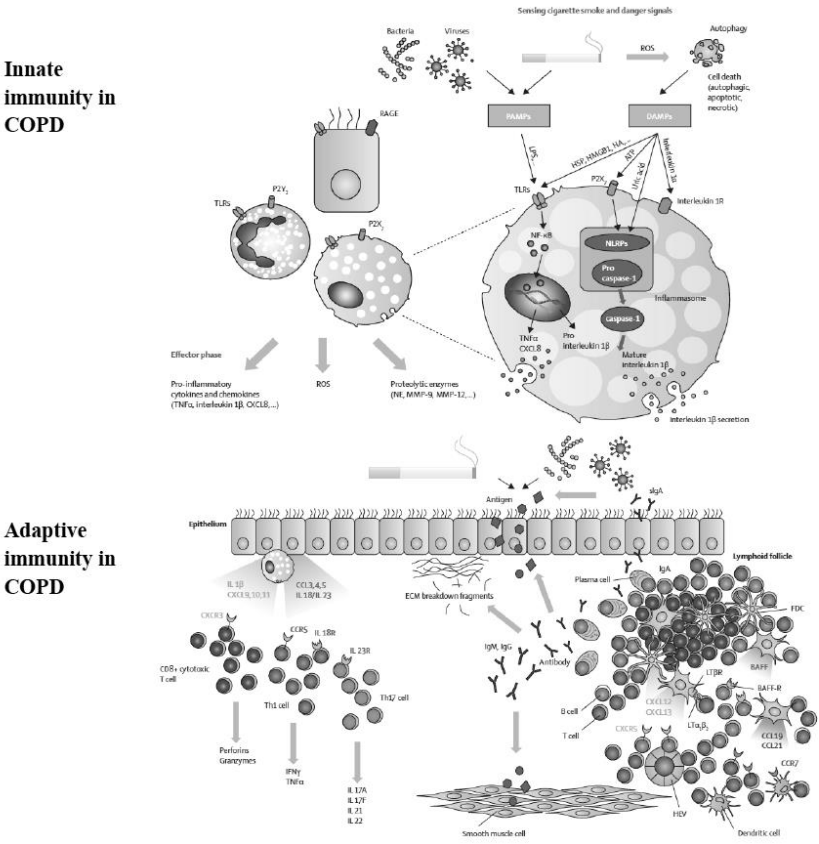

Figure 2. Re-used from Brusselle GG (26), with the permission of the publisher.

This view of the inflammatory cascade is valid for the more typical expression of COPD, where the Th1 and Th17 are the key patterns and neutrophils are predominant. However, in 10-40% of patients with COPD eosinophils could play a relevant role, and it has been recognized a specific eosinophilic pattern of COPD, characterized by a better response to inhaled corticosteroids and the presence of eosinophils in airways and blood (27).

1.3.1. Methods to assess airway inflammation

Airway inflammation can be evaluated sampling directly the airways or other biological media for biomarkers of airway inflammation. Beside bronchial biopsy,

very accurate but difficult to obtain in the routine clinical practice, there are many techniques used to have information about inflammation in the lung. BAL analysis, as bronchial biopsies requires bronchoscopy which is rather invasive and difficult to apply in routine clinical setting, even if remain one of the more important diagnostic tools in the subgroup of patients with refractory asthma, to ascertain whether the treatment was not successful (28).
Another, less invasive, technique, maybe the more informative after biopsies and BAL, is the analysis of induced sputum. Induced sputum has been used to characterize and phenotype obstructive lung disease. In asthma, differential cells count in induced sputum is a well-known diagnostic tool (29) and in the recent years it has been used a lot in clinical practice to monitor the disease control and the response to the treatment (30). In asthma research, induced sputum is used to define asthma endotypes by sputum inflammatory mediators analysis to better characterize different inflammatory pathways (31).
Induced sputum analysis has been recognized useful also in characterizing COPD inflammation (32). However, induced sputum is not completely non-invasive.
Blood eosinophilia, rather common in asthmatic subjects, have been used extensively, especially in the last years, to define a specific eosinophilic phenotype in COPD, which seems more responsive to inhaled corticosteroids (33).

1.3.1.1 Fractional exhaled nitric oxide (FeNO)

Fractional exhaled nitric oxide (FeNO) is a complete non-invasive biomarkers used in obstructive lung disease, in particular asthma.
Nitric oxide (NO) is an important molecule acting mainly as a vasodilator, but also as signalling/killing molecule in inflammation. Nitric oxide is produced in the whole body by specific enzymes named nitric oxide synthase (34). In the lung, nitric oxide plays an important role in vascular endothelial function and thus in pulmonary hypertension (35), but it is also released during airway inflammation as other nitrogen species, producing cell damage and enhancing inflammation, mainly through the production of reactive nitrogen species, such as peroxynitrite and the conversion of GTP in cGMP (36). Nitric oxide, produced endogenously in inflammation process, come mainly from inducible nitric oxide synthase (iNOS), which is overexpressed in asthma (37). In 1991, for the first time, the presence of exhaled nitric oxide in humans was demonstrated (38). Since that many article have been published about the use of exhaled nitric oxide as a biomarker of airway inflammation, including a joint official statement of ATS/ERS (ATS 2005) and an ATS clinical practice guideline (39). FeNO reflects mainly eosinophilic airway inflammation (40) and is correlated to sputum eosinophils, but not with lung function testing (41). The actual proposed use of exhaled nitric

oxide is monitoring obstructive airway disease in non-smoker, in fact current smoking is a strong inhibitor of iNOS (42) also in the case of environmental tobacco smoke (43). In former smokers FeNO concentration are not different from non-smokers after one year they quit smoking (44). Another strong inhibitor of FeNO are inhaled corticosteroids (45). These findings explain some limitation of FeNO as biomarker of obstructive lung disease, which can be used in particular in non-current smoker with a known eosinophilic inflammation (mainly asthmatics). However, the feasibility, the possibility to perform easily many measurement during the same day makes FeNO a good candidate biomarker for asthma treatment monitoring and, recently it has been proposed as a useful tool to discriminate patients with asthma-COPD overlap syndrome from those with COPD (46) and to assess the response to inhaled corticosteroids in COPD patients with an eosinophilic phenotype (47). As a matter of fact, a FeNO guided treatment is able to reduce exacerbation in asthmatics (48).
Procedures to standardize the collection of FeNO have been made long ago (49) and nowadays all the equipment are compliant with these standards. FeNO can be collected at different flow rates, the more used and known is 50 ml/s and therefore in this case FeNO is often expressed as FeNO50. Other flow rates have been used more seldom and may reflect distal airway production (50). ATS produced also a clinical practice guideline to interpret FeNO50 values in adults and children. For this recommendation, a, value of FeNO50 over 50 ppb is suggestive of eosinophilic airway inflammation, while a lower value between 25 and 50 ppb should be seen cautiously (39). Moreover, high concentration of FeNO50 have been recognized as predictor of wheeze in longitudinal studies (51). Furthermore, even if ATS statement does not recommend the use of reference values (39), differences in gender, age and ethnicity, especially in younger subject, pushed researchers to produce reference ranges for exhaled nitric oxide worldwide (52, 53, 54, 55).

1.4 Risk factors of obstructive lung disease

Risk factors for obstructive lung disease are many and may vary between different diseases. Some of them, such as smoking, occupational and environmental exposure, with different burden, could be cross sectional through the more common obstructive lung diseases (asthma and COPD).

1.4.1 Smoking

Smoking is the main risk factor for chronic obstructive lung disease (5) and a very important risk factors for asthma, especially if the exposure occurs in childhood,

both for mainstream or environmental tobacco smoke (56). The relationship between smoking and asthma was quite controversial and probably underestimated in cross sectional studies, while in recent longitudinal studies smoking has been recognized as a strong risk factors also for asthma (57). One explanation of many negative population based studies could be the so called "healthy smoker effect" that would select smoking habits by disease status, in other words a subject with childhood asthma because its disease would be less likely to start smoking (58). However, the same previous longitudinal study fails to show a healthy smoker effect (57), while in other this theory was proposed to explain the lack of association between smoking and asthma (59). Furthermore, the effect of maternal smoking on asthma incidence have been clearly demonstrated and there is increasing evidence that also the maternal exposure to environmental tobacco smoke could raise the incidence of asthmatic symptoms (wheeze) in childhood (60).
Even if the role of active smoking in COPD is clearly demonstrated, just recently the positive effect of smoking cessation was highlighted. Smoking cessation seems to be very effective in reducing the progression of COPD and mortality in the approximately 50% of current smokers with COPD (61).
In the other hand, smoking cessation in asthmatics could have beneficial effects (stopping disease progression toward ACOS, ameliorating symptoms and exacerbation rate) (62).
The pathogenesis of smoking induced airway damage and inflammation are now quite clear, while is still not completely clear the role of smoking in asthma pathogenesis. Recent evidences suggested a role of some compounds in cigarette smoke, such as polycyclic aromatic hydrocarbons, which are able to modulate the immune response polarizing immune system toward a stronger Th2 response (62). If the exposure to cigarette smoke will persist over time, some evidence suggests that also a Th1 response could be elicited, bringing to a more neutrophilic inflammation and thus to COPD and ACOS (63). The importance of smoking in asthma is now recognized and a phenotype of "smoking asthmatics", characterized by poorer asthma control, resistance to inhaled corticosteroids and accelerated lung function decline, have been created (64).
Nevertheless, the role of the interaction between smoking and other risk factor for obstructive lung disease remain still not completely clear.

1.4.2. Occupational exposure

The role of occupational exposures in causing asthma is already established and the population attributable risk of work related asthma, calculated in 2009, was 16.3% (65). Many agents are implicated in the pathogenesis of work-related asthma. Usually they can be divided in those acting with the involvement of the

immune system and those acting mainly as irritants. Among the first group is possible to recognize the so called high molecular weight agents (flour, enzymes, latex, etc.) which are causing asthma through an IgE mediated hypersensitivity, and the low molecular weight agents (isocyanates, acrylates, wood dust, etc.), which, apart from few exceptions (platinum salt, acid anhydrides, obeche wood, etc.) are causing asthma by a more complex immunological mechanism, not yet fully identified (66). Moreover, other occupational agents could cause work-related asthma for their generic irritant properties (cleaning agents, inorganic dust and fumes, etc.) capable to trigger an asthmatics response both in previously asthmatic patients, with bronchial hyperreactivity or in subjects never experiencing asthma (67). In this last group, the event triggering asthma is usually an acute exposure to high concentration of irritant substances, bringing to the so called reactive airways dysfunction syndrome (68). Work related asthma is usually divided two groups, the first group is occupational asthma, directly caused by a clear workplace exposure in subject usually non-asthmatics prior the work, in this category fall mostly work-related asthma forms with an immunological mechanism. The second group is represented by work-exacerbated asthma, where the occupational exposure could worsen the disease, but asthma is generally pre-existent, in this category fall other occupational risk factors, such as irritants, environmental tobacco smoke, etc. (67).

Occupational exposures are also an important risk factor for COPD and chronic bronchitis. Since the 19th century some authors reported that occupational exposures to gas, dust and fumes could cause airway obstruction and/or chronic bronchitis. Analysis of mortality showed also in the last century that workers with dusty occupations had an increased mortality due to ”bronchitis”. Furthermore, Fletcher wrote 1958 that ”men who work in dusty trades, especially coal miners, have higher prevalence of symptoms of bronchitis and emphysema….” (69). However, during the following decades the focus about risk factors for COPD or chronic bronchitis, was almost only on tobacco smoke as a risk factor, and the role of occupational exposures were not take into account as it was before. After 30 years, some new study highlights the importance of occupational exposure in causing deterioration of lung function tests, associated with dust exposure (70). Afterwards, given the reduction of smoking rates in western countries, a new interest in the role of occupational exposure in COPD causation raised up and during the last 20 years many studies, mostly positives, have been published on this topic. This allowed to produce systematic reviews which have concluded that occupational exposure to gas, dust and fumes impaired the pulmonary function and/or increased the risk for COPD (71,72). Given one of this review, PAR for COPD or chronic bronchitis is 15%(71).

During the last 10 years, additional studies have been published, supporting the association between occupational exposure to gas, dust and fume and development of COPD, this evidence was evaluated in depth recently by other systematic reviews (73,74).
In the latest review (74), risk factors for occupational COPD have been identified and classified. Exposure to coal mine dust, silica, asbestos, refractory ceramic fibers, flour, endotoxin, cadmium, carbon black, agricultural dusts (from poultry, animal and arable farming products and practices), dusts from rubber, cotton, wood, iron/steel and smelting, welding, fumes, isocyanates and other chemicals have been classified as risk factors for work related COPD. Occupation at risk of COPD by this review are farmers, cotton workers, welders, painters, railroad workers, coal miners and underground workers, carpenters, metal workers, construction workers, cement factory workers and gold miners.
In a more recent paper, considering the job title of a large sample of the general population (around 200000 subjects), also cleaners showed a rather high risk of developing COPD (75).
One open problem in all the study regarding COPD and work is the exposure assessment. In cohort studies, if the cohort is quite homogeneous (e.g. workers from the same factory), it could easier to obtain a cumulative exposure, in cross-sectional studies, especially population based studies, exposure assessment is based usually only on questionnaire answers. For this reason, beside a self-reported exposure to noxious agents, such as vapours, gas, dusts and fumes (VGDF), researchers ask the participants to report all their lifetime occupational history, which will be translated in job titles, ready to be used with a job exposure matrix (JEM). A JEM is a table where experts, in fair agreement, assign for each job title the likelihood of the exposure to a known agents/group of agents, founding their decision on the scientific evidence and their own experience. In COPD studies some specific job exposure matrix have been used, such as ALOHA JEM (76), ECOJEM (77) and the NIOSH JEM (78). Unfortunately, even if more objective than self-reported exposure, also JEM can be biased, especially when the same JEM is used across the countries, regardless of specific nation-based exposure patterns.
Beside exposure assessment, in work related COPD studies there is still a lack of data regarding dose-effect relations and interaction between occupational exposure to pollutants and tobacco smoke.

1.4.3. Other risk factors

Risk factors for obstructive lung disease more than smoking and workplace exposures are very many.

For asthma, an important risk factor is the complex genetic background which interacts with environmental exposures (79). Other risk factors for asthma, acting usually at early stage of life are a family history of asthma, low birth weight, economic and social disparities during childhood but also in adulthood, respiratory infections, resulting in wheezing (e.g. caused by syncytial virus) during childhood, overweight and obesity, nutrient/vitamin deficiency, urbanization, lack of rural exposure during childhood, exposure to dampness and biomass exposure (56).
One of the more important risk factor for asthma is atopy, especially in childhood asthma (80). Factors usually associated with asthma are gender and age, in an age dependent manner (56). Chronic rhinitis is also associated with asthma and if allergic usually come first (81). All these factors are playing mainly in young life, but some of them could also work lately in life as a risk factor of adult-onset asthma, in which obesity, psychosocial factors (depression), rhinosinusitis, smoking, occupational exposures, lower respiratory tract infections, sex hormones, alcohol are the main risk factors (82).
COPD has been considered for many years a disease or the elderly caused mostly by smoking. Recent evidences have shown that events early in life, as well genetic background, could play an important role in disease development and progression. As a matter of fact, having narrower airways in childhood predispose to COPD development late in life and factors that would influence airways are similar to those encountered in asthma: atopy, bronchial hyperresponsiveness, maternal exposure to smoking and environmental risk factor, preterm birth, respiratory tract infections, indoor and outdoor environmental exposures, including passive smoking (83).
Beside smoking, occupational exposures and factors acting early in life there is a group of factors, that would contribute in the pathogenesis of COPD. In this group, the exposure to outdoor and indoor pollution, especially from burning biomass indoor is one of the more important factor (83).

2. Aims

The overall aim of this thesis is to explore if a population-based study, mainly cross-sectional, is effective in evaluating the epidemiology of obstructive lung disease, airway inflammation and risk factors for airway obstruction.
Specific aims are:

- Evaluate the diagnostic accuracy of questionnaires in defining subjects with airway obstruction (paper I)
- Explore the possibility that elevated FeNO at the baseline could predict future wheezing and asthma (paper II)
- Set-up reference values for FeNO in the Western Sweden population (paper III)
- Assess risk factors for airway obstruction in Western Sweden population, focusing on smoking and occupational exposures (paper IV).

3. Population and methods

3.1 Population

This thesis is based on data of the ADONIX/INTERGENE cohort (ADult-Onset asthma and exhaled NItric oXide/INTERplay between GENEtic susceptibility and environmental factors for the risk of chronic diseases in West Sweden), a pooled cohort, studied between 2001 and 2008 by a baseline questionnaire, baseline lung function tests, baseline measurement of IgE for several allergens, baseline measurement of FENO at different flow rates and a follow-up questionnaire after 5 years from the baseline. The study was approved by the Ethical Committee of Göteborg University (No. 237/2000) and all subjects gave their informed consent. The total population enrolled in the study was a general population sample of 6685 subjects, men and women, 25–75 years old, randomly selected from the population register in Göteborg, Sweden, in 2001. To all of them a postal questionnaire and an invitation to undergo a clinical examination was sent. Because different aims and therefore study methods and exclusion criteria, population enrolled in different sub-studies will be described separately.

3.1.1 Study population –Paper I

The study population was recruited in 2001. 4520 subjects answered the key question on physician diagnosed COPD, introduced just in 2004 and have performed correctly a spirometry. Respondents (n=315) with physician-diagnosed asthma were excluded, as well the 21 not answering the question regarding asthma. Another 292 subject were excluded due to missing information about spirometry and smoking habits, yielding a final number of 3892 study subjects.

3.1.2 Study population –Paper II

The study population comprised subjects of ADONIX/INTERGENE cohort with complete anthropometric data, smoking data, spirometry data and FeNO levels at the exhalation flow rate of 50 mL/s and 270 ml/s. Subjects with affirmative answer to one or more items about asthma, physician-diagnosed asthma, wheeze, asthma symptoms (wheeze, cough, or dyspnoea), inhaled corticosteroids treatment, physician diagnosed COPD or chronic bronchitis symptoms at baseline were excluded. Participants were followed-up after four years for the presence of new

onset wheeze or/and asthma, the response rate to the follow-up questionnaire was 85%, resulting in a final study population of 3760 subjects.

3.1.3 Study population – Paper III

The study population comprised subjects of ADONIX/INTERGENE cohort with complete anthropometric data, smoking data, spirometry data and FeNO levels at the exhalation flow rate of 50 mL/s (n=5854). After exclusion of subject with asthma, chronic bronchitis, COPD, and present cold 3378 subjects were considered for the analysis.

3.1.4 Study population – Paper IV

The study population was based on all subjects of ADONIX/INTERGENE cohort who gave information about their occupation by the questionnaire, have answered to questions about smoking habits, asthma and other important covariates and have performed correctly spirometry. The final number of subject analysed was 6153. A subset analysis was performed on 4082 subjects who answered to the question on self-reported exposure to VGDF.

3.2 Questionnaires

A specific baseline questionnaire was mailed to the ADONIX cohort and another one, very similar, was sent to the INTERGENE cohort, allowing finally to merge the cohort together. Just few questions were missing in the INTERGENE population, such as the self-reported exposure to vapours, gas, dust and fumes.
The questionnaire was divided in different sections, one regarding job history and self-reported occupational exposures, one regarding respiratory diseases and symptoms, one regarding smoking habits and environmental tobacco smoke exposure, one regarding pharmacological treatment and finally one part regarding other information, such as educational level.
Key questions in the baseline questionnaire were:

- “Have you been diagnosed by a physician as having COPD or emphysema?”, which defined the doctor-diagnosed COPD category.
- “Have you been diagnosed by a physician as having asthma?”, which defined the doctor diagnosed asthma.
- Respondents reporting cough and phlegm for at least 3 months within 1 year for 2 consecutive years fell into the “chronic bronchitis” category.

- "Do you have or have you ever had asthma?" *or* "Have you been diagnosed by a physician as having asthma?", which defined asthma (paper III)
- "Do you have a cold now?" *and/or* "Do you have a sore throat now?", which defined the category "present cold" Paper III)

The questionnaire also included questions about smoking habits (never, ex, current smoking, years of smoking, cigarettes smoked), exposure to environmental tobacco smoke and other respiratory symptoms such as chronic non-infectious rhinitis and hay fever since the age of 15 (used in paper III to define atopy).
Sex and age were gathered by the Swedish Personal Number.
After four years participants were invited to answer to a follow-up questionnaire containing other key questions, (paper II):

- Wheeze at follow-up was defined as an affirmative answer to the item; "Have you ever noticed wheezing or whistling in your chest during the past five years?".
- Asthma at the follow-up was defined as physician diagnosed asthma or an affirmative answer to the question : "Have you at any time during the last five years had asthma (i.e. periodic or attacks of trouble breathing or shortness of breath. The problems can occur with or without cough and with or without wheezing)?"
- Asthma in the last 12 months at the follow-up was defined by an affirmative answer to the question: "Do you have had in the last 12 months asthma symptoms?"
- Chronic bronchitis at the follow-up was defined as having cough and phlegm for at least 3 months within 1 year for 2 consecutive years

The response rate to the questions regarding paper II was 85%.

3.3 Job exposure matrix

Information about occupation were gathered by the questionnaire and classified according to ISCO-88 (International Standard Classification of Occupations) (84). A specific job exposure matrix (JEM) to evaluate the exposure to vapours, dust, gas and fumes (VGDF) was developed. In details, all occupations were assessed as being exposed or not by one occupational hygienist and the assessments were discussed with three physicians, specialists in occupational medicine until consensus of this board was reached. An exposed occupation was defined based on the assumption that at least half of the subjects with this specific job title should have a probability of being exposed to vapours, or/and gas, or/and dust, or/and fumes.

To further classify the likelihood of exposure each occupation was rated as none, low (intermediate) or high likelihood of exposure, based on scientific literature and the specific experience of the board.
Each subject was then classified by the occupations hold during the entire working life as never exposed to VGDF, ever exposed to VGDF only in occupations characterized by low likelihood of exposure or ever exposed to VGDF in high exposure occupations. To assess exposure duration, starting and stopping years of any single occupation was used. Cut of points for exposure duration were chosen arbitrary in 5, 10, 15 years of exposure since percentiles of duration had different values between those with low and high likelihood of exposure.

3.4 Clinical examination and Spirometry

All the subjects underwent to a clinical examination and a spirometry, which allowed also to calculate, weight, height and thus BMI.
Before the spirometry, subjects were weighed and height was measured with subjects barefoot and wearing light clothes. Spirometry was performed with a dry wedge spirometer (Vitalograph; Buckingham, UK) according to the ATS/ERS standards (85). Forced expiratory volume in 1 second and FVC were expressed in litres. Percentages of predicted values of lung function variables (i.e., FEV_1, and FEV_1/FVC ratio) were calculated using the European Community for Steel and Coal (ECSC)/ERS equation (86) for the paper I, while a specific reference equation from the same population (18) was used in paper II, III and IV. No bronchoreversibility test with short-acting bronchodilators was performed. A FEV_1/FVC ratio <0.7 indicates airway obstruction according to GOLD criteria (5) (paper I and IV) and a FEV_1/FVC <1.645 × residual standard deviation (RSD) below the predicted value was used as estimation of the LLN, which is the criterion used by the ATS/ERS for defining airway obstruction (86,87) paper I, II, III and IV).

3.5 FeNO measurement and blood analysis

FeNO was measured with a chemiluminescence analyser (NIOX, Aerocrine AB, Stockholm, Sweden) calibrated every second week with a certified calibration gas. All procedures were performed in accordance with ATS recommendations (48). In details, the subjects exhaled against a mouth pressure of 5 cm H_2O at 50 mL/s (FeNO50) (paper II and III) and at 270 mL/s (FeNO 270) (paper II) for 10 seconds.

NO was measured between the 6th and 10th second. All measurements were performed in duplicate, and a deviation of not more than 10% between the values was defined as acceptable
All participant underwent to blood sampling. For the purposes of the studies included in this thesis, blood samples were analysed with Phadiatop® (Pharmacia; Uppsala; Sweden). Subjects with Phadiatop class 0 were classified as non atopic and those with class 1 or above as atopic (88).

3.6. Statistical analysis

Since the four paper used for this thesis have different aims, statistical analysis will be described separately.

3.6.1. Statistical analysis - Paper I

Continuous parameters were expressed as mean ± standard deviation (SD), while categorical data were expressed as numbers and percentages. Sensitivity, specificity, positive likelihood ratio (LR+), positive predictive values (PPVs), and negative predictive values (NPVs) have been used to define the diagnostic accuracy of the questions, indicating physician-diagnosed COPD against airway obstruction calculated by GOLD or ATS/ERS criteria, with 95% confidence intervals (CIs). Accuracy has been also assessed in a subgroup of the population >40 years of age, in subjects not reporting wheezing, and in subcategories by gender and smoking habits (never-smokers, former smokers, current smokers). All calculations were performed with SPSS 18.0 (IBM Corp., New York, NY, USA) and Simple Interactive Statistical Analysis **(**SISA) free software (89).

3.6.2. Statistical analysis - Paper II

In this paper the 90th and 80th percentiles for FeNO50 and FeNO270 concentrations at the baseline were used as cut-off values, calculated separately per smoking habits. The distribution of FeNO values was skewed, thus non-parametric test (Kruskal-Wallis) was used to highlight difference in FeNO in different categories. For other continuous variables, such as age and BMI, distributed normally, mean, standard deviation and t-student test were used. Categorical variables were expressed as numbers and percentages and difference studied by Chi-square test. The association between the 90th and 80th percentiles of FeNO values at baseline and onset of wheezing, asthma or chronic bronchitis at follow-up, was examined in logistic regression models adjusting for sex, height, age, smoking habits, airway

obstruction, atopy and rhinitis. The models were also separately run, according to smoking habits, atopy and rhinitis. All calculations were performed with SPSS 20.0 (IBM Corp., New York, NY, USA).

3.6.3. Statistical analysis - Paper III

Continuous variables were expressed as median and 5^{th} and 95^{th} percentile. The FeNO50 levels were not normally distributed, therefore non-parametric tests were used for univariate analyses. FeNO50 was then logarithmic (ln) transformed. Differences in age, height and atopy were significant for both females and males and therefore were introduced in the final nonparametric regressions (QUANTREG procedure in SAS). Non-parametric regression was used to obtain the estimated parameters for median, 5^{th} and 95^{th} percentiles. To test the influence of age, height and smoking on FeNO, we included smoking (former- or never-smoker), height, age and the interactions between smoking status and both age and height in the model. The statistical analyses were performed using SAS (Statistical Analysis System, version 9.3; SAS Institute Inc.; NC, USA).

3.6.3. Statistical analysis - Paper IV

Continuous data were expressed as mean and standard deviation (SD), whereas categorical data were presented as numbers and percentages. Difference in means were assessed by student t-test, while, differences in prevalence were assessed by Chi-square test. Logistic regression models were developed to assess risk factors of airway obstruction, stratifying for ever-smoking. The first model included age, gender, BMI, and exposure to VGDF (none, low, high). The second model was adjusted also for doctor diagnosed asthma, the third also for ETS. All models were replicated in a subset analysis on subjects over 50 years of age. In ever-smokers all models were adjusted also for current smoking. A sensitivity analysis on a subset of smokers reporting correctly information on pack/years was performed. Another model adjusted for age, gender, BMI, ETS in the last 12 months, doctor diagnosed asthma, VGDF exposure duration was used to check the influence of VGDF exposure duration on airway obstruction. A subset analysis was performed on self-reported VGDF exposure adjusting for age, gender, BMI; doctor diagnosed asthma, ETS, and in smokers, for current smoking. All calculations were performed with SPSS 20.0 (IBM Corp., New York, NY, USA).

4. Results

4.1. Epidemiology of airway obstruction in Sweden

In the sample of the Swedish population considered in this thesis with spirometry correctly performed (n=6153), 604 subjects (9.8%) had airway obstruction by GOLD criteria and 685 (10.5%) by ATS/ERS criteria. Those with obstruction were older, with lower BMI, more frequently ever smokers, smoking more than those not impaired and reported more an exposure to ETS in the last 12 months. Patient with doctor diagnosed asthma were more frequently obstructed. Considering the subset of who participated at the study in paper one, were subjects with doctor diagnosed asthma were excluded, prevalence of airway obstruction was lower (Table I, from paper I).

	FEV_1/FVC <0.7 n=366	FEV_1/FVC <1.645 SD below predicted n=163	All N=3,892
Physician-diagnosedCOPD %	5.7	9.8	0.8
Chronic bronchitis %	4.6	7.4	2.3
Women, %	49.2	66.9	52.5
Age (mean ± SD), yrs	58.3±9.5	54.9±10.3	51.7 ± 10.6
BMI (mean ± SD), kg/m^2	25.6±3.9	25±3.8	26.1± 4
Dyspnoea, %	6.3	9.2	6.3
Wheezing, %	24.6	31.3	14.6
Respiratory drugs	4.6	8	1.1
Smoking habits			
Never-smokers, %	29.8	27.6	47.7
Former smokers, %	38.8	35.6	36.2
Current smokers, %	31.4	36.8	16.1
Pack/years (mean ± SD)	22.6±15.5	22.2±13.8	15±12.2

Table 1. Characteristics of the subset of the study population used in paper I.

4.2 Diagnostic accuracy of questionnaire in defining airway obstruction

In the whole population of non-asthmatics subjects specificity and negative predictive value (NPV) of the question regarding physician diagnosed COPD or the questions about chronic bronchitis symptoms were high, whereas sensitivity and positive predictive value were low, especially for chronic bronchitis questions (table 2, from paper I)

		FEV_1/FVC <0.7 (GOLD)		FEV_1/FVC <1.645 SD below predicted (ATS/ERS)	
		Value	95% CI	Value	95% CI
Physician-diagnosed COPD	Sensitiv-	0.057	0.027–0.088	0.098	0.04–0.156
	Specific-	0.997	0.994–0.999	0.995	0.993–0.998
	LR+	16.859	6.894–	21.532	9.226–50.25
	PPV	0.636	0.427–0.846	0.485	0.267–0.702
	NPV	0.911	0.899–0.922	0.962	0.954–0.97
Chronic bronchitis symptoms	Sensitiv-	0.046	0.019–0.074	0.074	0.022–0.125
	Specific-	0.979	0.973–0.985	0.979	0.973–0.985
	LR+	2.244	1.161–4.337	3.520	1.664–7.443
	PPV	0.189	0.086–0.292	0.133	0.044–0.223
	NPV	0.908	0.896–0.920	0.960	0.952–0.968

Table 2. Diagnostic accuracy of the question "Have you been diagnosed by a physician as having COPD or emphysema?" and of self-reported, questionnaire-based chronic bronchitis symptoms to detect airway obstruction in non-asthmatics

The results of the analysis in subgroups by gender, by age >40 years old, and in those who did not report wheezing, were similar.

The sensitivity of the question "Have you been diagnosed by a physician as having COPD or emphysema?" was higher in smokers (0.087 by GOLD, 0.117 by ATS/ERS), compared to never-smokers (0.09 by GOLD, 0,022 by AR

4.3 Predictive value of FeNO for asthma and asthma related symptoms

At the follow-up, after four years, 188 subjects presented new-onset wheeze, 107 reported new-onset asthma or asthma symptoms and 40 new-onset chronic bronchitis. Higher concentrations (over 80th percentile and 90th percentile) of $FeNO_{50}$ and $FeNO_{270,}$ measured at baseline, were predictors of new onset wheezing in models adjusted for atopy, height, age, sex, rhinitis, airway obstruction and smoking at the baseline ($FeNO_{50}$ Table 3; $FeNO_{270}$ Table 4). Stratified models for smoking and atopy and rhinitis showed the highest relative risks for new onset wheezing among never-smokers; subjects with rhinitis showed a higher risk of wheezing when FeNO was higher; atopics had an increased risk of new onset wheeze if $FeNO_{270}$ was higher (Table 3 and Table 4). In all the stratified analyses the adjustment not include the variable of the stratum.

Population	$FeNO_{50}$	
	>90th percentile	>80th percentile
	OR§	OR°
All subjects	1.8 (1.1-2.8)*	1.5 (1.0-2.1)*
Stratified analyses		
Never-smokers	2.4 (1.3-4.4)*	2.0 (1.2-3.3)*
Ex-smokers	1.3 (0.6-3.0)	1.2 (0.6-2.2)
Current smokers	0.4 (0.1-3.0)	0.5 (0.1-2.0)
Atopics	2.2 (1.0-4.8)	2.0 (1.0-4.0)
Non-atopics	1.5 (0.9-2.6)	1.2 (0.8-2.9)
Rhinitis	2.1 (1.2-3.7)*	1.6 (1.0-2.7)
No rhinitis	1.2 (0.6-2.5)	1.1 (0.6-2.0)

*p <0.05; § OR compared to subjects < 90th percentile; °OR compared to subjects < 80th percentile

Table 3: Risk of new onset wheezing by having higher (> 90th and > 80 percentile) concentrations of FeNO50 at the baseline in all and in different strata

Population	$FeNO_{270}$	
	>90th percentile	>80th percentile
	OR§	OR°
All	2.0 (1.3-2.1)*	1.8 (1.2-2.5)*
Stratified analyses		
Smoking		
Never-smokers	3.3 (1.8-5.8)*	2.8 (1.7-4.7)*
Ex-smokers	1.0 (0.4-2.4)	1.0 (0.5-1.9)
Current smokers	0.6 (0.1-4.3)	1.1 (0.4-2.8)
Atopics	3.7 (1.7-8.0)*	3.5 (1.7-7.0)*
Non-atopics	1.4 (0.8-2.5)	1.4 (0.9-2.1)
Rhinitis	2.2 (1.2-3.9)*	2.4 (1.5-4.0)*
No rhinitis	1.5 (0.8-3.0)	1.2 (0.7-2.0)

*p <0.05; § OR compared to subjects < 90th percentile; °OR compared to subjects < 80th percentile

Table 4: Risk of new onset wheezing by having higher (> 90th and > 80 percentile) concentrations of FeNO270 at the baseline in all and in different strata

FeNO values over the 90th percentile at the baseline predict new-onset asthma, in particular in never smokers (table 5 and table 6).

Population	$FeNO_{50}$	
	>90th percentile	>80th percentile
	OR§	OR°
All	1.8 (1.1-3.2)*	1.7 (1.1-2.7)*
Stratified analyses		
Never-smokers	2.9 (1.4-6.0)*	2.3 (1.2-4.5)*
Ex-smokers	1.3 (0.5-3.6)	1.3 (0.6-2.8)
Current smokers	No cases	0.7 (0.1-5.8)
Atopics	1.8 (0.8-4.2)	1.5 (0.7-3.2)
Non-atopics	1.8 (0.8-3.8)	1.7 (0.9-3.1)
Rhinitis	1.8 (0.9-3.7)	1.5 (0.8-2.7)
No rhinitis	1.9 (0.8-4.6)	1.9 (0.9-3.8)

*p <0.05; § OR compared to subjects < 90th percentile; °OR compared to subjects < 80th percentile

Table 5: Risk of new onset asthma by having higher (> 90th and > 80 percentile) concentrations of $FeNO_{50}$ at the baseline in all and in different strata.

Population	$FeNO_{270}$	
	>90th percentile	>80th percentile
	OR§	OR°
All	2.1 (1.2-3.7)*	1.6 (1.0-2.5)
Stratified analyses		
Never-smokers	2.3 (1.1-5.1)*	1.9 (1.0-3.7)
Ex-smokers	1.8 (0.7-4.4)	1.1 (0.5-2.6)
Current smokers	1.9 (0.2-16.0)	1.6 (0.4-5.8)
Atopics	2.3 (1.0-5.5)	1.6 (0.8-3.4)
Non-atopics	1.9 (0.9-4.0)	1.4 (0.8-2.7)
Rhinitis	2.1 (1.0-4.3)	1.6 (0.8-3.0)
No rhinitis	2.0 (0.8-5.0)	1.4 (0.6-3.0)

*p <0.05; § OR compared to subjects < 90th percentile; °OR compared to subjects < 80th percentile

Table 6: Risk of new onset asthma by having higher (> 90th and > 80 percentile) concentrations of $FeNO_{270}$ at the baseline in all and in different strata.

FeNO higher values at the baseline did not predict new-onset chronic bronchitis.

4.4 Reference values of FeNO in Western Sweden

$FeNO_{50}$ concentrations in this sample of the Swedish population were lower in current smokers, compared to non-smokers or former smokers and higher in males than in females. Numbers are presented in table 7 (modified from table 2 of paper 3).

	Females (n=1741)				Males (n=1637)				p value
	n	Median	5th %tile	95th %tile	N	Median	5th %tile	95th %tile	
Never smokers	868	15.7	7.8	35.7	817	19.0	9.0	44.2	<0.0001
Ex-smokers	581	16.3	7.6	35.6	615	18.9	9.2	39.9	<0.0001
Current smokers	292	10.4	4.4	29.4	205	13.2	6.2	34.3	<0.0001
All	1741	15.0	6.6	35.3	1637	18.2	8.2	41.3	<0.001.

Table 7. FeNO values by gender and smoking.

Subjects with atopy have higher levels of FeNO and in non-atopics FeNO increased with age. As a result of quantile regression analysis, constants to predict FeNO values in non smokers, stratified for gender, are presented in Table 8 (From table 4 of paper III).

	Females	**Males**
Intercept	0.818	1.315
Age (years)(A)	0.0121	0.00732
Height (cm)(H)	0.00787	0.00732
Atopy (Yes=1, No=0)(At)	0.189	0.192
Residual standard deviation (RSD)	0.46	0.47
Adjusted R^2	0.09	0.05

Table 8. estimated normal values of FeNO by gender

And the equation to obtain the normal value of ln FeNO50 is
Intercept + (A) × (age in years) + (H)×(height in cm) + At×(atopy:0=No; 1=Yes).
The regression analysis among current smokers was not stable, thus the proposed reference values for current smokers are the outlined 95th and 5th percentiles shown in Table 7. The proposed upper normal value for FE_{NO} is 29.4 ppb for female current smokers and 34.3 ppb for male current smokers.

4.5 Risk factors for airway obstruction

In the study population, subjects with airway obstruction, defined using both the definition (GOLD and ATS/ERS) were older, more frequently ever-smoker, exposed to ETS, asthmatics and underweight. Prevalence of occupational exposures to VGDF and airway obstruction defined by GOLD or ATS/ERS criteria, stratified for smoking habits and asthma are presented in table 9 and table 10 (from table 2 and 3 of paper IV). The exposure is expressed at any level of exposure (Exposure column) and by exposure level.

Non smokers (n=2836)					
Exposure	**GOLD**	**ATS/ERS**	**Exposure Level**	**GOLD**	**ATS/ERS**
Exposed to VGDF by JEM	84/1478 (5.7%)	108/1478 (7.3%)	Only low	75/1354 (5.5%)	96/1354 (7.1%)
			High	9/124 (7.3%)	12/124 (9.7%)
Unexposed	90/1358 (6.6%)	104/1358 (7.7%)		90/1358 (6.6%)	104/1358 (7.7%)
Ever-smokers (n=3317)					
Exposure	**GOLD**	**ATS/ERS**	**Exposure Level**	**GOLD§**	**ATS/ERS**
Exposed to VGDF by JEM	269/2049 (13.1%)	299/2049 (14.6%)	Only low	227/1825 (12.4%)	258/1825 (14.1%)
			High	42/224 (18.8%)	41/224 (18.3%)
Unexposed	161/1268 (12.7%)	174/1268 (13.7%)		161/1268 (12.7%)	174/1268 (13.7%)

Table 9. Prevalence of subjects with airway obstruction and VGDF exposure by JEM, stratified by smoking

Non asthmatics (n= 5659)					
Exposure*	**GOLD**	**ATS/ ERS**	**Exposure Level**	**GOLD**	**ATS/ ERS**
Exposed to VGDF by JEM	303/3252 (9.3%)	350/3252 (10.8%)	Only low	257/2929 (8.8%)	302/2929 (10.3%)
			High	46/323 (14.2%)	48/323 (14.9%)
Unexposed	207/2407 (8.6%)	226/2407 (9.4%)		207/2407 (8.6%)	226/2407 (9.4%)
Asthmatics (n= 416)					
Exposure	**GOLD**	**ATS/ ERS**	**Exposure Level**	**GOLD**	**ATS/ ERS**
Exposed to VGDF by JEM	47/250 (18.8%)	55/250 (22%)	Only low	42/226 (18.6%)	50/226 (22.1%)
			High	5/24 (20.8%)	5/24 (20.8%)
Unexposed	39/166 (23.5%)	45/166 (27.1%)		39/166 (23.5%)	45/166 (27.1%)

Table 10. Prevalence of subjects with airway obstruction and VGDF exposure by JEM, stratified by doctor diagnosed asthma

The results of the regression analysis, for the risk of airway obstruction related to VGDF exposure, adjusted for sex, age, BMI in non smokers is presented in table 11 (extracted from table 4 of paper IV)

Exposure to VGDF	**Smoking NO (n= 2836)**	
	GOLD	**LLN**
	OR (95%CI)	OR (95%CI)
No	1	1
Only low	0.95 (0.68-1.32)	0.93 (0.69-1.25)
High	1.16 (0.58-2.42)	1.31 (0.69-2.47)

Table 11. risk estimates for airway obstruction of have been exposed to VGDF in non smokers.

The adjustment for asthma and exposure to environmental tobacco smoke did not change the results.

In ever-smokers, the results of the regression analysis, for the risk of airway obstruction related to VGDF exposure, adjusted for sex, age, BMI, ever smoking, is displayed in table 12 (extracted from table 4 of paper IV)

Exposure to VGDF	**Smoking YES (n=3317)**	
	GOLD	**LLN**
	OR (95%CI)	OR (95%CI)
No	1	1
Only low	1.09 (0.86-1.37)	1.14 (0.92-1.42)
High	***1.74 (1.15-2.62)***	***1.59 (1.07-2.37)***

Table 13. risk estimates for airway obstruction of have been exposed to VGDF in ever-smokers.

Also in ever smokers the adjustment for asthma and exposure to environmental tobacco smoke did not change the results.
Stratification for age over 50 years did not change significantly the results, even if for those aged > 50 years risk estimates for VGDF exposure were slightly higher.
In smokers, after adjustment for sex, age, BMI, doctor diagnosed asthma, ETS in the last year and current smoking the risk of airway obstruction related to high levels of VGDF exposure was higher among those exposed for more than 5 years (OR 2.09, 95%CI 1.26-3.47 by GOLD definition; OR 2.06, 95%CI 1.26-3.37 by ATS/ERS definition). A subset sensitivity analysis in smokers, adjusting also for pack years, confirmed these findings just on those > 50 years. Using other cut-off points (> 10 years, > 15 years, did not change the results).
Using a stricter definition of airway obstruction (reduced FEV1/FVC ratio and FEV1 < 80% of the predicted value) did not change significantly risk estimates and statistics.
The subset analysis, where the exposure to VGDF was self reported, confirmed that in never-smokers the risk was not significant (OR 1.21, 95%CI 0.78-1.88 by GOLD definition; OR 1.13, 95%CI 0.76-1.70 by ATS/ERS definition), whereas in ever-smokers the risk was significant (OR 1.43, 95%CI 1.09-1.89 by GOLD definition; OR 1.45, 95%CI 1.12-1.88 by ATS/ERS definition)

5. Discussion

5.1 Epidemiology of airway obstruction and diagnostic accuracy of questionnaire to detect airway obstruction

In this population, the prevalence of airway obstruction is similar to other studies performed in Northern Europe and Europe (90,91).
The question "Have you been diagnosed by a physician as having COPD or emphysema?", commonly used in population-based epidemiological studies, in this population had a low sensitivity and high specificity to detect significant airflow obstruction according to both definition of airway obstruction (GOLD and ATS/ERS), as well as a rather high positive likelihood ratio and reasonable positive predictive value. These results suggest that if a subject answer affirmatively to this question, the risk of not having an airway obstruction in this subject is very low. Unfortunately, the very low sensitivity suggests that this question cannot be used to catch all the subjects with airway obstruction. This finding depends mostly the well-known underdiagnosis of COPD by physicians (92). The sensitivity of COPD question in catching airway obstruction is lower than the question "Have you been diagnosed by a physician as having asthma?" in defining asthma cases (93). However, the high specificity could be useful in epidemiological study, because those reporting a diagnosis of COPD or emphysema present a very high likelihood to have an airway obstruction. because asthma is more widely known to patients and physicians than COPD. Other studies have used mixed questions do detect subjects at risk of airway obstruction, showing better accuracy than the single question used in our study, but the specific aim of those studies was just defining a set of questions to define subjects with airway obstruction (95/96), while we had just that question, making any comparison impossible. The accuracy of questionnaire questions in detecting airflow obstruction did not differ between men and women. The question used to detect airway obstruction in this study showed higher sensitivity in detecting COPD in smokers. This result is expected and could be related to the tendency of physicians to make a COPD diagnosis if the patient is a smoker (97). Even if subjects with a diagnosis of COPD overlap poorly with those reporting chronic bronchitis symptoms, the diagnostic accuracy of chronic bronchitis symptoms was similar to the questionnaire question, suggesting that physicians have made the diagnosis of COPD only in the few with chronic bronchitis symptoms who had also a clear airway obstruction, but also in this case questions about chronic bronchitis cannot be used to detect airway obstruction. Given the poor overlap, also a combined mix of questions containing the question on COPD diagnosis and chronic bronchitis symptoms is not useful in this population.

5.2 Role of FeNO in predicting wheezing and asthma

In this thesis baseline FeNO, at different flow rates (50 ml/s and 270 ml/s), predicted the onset of wheezing and asthma after a follow-up period of 4 years. This finding is a confirm of a previous smaller study made in the same population. In that study, given the smaller size of the population, asthma was not considered and data on FeNO at higher flow rate was not available (51).
The association of previous high levels of FENO at high flow rate ($FENO_{270}$) and a further development of wheezing suggests that the early inflammatory changes, leading finally to airway obstruction, would happen also in the distal airways. As a matter of fact, FeNO at higher flow rate, in non-symptomatic, non- obstructed subjects reflects the part of exhaled nitric oxide not influenced by the larger proximal airways (98). Moreover, although wheezing could be expression of an obstruction in any section of the airways, in children it is often a hallmark of non-asthmatics small airways diseases, such as bronchiolitis (99). In smokers, given the strong influence of smoking on iNOS (100), FeNO was not a significant predictor of new onset wheeze or asthma at any flow rate. However, since the effect of smoking could be reversible after quitting smoking habits (44), it could be very interesting in the future, given a larger population, to repeat the analysis separating former smokers from current smokers.
In this study, FeNO is better predictor of wheezing in atopics than in non-atopics, especially at higher flow rates. This a confirm that FeNO is a good predictor of many condition related to atopy, such as allergic rhinitis (101). Adjusting for rhinitis in the regression model reduced the risk that rhinitis could be a confounder of the effect of FeNO on new-onset wheezing and asthma. However,
the higher risk estimates for wheezing in subjects with higher levels of FeNO and rhinitis confirm the interaction between upper and lower airways found in longitudinal studies, also in adults. Higher $FENO_{50}$ is associated to higher risk of more symptomatic new-onset asthma, confirming that higher FeNO is correlated to less controlled eosinophilic asthma (102).

5.3 Reference values for FeNO

In this thesis, given the influence of sex, age and height on FeNO, upper and lower limits of the normal were set up, based on sex, height and age.
The normal values proposed for non-smokers and former smokers were the same, because these two categories showed the same distribution of FeNO levels. The influence of smoking on FeNO was significant, this expected finding and the number of current smokers made the regression model in current smokers very unstable and therefore the proposed cut-off limits were based on the univariate distribution of FeNO. This could be important, since evidence of a role of high level of FeNO in respiratory disease is increasing (103). Even if the ATS document on FeNO interpretation (39), is suggesting to use cut-off points than reference values, this recommendation was defined weak and had a low quality of evidence, because the cut-of points for children and adults were not based on very strong studies. For this reason, defining normal values will help to determine what is not normal, beyond a simple cut-off value. The results indicate that the upper limits of normality in this sample of the general population can be very different, for example, between a young woman without atopy (around 22 ppb) and an elderly man (over 50 ppb). The upper limit of normal calculated in this study are substantially lower than the upper limits for diagnosing eosinophilic airways inflammation, suggested by The American Thoracic Society, which is 50 ppb (39). Because our cut-off limits are based on the upper 95^{th} percentiles, it is normal that our limits are lower. Other studies have assessed reference values for FeNO, the National Health and Nutrition Examination Survey (NHANES) 2007-2010 recommended cut-off values for diagnosing asthma for persons aged 12 to 80 years of 39 ppb; also in this case this value is based on the 95^{th} percentile (39). The methodology used in NHANES was different from the present thesis, and the final regression model included also race, smoking and passive smoking.
From the present results, we can also conclude that there are consistent differences between males and females, which differ from our previous findings based on fewer subjects (51), probably because of the smaller sample size.
In current smokers, we proposed reference values, which, given the influence of smoking on FeNO, was seldom a matter of studies, giving to our findings originality. In our proposed sex-specific fixed cut-off limits based on a univariate analysis, the upper 95^{th} percentile for female and male current smokers was 29.4 ppb and males 34.3 ppb, respectively, meaning that high a FE_{NO} level among current smokers may be of clinical relevant, as previously proposed (103).

5.4 Smoking and occupational risk factors for airway obstruction.

In this thesis, performed on a large sample of the Swedish population, in ever smokers with a high likelihood of exposure to VGDF, the risk of airway obstruction was higher, in particular in older workers. However, in non smokers, an effect of occupational exposure was not observed. These findings are analogous to those reported on a similar smaller population of Norwegians by Bakke et al in early nineties (104). Other studies, collected in recent reviews (73,74), have addressed the role of workplace exposure in synergy with smoking on airway obstruction. In other study, performed in an older population, there was not an association between airway obstruction and VGDF exposure in non-smokers, while in smokers the risk of airway obstruction in those exposed to VGDF was high and almost twice compared to smokers not exposed (105). The interaction between smoking and occupational exposure to VGDF has been already demonstrated to be more than additional (106). Maybe, this finding could be explained by the possible interaction between smoking and occupational exposure in enhancing specific networks, as the matrix metalloproteinases. As a matter of fact, MMP-8 and MMP-9, two important collagenases, are considered two key metalloproteinases in COPD pathogenesis, proposed as smoking induced damage biomarkers in blood and induced sputum (107); recently MMP-8 and MMP-9 have been found elevated in sputum and blood of smelters exposed to fumes and mineral dust, also in non smokers, compared to a control of non-exposed subjects (108). In this study, in smokers, a longer exposure duration at workplaces at high likelihood of VGDF exposure was associated with a higher risk of airway obstruction. Nevertheless, in tunnel workers, heavily exposed to mineral dust, the exposure duration, especially between 10-20 years, more than > 20 years, has a significant impact on the prevalence of airway obstruction (109). This finding, more consistent in older subjects (data nor shown), could help to strengthen the role of occupational exposure in contributing, beside tobacco smoke, to airway obstruction. This finding of the increased risk of airway obstruction in older smokers exposed to VGDF, could suggest that older workers had longer exposure, but also more time for disease progression up to became a clear obstruction at lung function test. Furthermore, older workers could have been exposed to VGDF when exposure levels were probably well above the concentrations which younger workers have experienced in the last decades. In an older study Davison et al. shown that airway obstruction was significantly associated with year when started the exposure to cadmium (pre-1951, 1951-1970, post-1970) (110)

Whereas the risk of airway obstruction, exposed to occupational risk factors, have been demonstrated in other population, another more recent Northern European study, where the exposure was self-reported, the risk of airway obstruction related to VGDF in non-smokers was demonstrated (111). Unfortunately, in our survey just 2/3 of the entire sample answered to the specific question on self-reported VGDF exposure, making arguable this information. Anyway, a subset analysis on those answering to that question, did not revealed an increased risk of airway obstruction in non-smokers self-reporting VGDF exposure. Moreover, in this sub-analysis, in ever smokers, an increased risk of airway obstruction in those reporting VGDF exposure was present, confirming the finding of our previous analysis based on job exposure matrix.

5.5 Validity issues

In this study, only pre-bronchodilator spirometric data was available. Because the GOLD guidelines give a FEV_1/FVC ratio of <0.7 as cut-off point to diagnose COPD, based on post-bronchodilator data, and the American College of Chest Physicians, American College of Physicians, ATS, and ERS define COPD as a disease characterized by an airflow obstruction not fully reversible (112), this could be considered one limitation of this thesis if our results would be used also to study the diagnostic accuracy of the question regarding COPD, bearing to an overdiagnosis of COPD. Nevertheless, in another, similar study, a pre-bronchodilator FEV_1/FVC ratio was used (113) and it is known that the bronchodilator response suffers from a lack of reproducibility (114,115), being influenced by smoking habits and other parameters (116), and failed to discriminate between asthma and COPD (115, 117). The choice of using the European Community for Steel and Coal (ECSC)/ERS equation (86) to calculate LLN could be another limitation, since it will not take in consideration the non-linear decline of FEV1 related to the age. Unfortunately, reference values for this population were not available at the time the study. However, the aim of this study was to assess the diagnostic accuracy of a questionnaire and, to make our results comparable and applicable in clinical and epidemiological practice, we had to rely on those methods still most widely used, despite some limitations.

In the analysis of the predictive value of FeNO, the choice of using the 80^{th} and the 90^{th} percentile to define subjects with higher levels of FeNO is based on our previous experience (51) where 90^{th} percentile was enough to clear cut a higher risk of new onset wheezing. Another cut point was set at 80^{th} percentile, finding almost the same results of the 90^{th} percentile. Another point, reported also in our previous smaller survey, was the choice of the follow-up time. Maybe it would have been better to use several follow-up point, for example yearly, to avoid recall

bias in those with mild wheezing after a rather long times (4 years) who can forget to report mild wheezing episodes. However, it seems very difficult to find a reason why those who are underreporting will differ in FeNO value with those reporting wheezing. The rationale for asking about wheeze the last five years instead of the last 4 years was to catch all new-onset wheeze, even if the subjects may be uncertain of the exact year for the onset. In any case those who were reporting wheezing, asthma-symptoms, asthma at the baseline were excluded, reducing the risk of misclassification.

There could have been some false negative, who not report asthma, asthma-symptoms and wheezing at the baseline. They could influence further analysis if they report new onset wheeze, since wheezing at the baseline is associated with higher FeNO (44). We presumed that this bias would be more prominent for those reporting new onset wheezing the first year after the baseline. For this reason, we performed a separate analysis without those reporting new onset wheeze the first year after the baseline, obtaining the same results with wheezing predicted by FeNO at different flow rates.

The main limitation of the study about risk factors for airway obstruction is the lack of information about other environmental exposure than the occupational.

Unfortunately we had no enough data on modelled exposure to toxic gases or particulate matter. The geocoded information on distance to dense traffic road, used in the same population previously (118), were referred just to current address, whereas airway obstruction could be also related to a more chronic exposure, dated back in the past, when we do not have information.

Another limitation of this thesis in the part regarding causation of airway obstruction is the high number of missing information regarding amount and duration of smoking exposure and self-reported exposure to VGDF. However, the sensitivity analyses regarding these aspects have shown similar results when the analysis have been adjusted for pack/years and similar risk estimates and statistical significance when JEM derived VGDF exposure was replaced by the self-reported one.

Furthermore, another limitation could be considered the use of pre-bronchodilator spirometry to define airway obstruction. It is clear that pre-bronchodilation spirometry could overestimate the number of subjects with airway obstruction. Anyway, the objective of this study was not to identify a pure COPD phenotype, but a broader category of airway obstruction, including also those with a previous physician diagnosis of asthma. As a matter of fact, many of the most important studies considered in the main recent reviews on COPD (73, 74) were done without post-bronchodilator data. Recently, it was demonstrated that pre-bronchodilator airway obstruction can be used to identify the large majority of post-bronchodilator airway obstructions (119).

6. Conclusions

This thesis showed that population based studies, despite some limitations, are still suitable to answer questions regarding the epidemiology of obstructive lung diseases, airway inflammation and causation of airway obstruction.

One main finding is that epidemiological questionnaires, when is used a single question to define COPD, are not useful to define subjects with airway obstruction, because the known underestimation of obstructive lung diseases among the population and the medical community. However, for epidemiological purposes, a subject with a physician diagnosis of COPD reported in the questionnaire has a very high likelihood of airway obstruction.

Another interesting finding is the role of FeNO in detecting early airway inflammatory diseases and airway associated symptoms such as wheezing. It is rather clear that, elevated levels of FeNO have to raise the attention of health care personnel on further eosinophilic airway diseases, even if the subject, at present, is not symptomatic. Therefore, in this thesis, reference values for nitric oxide were set-up, with the definition of abnormal values for our population, also in current smokers, covering the grey area of the use of FeNO in smokers.

Finally, in this population occupational exposure to vapours, dust, gas and fumes, seems to have an effect just when in combination with current or former smoking. This effect was more prominent in those working longer exposed to VGDF.

7. Future perspectives

Large population-based epidemiological studies, despite some limitations and considerable costs, are still very useful to have a picture of the population, which is needed to plan further preventive and therapeutical intervention.
In this viewpoint, follow-up of these large cohorts from rather small catchment areas, such as ADONIX-INTERGENE cohort, are very interesting and could strengthen the role of risk factors in causing disease, adding the longitudinal view that in causation perspective is more robust than cross-sectional design, especially for chronic multifactorial disease. Moreover, follow-up could also give important information regarding intervention made at the population level (e.g. smoking cessation campaigns) or at the individual level (e.g. prevention of an occupational exposure, treatment of a disease).
However, the typical epidemiological approach which was used in this thesis, has to take into account newer methodologies available, such as gene-environment interactions. As a matter of facts, many genes have been associated to a higher susceptibility to respiratory diseases and other chronic diseases. Furthermore, a key point to consider in the development of a chronic multifactorial disease, is also the socio-economical background of the population under study. The socio-economical background is not only different education and wealth status, but also social inequalities, gender issues, language barrier and differences in health care access, which have always to be considered. In this perspective the role of actual and previous occupational exposure is still very important and partially neglected, because of the rapid economic changes in Europe in the last decades, which brought the public opinion and health care providers to consider actual workplace safe. Unfortunately, new occupational risk factors, aging workforce and progressive reduction of surveillance need an immediate action to prevent old and new work-related diseases. Large population based studies could help to highlight this trends, helping to focus preventive intervention.

Acknowledgments

I wish to express my sincere gratitude and appreciation to:

Professor Kjell Torén, my supervisor and my mentor, for his constant and unwavering support, for skillful scientific advice, patience, perseverance, generosity, help and for creating a stimulating scientific environment, which was the base for this thesis and for future collaborations.

Anna-Carin Olin and Jonas Brisman, for valuable collaboration, generous encouragement, constructive and stimulating discussion.

Eva Andersson, for introducing me in the field of respiratory epidemiology and survival analysis, to trust me at early stage of my career and overall to make this thesis possible. Without her my growth as researcher would not be possible.

My other co-authors Björn Bake, Annika Claesson, Anna Dahlman-Höglund, Linus Schiöler for valuable cooperation and stimulating discussion.

All my other colleagues at the Department of Occupational and Environmental Medicine of the University of Gothenburg, for continuous encouragement, discussion and friendly support.

Professor Giacomo Muzi and Professor Giuseppe Abbritti, Heads of the Section of Occupational Medicine of the University of Perugia for their mentorship, the continuous and enthusiastic support in keeping on the collaboration with the University of Gothenburg.

All my colleagues at the Section of Occupational Medicine of the University of Perugia, who supported me and replaced me in clinical and academic duties when I was abroad for my education.

Professor Olav Axelson and my colleagues Said Havarinasab, Per Leanderson and Bengt Ståhlbom, who supported me at early stage of my career in Linköping.

My friend Professor Giovanni Ferrara, for invaluable support, friendship and nice discussion on football, food, politics and other interesting aspects of life.

My mother, for her never-ending support and love, my father and my sister for their love and for tolerating my frequent “escapes” to Sweden

Mabel, to being there in this late stage of my education, supporting me always, also in tough times and helping to make my life better.

References

1. *Papanikolaou I, Kagouridis K, Papiris SA. Patterns of airway involvement in inflammatory bowel diseases. World J Gastrointest Pathophysiol. 2014 Nov 15;5(4):560-9.*

2. *Sekiguchi H, Suzuki J, Utz JP. Sarcoidosis with major airway, vascular and nerve compromise. Can Respir J. 2013 Jul-Aug;20(4):256-8.*

3. *Martinez FD, Vercelli D. Asthma. Lancet. 2013 Oct 19;382(9901):1360-72.*

4. *D'Amato G, Vitale C, Molino A, Stanziola A, Sanduzzi A, Vatrella A, Mormile M, Lanza M, Calabrese G, Antonicelli L, D'Amato M. Asthma-related deaths. Multidiscip Respir Med. 2016 Oct 12;11:37.*

5. *GOLD- Global Strategy for the Diagnosis, Management and Prevention of COPD. Global Initiative for Chronic Obstructive Lung Disease (GOLD) (2017)*

6. *Eder W, Ege MJ, von Mutius E. The asthma epidemic. N Engl J Med. 2006 Nov 23;355(21):2226-35.*

7. *Elborn JS. Cystic fibrosis. Lancet. 2016 Nov 19;388(10059):2519-2531.*

8. *Monteagudo M, Rodríguez-Blanco T, Barrecheguren M, Simonet P, Miravitlles M. Prevalence and incidence of bronchiectasis in Catalonia, Spain: A population-based study. Respir Med. 2016 Dec;121:26-31*

9. *Global Initiative for Asthma. The global strategy for asthma man- agement and prevention.: 2016 GINA Report, Global Strategy for Asthma Management and Prevention; 2016*

10. *Postma DS, Weiss ST, van den Berge M, Kerstjens HA, Koppelman GH. Revisiting the Dutch hypothesis. J Allergy Clin Immunol. 2015 Sep;136(3):521-9.*

11. *Kistemaker LE, Gosens R. Acetylcholine beyond bronchoconstriction: roles in inflammation and remodeling. Trends Pharmacol Sci. 2015 Mar;36(3):164-71.*

12. *Abramson MJ, Perret JL, Dharmage SC, McDonald VM, McDonald CF. Distinguishing adult-onset asthma from COPD: a review and a new approach. Int J Chron Obstruct Pulmon Dis. 2014 Sep 9;9:945-62.*

13. *Postma DS, Reddel HK, ten Hacken NH, van den Berge M. Asthma and chronic obstructive pulmonary disease: similarities and differences. Clin Chest Med. 2014 Mar;35(1):143-56.*

14. *Pain M, Bermudez O, Lacoste P, Royer PJ, Botturi K, Tissot A, Brouard S, Eickelberg O, Magnan A. Tissue remodelling in chronic bronchial diseases: from the epithelial to mesenchymal phenotype. Eur Respir Rev. 2014 Mar 1;23(131):118-30.*

15. *Quanjer PH, Weiner DJ, Pretto JJ, Brazzale DJ, Boros PW. Measurement of FEF25-75% and FEF75% does not contribute to clinical decision making. Eur Respir J. 2014 Apr;43(4):1051-8.*

16. *Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, Enright PL, Hankinson JL, Ip MS, Zheng J, Stocks J; ERS Global Lung Function Initiative.Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J. 2012 Dec;40(6):1324-43.*

17. *Backman H, Lindberg A, Sovijärvi A, Larsson K, Lundbäck B, Rönmark E. Evaluation of the global lung function initiative 2012 reference values for spirometry in a Swedish population sample. BMC Pulm Med. 2015 Mar 25;15:26.*

18. *Brisman J, Kim JL, Olin AC, Torén K, Bake B. Spirometric reference equations for Swedish adults. Clin Physiol Funct Imaging. 2016 Feb 10. doi: 10.1111/cpf.12349. [Epub ahead of print] PubMed PMID: 26865107.*

19. *Di Marco F, Tantucci C, Pellegrino G, Centanni S. Chronic obstructive pulmonary disease diagnosis: the simpler the better? Not always. Eur J Intern Med. 2013 Apr;24(3):199-202.*

20. *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 Nov;26(5):948-68.*

21. *Fabbri LM, Rabe KF. From COPD to chronic systemic inflammatory syndrome? Lancet. 2007 Sep 1;370(9589):797-9.*

22. *de Queiroz MC, Moreira MA, Jardim JR, Barbosa MA, Minamisava R, Gondim Hdel C, Velasco FC, Penhavel MV. Knowledge about COPD among users of primary health care services. Int J Chron Obstruct Pulmon Dis. 2014 Dec 17;10:1-6.*

23. *Weiss G, Steinacher I, Lamprecht B, Schirnhofer L, Kaiser B, Sönnichsen A, Studnicka M. Detection of chronic obstructive pulmonary disease in primary care in Salzburg, Austria: findings from the real world. Respiration. 2014;87(2):136-43.*

24. *Chung KF. Targeting the interleukin pathway in the treatment of asthma. Lancet. 2015 Sep 12;386(9998):1086-96. doi: 10.1016/S0140-6736(15)00157-9.*

25. *Pelaia G, Vatrella A, Busceti MT, Gallelli L, Calabrese C, Terracciano R, Maselli R. Cellular mechanisms underlying eosinophilic and neutrophilic airway inflammation in asthma. Mediators Inflamm. 2015;2015:879783.*

26. *Brusselle GG, Joos GF, Bracke KR. New insights into the immunology of chronic obstructive pulmonary disease. Lancet. 2011 Sep 10;378(9795):1015-26.*

27. *George L, Brightling CE. Eosinophilic airway inflammation: role in asthma and chronic obstructive pulmonary disease. Ther Adv Chronic Dis. 2016 Jan;7(1):34-51.*

28. *Lommatzsch SE, Martin RJ, Good JT Jr. Importance of fiberoptic bronchoscopy in identifying asthma phenotypes to direct personalized therapy. Curr Opin Pulm Med. 2013 Jan;19(1):42-8.*

29. *Pavord ID, Pizzichini MM, Pizzichini E, Hargreave FE. The use of induced sputum to investigate airway inflammation. Thorax. 1997 Jun;52(6):498-501.*

30. *Moeller A, Carlsen KH, Sly PD, Baraldi E, Piacentini G, Pavord I, Lex C, Saglani S; ERS Task Force Monitoring Asthma in Children.. Monitoring asthma in childhood: lung function, bronchial responsiveness and inflammation. Eur Respir Rev. 2015 Jun;24(136):204-15*

31. *Wesolowska-Andersen A, Seibold MA. Airway molecular endotypes of asthma:dissecting the heterogeneity. Curr Opin Allergy Clin Immunol. 2015 Apr;15(2):163-8.*

32. *Shaw JG, Vaughan A, Dent AG, O'Hare PE, Goh F, Bowman RV, Fong KM, Yang IA.Biomarkers of progression of chronic obstructive pulmonary disease (COPD). J Thorac Dis. 2014 Nov;6(11):1532-47.*

33. *Barnes CB, Ulrik CS. Asthma and adherence to inhaled corticosteroids: current status and future perspectives. Respir Care. 2015 Mar;60(3):455-68.*

34. *Anggård EE. Endogenous and exogenous nitrates. Acta Anaesthesiol Scand Suppl. 1992;97:7-10.*

35. *Kylhammar D, Rådegran G. The principal pathways involved in the in vivo modulation of hypoxic pulmonary vasoconstriction, pulmonary arterial remodelling and pulmonary hypertension. Acta Physiol (Oxf). 2016 Jul 6. doi:10.1111/apha.12749. [Epub ahead of print]*

36. *Wallace JL, Ianaro A, Flannigan KL, Cirino G. Gaseous mediators in resolution of inflammation. Semin Immunol. 2015 May;27(3):227-33.*

37. *Tufvesson E, Andersson C, Weidner J, Erjefält JS, Bjermer L. Inducible nitric oxide synthase expression is increased in the alveolar compartment of asthmatic patients. Allergy. 2016 Sep 20. doi: 10.1111/all.13052. [Epub ahead of print]*

38. *Gustafsson LE, Leone AM, Persson MG, Wiklund NP, Moncada S. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs*

and humans. Biochem Biophys Res Commun. 1991 Dec 16;181(2):852-7.

39. *Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, Olin AC, Plummer AL, Taylor DR; American Thoracic Society Committee on Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications.. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO)for clinical applications. Am J Respir Crit Care Med. 2011 Sep 1;184(5):602-15.*

40. *Yamamoto M, Tochino Y, Chibana K, Trudeau JB, Holguin F, Wenzel SE. Nitric oxide and related enzymes in asthma: relation to severity, enzyme function and inflammation. Clin Exp Allergy. 2012 May;42(5):760-8.*

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

42. *Xu X, Hu H, Kearney GD, Kan H, Carrillo G, Chen X. A population-based study of smoking, serum cotinine and exhaled nitric oxide among asthmatics and a healthy population in the USA. Inhal Toxicol. 2016 Dec;28(14):724-730.*

43. *de la Riva-Velasco E, Krishnan S, Dozor AJ. Relationship between exhaled nitric oxide and exposure to low-level environmental tobacco smoke in children with asthma on inhaled corticosteroids. J Asthma. 2012 Sep;49(7):673-8.*

44. *Olin AC, Rosengren A, Thelle DS, Lissner L, Bake B, Torén K. Height, age, and atopy are associated with fraction of exhaled nitric oxide in a large adult general population sample. Chest. 2006 Nov;130(5):1319-25.*

45. *Lim CS, Rani FA, Tan LE. Response of exhaled nitric oxide to inhaled corticosteroids in patients with stable COPD: A systematic review and meta-analysis. Clin Respir J. 2016 Jun 22. doi: 10.1111/crj.12518. Epub ahead of print]*

46. *Chen FJ, Huang XY, Liu YL, Lin GP, Xie CM. Importance of fractional exhaled nitric oxide in the differentiation of asthma-COPD overlap syndrome, asthma, and COPD. Int J Chron Obstruct Pulmon Dis. 2016 Sep 26;11:2385-2390.*

47. *Malinovschi A, Ludviksdottir D, Tufvesson E, Rolla G, Bjermer L, Alving K, Diamant Z. Application of nitric oxide measurements in clinical conditions beyond asthma. Eur Clin Respir J. 2015 Aug 17;2:28517.*

48. *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 Apr 15;171(8):912-30.*

49. *Essat M, Harnan S, Gomersall T, Tappenden P, Wong R, Pavord I, Lawson R, Everard ML. Fractional exhaled nitric oxide for the management of asthma in adults: a systematic review. Eur Respir J. 2016 Mar;47(3):751-68.*

50. *Bake B, Viklund E, Olin AC. Effects of pollen season on central and peripheral nitric oxide production in subjects with pollen asthma. Respir Med. 2014 Sep;108(9):1277-83.*

51. *Olin AC, Rosengren A, Thelle DS, Lissner L, Torén K. Increased fraction of exhaled nitric oxide predicts new-onset wheeze in a general population. Am J Respir Crit Care Med. 2010 Feb 15;181(4):324-7.*

52. *Rouatbi S, Chouchene MA, Sfaxi I, Ben Rejeb M, Tabka Z, Ben Saad H. Fraction of exhaled nitric oxide (FeNO) norms in healthy Tunisian adults. Biomed Res Int. 2014;2014:269670.*

53. *Brody DJ, Zhang X, Kit BK, Dillon CF. Reference values and factors associated with exhaled nitric oxide: U.S. youth and adults. Respir Med. 2013 Nov;107(11):1682-91.*

54. *Ko FW, Leung TF, Wong GW, Chu JH, Sy HY, Hui DS. Determinants of, and reference equation for, exhaled nitric oxide in the Chinese population. Eur Respir J. 2013 Sep;42(3):767-75.*

55. *Olin AC, Bake B, Torén K. Fraction of exhaled nitric oxide at 50 mL/s: reference values for adult lifelong never-smokers. Chest. 2007 Jun;131(6):1852-6.*

56. *Beasley R, Semprini A, Mitchell EA. Risk factors for asthma: is prevention possible? Lancet. 2015 Sep 12;386(9998):1075-85.*

57. *Vignoud L, Pin I, Boudier A, Pison C, Nadif R, Le Moual N, Slama R, Makao MN, Kauffmann F, Siroux V. Smoking and asthma: disentangling their mutual influences using a longitudinal approach. Respir Med. 2011 Dec;105(12):1805-14.*

58. *Eisner MD. Smoking and adult asthma: a healthy smoker effect? Am J Respir Crit Care Med. 2002 Jun 1;165(11):1566; author reply 1566-7.*

59. *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(2):175-83.*

60. *Vardavas CI, Hohmann C, Patelarou E, Martinez D, Henderson AJ, Granell R, Sunyer J, Torrent M, Fantini MP, Gori D, Annesi-Maesano I, Slama R, Duijts L, de Jongste JC, Aurrekoetxea JJ, Basterrechea M, Morales E, Ballester F, Murcia M,Thijs C, Mommers M, Kuehni CE, Gaillard EA, Tischer C, Heinrich J, Pizzi C, ZugnaD, Gehring U, Wijga A, Chatzi L, Vassilaki M, Bergström A, Eller E, Lau S, KeilT, Nieuwenhuijsen M, Kogevinas M. The independent role of prenatal and postnatal exposure to active and passive smoking on the development of early wheeze inchildren. Eur Respir J. 2016 Jul;48(1):115-24.*

61. *Tashkin DP. Smoking Cessation in Chronic Obstructive Pulmonary Disease. Semin Respir Crit Care Med. 2015 Aug;36(4):491-507.*

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

63. *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 Mar;129(3):661-8.*

64. *Bakakos P, Kostikas K, Loukides S. Smoking asthma phenotype: diagnostic and management challenges. Curr Opin Pulm Med. 2016 Jan;22(1):53-8.*

65. *Torén K, Blanc PD. Asthma caused by occupational exposures is common – a systematic analysis of estimates of the population-attributable fraction. BMC Pulm Med. 2009 Jan 29;9:7.*

66. *Vandenplas O, Suojalehto H, Cullinan P. Diagnosing occupational asthma. Clin Exp Allergy. 2017 Jan;47(1):6-18.*

67. *Tarlo SM, Balmes J, Balkissoon R, Beach J, Beckett W, Bernstein D, Blanc PD, Brooks SM, Cowl CT, Daroowalla F, Harber P, Lemiere C, Liss GM, Pacheco KA, Redlich CA, Rowe B, Heitzer J. Diagnosis and management of work-related asthma: American College Of Chest Physicians Consensus Statement. Chest. 2008 Sep;134(3 Suppl):1S-41S.*

68. *Tarlo SM. Irritant-induced asthma in the workplace. Curr Allergy Asthma Rep. 2014 Jan;14(1):406.*

69. *Fletcher CM. Disability and mortality from chronic bronchitis in relation to dust exposure. AMA Arch Ind Health. 1958 Nov;18(5):368-73.*

70. *Becklake MR. Occupational exposures: evidence for a causal association with chronic obstructive pulmonary disease. Am Rev Respir Dis 1989;140:S85-S91.*

71. *Blanc PD, Torén K. Occupation in chronic obstructive pulmonary disease and chronic bronchitis: an update. Int J Tuberc Lung Dis. 2007 Mar;11(3):251-7.*

72. *Balmes J, Becklake M, Blanc P, et al. American Thoracic Society Statement: Occupational contribution to the burden of airway disease. Am J Respir Crit Care Med 2003;167:787-797.*

73. *Omland O, Würtz ET, Aasen TB, Blanc P, Brisman JB, Miller MR, Pedersen OF,Schlünssen V, Sigsgaard T, Ulrik CS, Viskum S. Occupational chronic obstructive pulmonary disease: a systematic literature review. Scand J Work Environ Health. 2014 Jan;40(1):19-35.*

74. *Fishwick D, Sen D, Barber C, Bradshaw L, Robinson E, Sumner J; COPD Standard Collaboration Group.. Occupational chronic obstructive pulmonary disease: a standard of care. Occup Med (Lond). 2015 Jun;65(4):270-82.*

75. *De Matteis S, Jarvis D, Hutchings S, Darnton A, Fishwick D, Sadhra S, Rushton L, Cullinan P. Occupations associated with COPD risk in the large population-based UK Biobank cohort study. Occup Environ Med. 2016 Jun;73(6):378-84.*

76. *Sunyer J, Kogevinas M, Kromhout H, Antó JM, Roca J, Tobias A, Vermeulen R, Payo F, Maldonado JA, Martinez-Moratalla J, Muniozguren N. Pulmonary ventilatory defects and occupational exposures in a population-based study in Spain. Spanish Group of the European Community Respiratory Health Survey. Am J Respir Crit Care Med. 1998 Feb;157(2):512-7.*

77. *Le Moual N, Bakke P, Orlowski E, Heederik D, Kromhout H, Kennedy SM, Rijcken B, Kauffmann F. Performance of population specific job exposure matrices (JEMs): European collaborative analyses on occupational risk factors for chronic obstructive pulmonary disease with job exposure matrices (ECOJEM). Occup Environ Med. 2000 Feb;57(2):126-32.*

78. *Kurth L, Doney B, Weinmann S. Occupational exposures and chronic obstructive pulmonary disease (COPD): comparison of a COPD-specific job exposure matrix and expert-evaluated occupational exposures. Occup Environ Med. 2016 Oct 24. pii: oemed-2016-103753.*

79. *Guerra S, Martinez FD. Asthma genetics: from linear to multifactorial approaches. Annu Rev Med. 2008;59:327-41.*

80. *Sly PD, Boner AL, Björksten B, Bush A, Custovic A, Eigenmann PA, Gern JE, Gerritsen J, Hamelmann E, Helms PJ, Lemanske RF, Martinez F, Pedersen S, Renz H, Sampson H, von Mutius E, Wahn U, Holt PG. Early identification of atopy in the prediction of persistent asthma in children. Lancet. 2008 Sep 20;372(9643):1100-6.*

81. *Bachert C, Gevaert E. Advances in rhinitis and rhinosinusitis in 2015. J Allergy Clin Immunol. 2016 ov;138(5):1277-1283.*

82. *Ilmarinen P, Tuomisto LE, Kankaanranta H. Phenotypes, Risk Factors, and Mechanisms of Adult-Onset Asthma. Mediators Inflamm. 2015;2015:514868.*

83. *Postma DS, Bush A, van den Berge M. Risk factors and early origins of chronic obstructive pulmonary disease. Lancet. 2015 Mar 7;385(9971):899-909.*

84. *ILO. (1991) International Standard Classification of Occupations ISCO 88. Geneva, Switzerland: ILO. ISBN 92-2-106438-7.*

85. *Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J 2005;26:319-38.*

86. *Quanjer PH, Tammeling GJ, Cotes JE Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl. 1993;16:5–40.*

87. *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(5):948–968.*

88. *Matricardi PM, Nisini R, Pizzolo JG, d´Amelio R. The use of Phadiatop in mass-screening programmes of inhalant allergies: Advantages and limitations. Clin Exp Allergy 1990;20:151-155*

89. *Simple Interactive Statistical Analysis (SISA) free software,Southampton,UK. Cited 2013 May 18, Available at [http://www.quantitativeskills.com/sisa/statistics/diagnos.htm].*

90. *Aaron SD, Gershon AS, Gao Y, Yang J, Whitmore GA. Influence of country-level differences on COPD prevalence. Int J Chron Obstruct Pulmon Dis. 2016 Sep 19;11:2305-2313.*

91. *Backman H, Eriksson B, Rönmark E, Hedman L, Stridsman C, Jansson*

SA, Lindberg A, Lundbäck B. Decreased prevalence of moderate to severe COPD over 15 years in northern Sweden. Respir Med. 2016 May;114:103-10.

92. *Viegi G, Pistelli F, Sherrill DL Maio S, Baldacci S, Carrozzi L. Definition, epidemiology and natural history of COPD. Eur Respir J. 2007;30(5):993–1013*

93. *Torén K, Brisman J, Järvholm B. Asthma and asthma-like symptoms in adults assessed by questionnaires. A literature review. Chest. 1993;104(2):600–608.*

94. *Barr RG, Herbstman J, Speizer FE Camargo CA Jr. Validation of self-reported chronic obstructive pulmonary disease in a cohort study of nurses. Am J Epidemiol. 2002;155(10):965–971.*

95. *Price DB, Tinkelman DG, Nordyke RJ Isonaka S, Halbert RJ; COPD Questionnaire Study Group. Scoring system and clinical application of COPD diagnostic questionnaires. Chest. 2006;129(6):1531–1539.*
96. *Lyngsø AM, Backer V, Gottlieb V, Nybo B, Ostergaard MS, Frølich A. Early detection of COPD in primary care--the Copenhagen COPD Screening Project. BMC Public Health. 2010 Sep 1;10:524.*

97. *Bednarek M, Maciejewski J, Wozniak M Kuca P, Zielinski J. Prevalence, severity and underdiagnosis of COPD in the primary care setting. Thorax. 2008;63(5):402–407*

98. *Jörres RA. Modelling the production of nitric oxide within the human airways. Eur Respir J. 2000 Sep;16(3):555-60.*

99. *Hall CB, Simőes EA, Anderson LJ. Clinical and epidemiologic features of respiratory syncytial virus. Curr Top Microbiol Immunol. 2013;372:39-57.*

100. *Malerba M, Montuschi P. Non-invasive biomarkers of lung inflammation in smoking subjects. Curr Med Chem. 2012;19(2):187-96.*

101. *Malinovschi A, Alving K, Kalm-Stephens P, Janson C, Nordvall L. Increased exhaled nitric oxide predicts new-onset rhinitis and persistent*

rhinitis in adolescents without allergic symptoms. Clin Exp Allergy. 2012 Mar;42(3):433-40.

102. 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 Aug;132(2):336-41

103. Malinovschi A, Backer V, Harving H, Porsbjerg C. The value of exhaled nitric oxide to identify asthma in smoking patients with asthma-like symptoms. Respir Med 2012;106:794-801.

104. Bakke PS, Baste V, Hanoa R, Gulsvik A. Prevalence of obstructive lung disease in a general population: relation to occupational title and exposure to some airborne agents. Thorax. 1991 Dec;46(12):863-70.

105. Blanc PD, Eisner MD, Earnest G, Trupin L, Balmes JR, Yelin EH, Gregorich SE, Katz PP. Further exploration of the links between occupational exposure and chronic obstructive pulmonary disease. J Occup Environ Med. 2009 Jul;51(7):804-10.

106. Boggia B, Farinaro E, Grieco L, Lucariello A, Carbone U. Burden of smoking and occupational exposure on etiology of chronic obstructive pulmonary disease in workers of Southern Italy. J Occup Environ Med. 2008 Mar;50(3):366-70.

107. Comandini A, Rogliani P, Nunziata A, Cazzola M, Curradi G, Saltini C. Biomarkers of lung damage associated with tobacco smoke in induced sputum. Respir Med. 2009 Nov;103(11):1592-613. doi: 10.1016/j.rmed.2009.06.002

108. Sikkeland LI, Johnsen HL, Riste TB, Alexis NE, Halvorsen B, Søyseth V, Kongerud J. Sputum neutrophils are elevated in smelter workers, and systemic neutrophils are associated with rapid decline in FEV1. Occup Environ Med. 2016 Jul;73(7):459-66.

109. Ulvestad B, Bakke B, Melbostad E, Fuglerud P, Kongerud J, Lund MB. Increased risk of obstructive pulmonary disease in tunnel workers. Thorax. 2000 Apr;55(4):277-82.

110. *Davison AG, Fayers PM, Taylor AJ, Venables KM, Darbyshire J, Pickering CA, et al. Cadmium fume inhalation and emphysema. Lancet. 1988 Mar 26;1(8587):663–7*

111. *Hagstad S, Backman H, Bjerg A, Ekerljung L, Ye X, Hedman L, Lindberg A, Torén K, Lötvall J, Rönmark E, Lundbäck B. Prevalence and risk factors of COPD among never-smokers in two areas of Sweden - Occupational exposure to gas, dust or fumes is an important risk factor. Respir Med. 2015 Nov;109(11):1439-45.*

112. *Qaseem A, Wilt TJ, Weinberger SE Hanania NA, Criner G, van der Molen T, Marciniuk DD, Denberg T, Schünemann H, Wedzicha W, MacDonald R, Shekelle P; American College of Physicians; American College of Chest Physicians; American Thoracic Society; European Respiratory Society. Diagnosis and management of stable chronic obstructive pulmonary disease: a clinical practice guideline update from the American College of Physicians, American College of Chest Physicians, American Thoracic Society, and European Respiratory Society. Ann Intern Med. 2011;155(3):179–191.*

113. *Ulrik CS, Løkke A, Dahl R Dollerup J, Hansen G, Cording PH, Andersen KK; TOP study group. Early detection of COPD in general practice. Int J Chron Obstruct Pulmon Dis. 2011;6:123–127.*

114. *Levy ML, Quanjer PH, Booker R Cooper BG, Holmes S, Small I; General Practice Airways Group. Diagnostic spirometry in primary care: Proposed standards for general practice compliant with American Thoracic Society and European Respiratory Society recommendations: a General Practice Airways Group (GPIAG). Prim Care Respir J. 2009;18(3):130–147.*

115. *Calverley PM, Burge PS, Spencer S Anderson JA, Jones PW. Bronchodilator reversibility testing in chronic obstructive pulmonary disease. Thorax. 2003;58(8):659–664.*

116. *Lehmann S, Bakke PS, Eide GE Humerfelt S, Gulsvik A. Bronchodilator reversibility testing in an adult general population; The importance of smoking and anthropometrical variables on the response to a B2-agonist. Pulm Pharmacol Ther. 2006;19(4):272–280*

117. *Meslier N, Racineux JL, Six P Lockhart A. Diagnostic value of reversibility of chronic airway obstruction to separate asthma from chronic bronchitis: a statistical approach. Eur Respir J. 1989;2(6):497–505.*

118. *Marcon A, Anto JM, Cerveri I, Gislason T, Heinrich J, Janson C, Jarvis D, Kuenzli N, Leynaert B, Probst-Hensch N, Svanes C, Toren K, Weyler J, Zock JP, Burney P, De Marco R.Can we use pre-bronchodilator spirometry to define post-bronchodilator airflow obstruction? European Respiratory Journal 2015 46: PA5065;*

119. *Carlsen HK, Modig L, Levinsson A, Kim JL, Toren K, Nyberg F, Olin AC. Exposure to traffic and lung function in adults: a general population cohort study. BMJ Open. 2015 Jun 24;5(6):e007624.*