Lung function and the prediction of health outcomes in an urban population

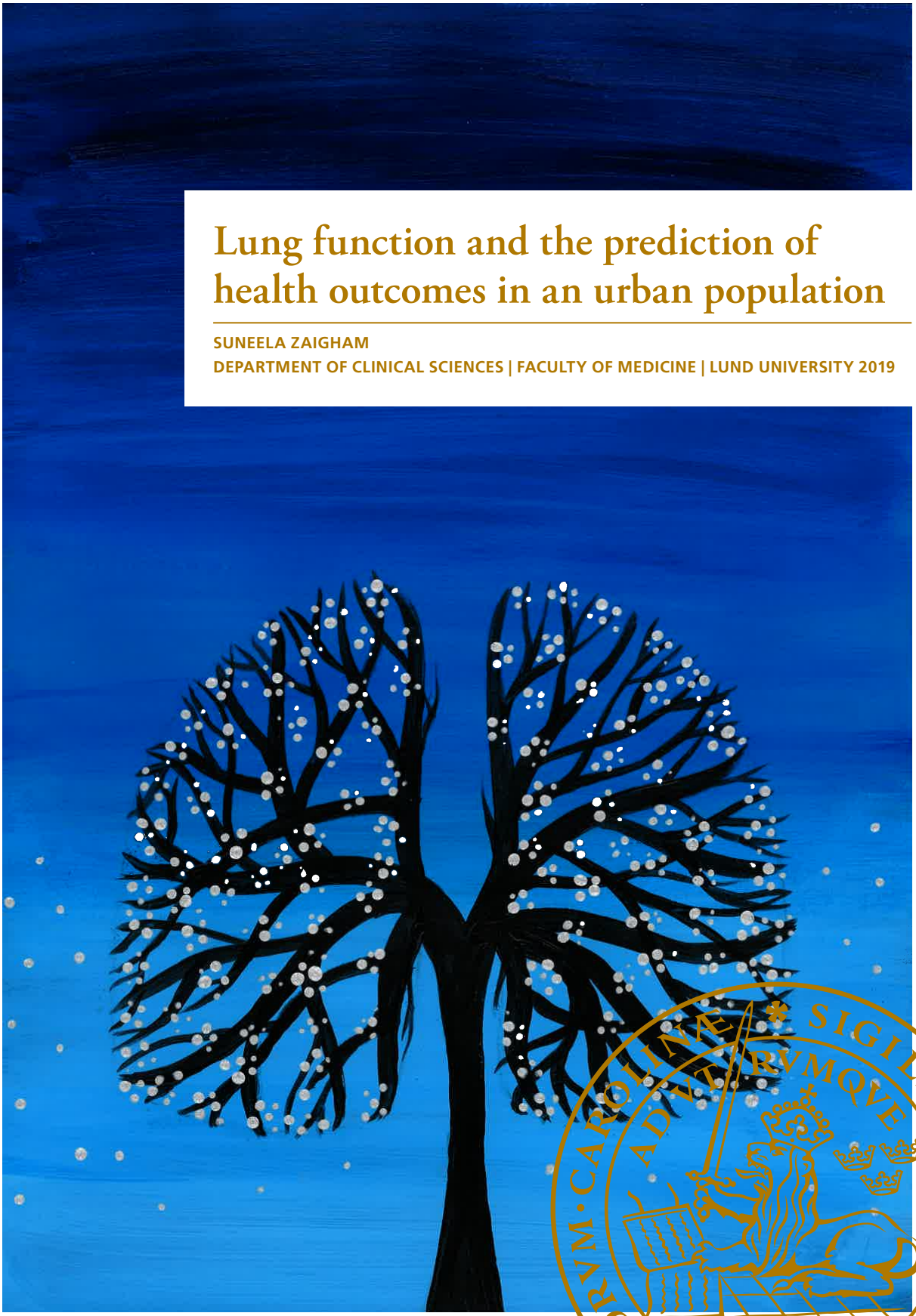
Zaigham, Suneela

2019

Document Version:
Publisher's PDF, also known as Version of record

Lung function and the prediction of health outcomes in an urban population

SUNEELA ZAIGHAM
DEPARTMENT OF CLINICAL SCIENCES | FACULTY OF MEDICINE | LUND UNIVERSITY 2019



Lung function and the prediction of health outcomes
in an urban population

Lung function and the prediction of health outcomes in an urban population

Suneela Zaigham

LUND
UNIVERSITY

DOCTORAL DISSERTATION
by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended at Agardhsalen Clinical Research Centre, Jan Waldenströms gata 35, Skånes Universitetssjukhus, Malmö, Friday 18th January 2019 at 09.00

Faculty opponent
Professor Massimo Pistolesi
University of Firenze, Florence, Italy

Organization LUND UNIVERSITY	Document name DOCTORAL DISSERTATION
	Date of issue 18 January 2019
Author(s): Suneela Zaigham	Sponsoring organization

Title and subtitle: Lung function and the prediction of health outcomes in an urban population

Low lung function is a known predictor of various cardiovascular outcomes and all-cause mortality, beyond the effect of smoking. This association has been found using various measures of spirometry such as low FEV_1, FVC and the ratio of FEV_1/FVC. The mechanism for this association is still debated. There is a known co-morbidity between chronic obstructive pulmonary disease (COPD) and cardiovascular outcomes. Additionally, diabetes mellitus (DM) is a well-known risk factor for cardiovascular disease (CVD). However, the role of low levels of lung function as a predictor for incident COPD outcomes and DM has not been well established. This could potentially add clarity to the relationship between lung function and health outcomes such as CVD and premature mortality along with giving new insights into disease prediction using lung function in otherwise healthy individuals. Furthermore, the use of measures of ventilation inhomogeneity such as lung clearance index (LCI) for disease prediction has not been widely explored in the adult population, and could potentially add value as an early marker of changes in small airways when spirometry may not be affected.

This doctoral thesis utilised two Malmö-based cohorts to assess baseline lung function in relation to various health outcomes in a prospective follow-up design. The “Men Born in 1914” cohort consisted of 55 year old men living in Malmö with lung function measured in 1969 and repeat measurements in 1982 when subjects were 68 years old. The Malmö Preventive Project (MPP) cohort measured spirometry in middle-aged men and women living in Malmö between 1974 and 1992. Incident COPD hospitalisations, COPD-related mortality, incident coronary events, all-cause mortality and FEV_1 decline were the outcomes assessed using the “Men Born in 1914” cohort in relation to baseline spirometry and LCI. Incident DM was the outcome of interest in relation to baseline spirometry in the MPP cohort. Cox proportional hazards regression was used to assess incidence of the various outcomes according to baseline lung function.

Poor lung function (as defined by FEV_1/VC ratio < lower limit of normal (LLN), a low FEV_1 or VC and high LCI at baseline) is a strong risk factor for the prediction of COPD hospitalisations, COPD-related mortality and all-cause mortality even after adjustment for baseline smoking. The risk of incident COPD outcomes was present even for those subjects with an FEV_1/VC ratio < 0.70 but > LLN at baseline. These measures had a stronger association with COPD outcomes than they did for coronary events. VC had a weaker association with future COPD outcomes than other measures, and similarly predicted incident COPD and coronary events. A low FEV_1 and FVC preceded and significantly predicted the risk of DM even after many years of follow-up, particularly in middle-aged men.

This thesis shows that low levels of lung function at baseline can potentially identify high-risk subjects where early identification using screening could allow early intervention strategies to put be in place and subsequently alter the disease course and prognosis. The use of spirometry in general health screening can therefore potentially be of major individual and societal benefit. MBW measures such as LCI can be used in addition to spirometry to find those at highest risk of developing severe COPD outcomes later in life. Lung function screening should be considered as part of the general health assessment in the population. Population-wide screening of not only smokers can potentially provide vital information to guide disease prevention strategies.

Key words: Spirometry, Multiple-breath washout, Chronic Obstructive Pulmonary Disease, Coronary events, Diabetes Mellitus, Incidence

Classification system and/or index terms (if any)

Supplementary bibliographical information		Language: English
ISSN and key title 1652-8220		ISBN 978-91-7619-718-9
Recipient’s notes	Number of pages 109	Price
	Security classification	

Signature  Date 2018-11-23

Lung function and the prediction of health outcomes in an urban population

Suneela Zaigham

LUND
UNIVERSITY

Coverphoto by Suneela Zaigham

Faculty of Medicine
Department of Clinical Sciences Malmö

ISBN 978-91-7619-718-9
ISSN 1652-8220
Lund University, Faculty of Medicine Doctoral Dissertation Series 2019: 1

Printed in Sweden by Media-Tryck, Lund University
Lund 2018

Media-Tryck is an environmentally certified and ISO 14001 certified provider of printed material. Read more about our environmental work at www.mediatryck.lu.se

"God does not burden any soul with more than it can bear"
(Holy Quran, Surah Bakarah, verse 286)

To my children, Zainab and Mustafa

Table of Contents

List of papers

This doctoral thesis is based on the following original papers. The papers are reproduced with permission from the publishers and are referred to in the text by their roman numerals:

Paper I: Zaigham S, Wollmer P, Engström G. Lung function, forced expiratory volume in 1s decline and COPD hospitalisations over 44 years of follow-up. European Respiratory Journal 2016; 47(3): 742-750. © ERS 2018.

Paper II: Zaigham S, Wollmer P, Engström G. The association of lung clearance index with COPD and FEV1 reduction in "Men born in 1914". COPD: Journal of Chronic Obstructive Pulmonary Disease 2017; 14(3): 324-329. © 2017 Taylor & Francis Group, LLC.

Paper III: Zaigham S, Nilsson PM, Wollmer P, Engström G. The temporal relationship between poor lung function and the risk of diabetes. BMC Pulmonary Medicine 2016; 16(1): 75. © Zaigham et al.

Paper IV: Zaigham S, Johnson L, Wollmer P, Engström G. Measures of low lung function and the prediction of incident COPD and coronary heart disease. Respiratory Medicine 2018; 144: 68-73. © 2018 Elsevier

Abstract

Low lung function is a known predictor of various cardiovascular outcomes and all-cause mortality, beyond the effect of smoking. This association has been found using various measures of spirometry such as low FEV_1, FVC and the ratio of FEV_1/FVC. The mechanism for this association is still debated. There is a known co-morbidity between chronic obstructive pulmonary disease (COPD) and cardiovascular outcomes. Additionally, diabetes mellitus (DM) is a well-known risk factor for cardiovascular disease (CVD). However, the role of low levels of lung function as a predictor for incident COPD outcomes and DM has not been well established. This could potentially add clarity to the relationship between lung function and health outcomes such as CVD and premature mortality along with giving new insights into disease prediction using lung function in otherwise healthy individuals. Furthermore, the use of measures of ventilation inhomogeneity such as lung clearance index (LCI) for disease prediction has not been widely explored in the adult population, and could potentially add value as an early marker of changes in small airways when spirometry may not be affected.

This doctoral thesis utilised two Malmö-based cohorts to assess baseline lung function in relation to various health outcomes in a prospective follow-up design. The "Men Born in 1914" cohort consisted of 55 year old men living in Malmö with lung function measured in 1969 and repeat measurements in 1982 when subjects were 68 years old. The Malmö Preventive Project (MPP) cohort measured spirometry in middle-aged men and women living in Malmö between 1974 and 1992. Incident COPD hospitalisations, COPD-related mortality, incident coronary events, all-cause mortality and FEV_1 decline were the outcomes assessed using the "Men Born in 1914" cohort in relation to baseline spirometry and LCI. Incident DM was the outcome of interest in relation to baseline spirometry in the MPP cohort. Cox proportional hazards regression was used to assess incidence of the various outcomes according to baseline lung function.

Poor lung function (as defined by FEV_1/VC ratio < lower limit of normal (LLN), a low FEV_1 or VC and high LCI at baseline) is a strong risk factor for the prediction of COPD hospitalisations, COPD-related mortality and all-cause mortality even after adjustment for baseline smoking. The risk of incident COPD outcomes was present even for those subjects with an FEV_1/VC ratio < 0.70 but > LLN at baseline. These measures had a stronger association with COPD outcomes than they did for coronary events. VC had a weaker association with future COPD outcomes than other measures, and similarly predicted incident COPD and coronary events. A low FEV_1 and FVC preceded and significantly predicted the risk of DM even after many years of follow-up, particularly in middle-aged men.

This thesis shows that low levels of lung function at baseline can potentially identify high-risk subjects where early identification using screening could allow early intervention strategies to put be in place and subsequently alter the disease course and prognosis. The use of spirometry in general health screening can therefore potentially be of major individual and societal benefit. MBW measures such as LCI can be used in addition to spirometry to find those at highest risk of developing severe COPD outcomes later in life. Lung function screening should be considered as part of the general health assessment in the population. Population-wide screening of not only smokers can potentially provide vital information to guide disease prevention strategies.

Populärvetenskaplig sammanfattning på svenska

Nedsatt lungfunktion i medelåldern har visat sig vara en riskfaktor för hjärt-kärlsjukdom. Detta gäller för både rökare och icke-rökare. Det är dock oklart ifall sänkt lungfunktion även ökar risken för andra allvarliga händelser relaterade till lungsjukdomar, som exempelvis inläggningar på sjukhus eller dödsfall på grund av kroniskt obstruktiv lungsjukdom (KOL). KOL är en kronisk lungsjukdom som gör det svårare att andas. Sjukdomen orsakas framförallt av tobaksrökning, men även icke-rökare kan drabbas. Vid tidig diagnos kan man förhindra utvecklingen av sjukdomen. KOL har visat sig ha ett samband med hjärt-kärl sjukdom. Om lungfunktion kan användas för att identifiera de med ökad risk för att utveckla både KOL och hjärt-kärlsjukdom skulle detta kunna vara av stor nytta. Diabetes är ytterligare en viktig folksjukdom som ökar risken för hjärt-kärlsjukdom. Det har visat sig att diabetiker har sämre lungfunktion än icke-diabetiker. Däremot vet man inte om diabetes orsakar försämringen i lungfunktion, eller om nedsatt lungfunktion orsakar diabetes. Om man vet mer om vilket samband sänkt lungfunktion har med diabetes och allvarliga KOL händelser kan detta förhoppningsvis på sikt öka möjligheterna att identifiera patienter som har ökad risk för hjärt-kärlsjukdom och även leda till att dessa sjukdomars naturliga förlopp kan förändras.

Syftet med denna avhandling är att utreda ifall nedsatt lungfunktion påverkar risken för allvarlig KOL, diabetes och dödlighet oavsett orsak – utöver den risk som orsakas av tobaksrökning. Vi avser dessutom att utreda ifall vissa mått för lungfunktion är mer relaterade till utvecklandet av allvarliga KOL händelser eller framtida hjärt-kärlsjukdom. För att kunna besvara dessa frågeställningar har vi använt oss av information från två befolkningsstudier. Den ena består endast av män i medelåldern (1914-års män i Malmö) och den andra består av information från både män och kvinnor i medelåldern (Malmö Förebyggande Medicin).

Studiedeltagarnas lungfunktion mättes vid början av studien. Lungfunktion kan enkelt mätas sittandes på en stol med hjälp av en undersökning som kallas för spirometri. Spirometern mäter hur mycket och hur snabbt man kan andas in och ut. Kvävgasutsköljning mäter hur väl lungan ventileras och är en annan mätmetod som är enkel att utföra. Man andas 100% syrgas med vanliga andetag och mäter hur mycket man måste andas för att kvävgasen ska ersättas. Efter lungfunktionsundersökningarna följdes studiedeltagarna upp under lång tid (44 års uppföljning respektive 37 års uppföljning för 1914-års män i Malmö och Malmö Förebyggande Medicin).

Studien 1914-års män i Malmö består av 703 55-åriga män, varav 689 undersöktes med spirometri. Studien visade att män med låg lungfunktion vid undersökningarna 1969-1970 hade ökad risk att bli drabbas av KOL under uppföljningsperioden. Detta samband kvarstod trots att man justerade för effekten av tobaksrökning. Nedsatt lungfunktion tidigt i livet ökade även risken för framtida försämring i lungfunktionen,

och medförde en ökad risk för död oavsett orsak senare i livet. Vissa mått av lungfunktion visade sig ha starkare samband med risken att utveckla allvarliga KOL händelser än risken att utveckla akuta hjärt- kärlsjukdomar exempelvis hjärtinfarkt.

Mer än 27000 män och kvinnor mellan 40- och 50-års ålder deltog i studien Malmö Förebyggande Medicin. Denna studie visade att sänkt lungfunktion (enligt spirometri) kunde förutsäga framtida risk att utveckla diabetes och det var tydligt att ju lägre lungfunktionen var, desto högre var risken att utveckla diabetes senare i livet. Man fann att nedsatt lungfunktion mellan 40- och 50-års ålder ledde till en ökad risk att utveckla diabetes mer än 30 år senare. Risken var ökad bland både rökare och icke-rökare, men framför allt hos män.

Från dessa fynd kan man dra slutsatsen att mätning av lungfunktion som del av en allmän hälsoundersökning i medelåldern kan vara av nytta för att identifiera de med ökad risk för flera viktiga hälsoutfall senare i livet. Att identifiera dessa personer tidigt i livet är av stor betydelse för våra möjligheter att förändra dessa sjukdomars förlopp och på så sätt minska bördan av kroniska sjukdomar i befolkningen.

Abbreviations

ANOVA= Analysis of Variance

BMI= Body mass index

CE= Coronary events

CEV= Cumulative expired volume

CF= Cystic Fibrosis

CHD= Coronary heart disease

CI= Confidence interval

CIF= Cumulative incidence function

COPD= Chronic Obstructive Pulmonary Disease

CT = Computerised Tomography

CVD= Cardiovascular disease

DAG= Directed Acyclic Graphs

DM= Diabetes Mellitus

ECG= Electrocardiography

ERV= Expiratory reserve volume

ESR= Erythrocyte sedimentation rate

FEF= Forced expiratory flow

FET= Forced expiratory time

FEV_1= Forced Expiratory Volume in 1 second

FR= Fixed ratio

FRC= Functional residual capacity

FVC= Forced vital capacity

GOLD= Global Initiative for Chronic Obstructive Pulmonary Disease

HR= Hazard ratio

IC= Inspiratory capacity

ICD = International Classification of Diseases

IHD= Ischaemic heart disease

IRV= Inspiratory reserve volume

LCI= Lung clearance index

LLN= Lower limit of normal

LR test= Likelihood ratio test

MBW= Multiple Breath Washout

MDC-CC= Malmö diet and Cancer study- Cardiovascular cohort

MDCS= Malmö diet and cancer study

MI= Myocardial infarction

MPP= Malmö Preventive Project

NDR= Swedish National Diabetes Register

OR= Odds ratio

PEFR= Peak expiratory flow rate

Q= Quartile

ROC = Receiver operating characteristic

RV= Residual volume

SBW= Single Breath Washout

SD= Standard deviation

SEI= Statistics Sweden Socio-economic Index

SES= Socio-economic status

SHR = Sub-hazard ratio

SVC= Slow vital capacity

TLC= Total lung capacity

TV= Tidal volume

ULN = Upper limit of normal

VC = Vital Capacity

WHO = World Health Organization

1. Introduction

1.1. The history of lung function testing

An interest in measuring lung function has been around for many centuries. Spirometry is the most commonly utilised test and provides a quantifiable measure of lung function. Although various methods of measuring lung volume had existed for many years prior the invention of spirometry, it was in 1846 that an English surgeon - John Hutchinson, first invented the original spirometer; deriving from the words "to breath" (spiro) and "to measure" (meter). It was also at this time he discovered that vital capacity (VC) also termed "the capacity for life" was proportional to one's height and inversely associated with one's age [1]. Based on his findings, he concluded that the amount of air expelled from a fully inflated lung was a powerful indicator of longevity[2] and reductions in the VC predicted premature morbidity and mortality[1]. The relationship between the VC of the lungs and future health has therefore been recognised for over 170 years.

In 1947, approximately 100 years after the discovery of VC using spirometry, it became apparent that the VC measurement needed a time component added. The purpose of this was to be able to evaluate airflow defects in specific diseases in which the rate of exhalation was affected, such as that in emphysema and asthma[3]. Subsequently, measurement of the "maximal volume of air expelled in one second after a deep inspiration"[4] was developed which was later named the forced expiratory volume in 1 second (FEV_1) by the British Thoracic Society in 1957[4]. Thus formed the two central elements of spirometry; the amount of air forcibly exhaled from the lungs after full inspiration – the forced vital capacity (FVC) and the airflow during the first second of this manoeuvre – the FEV_1.

Inert gas washout tests- single or multiple breath washout (SBW or MBW respectively) tests are less commonly used relative to spirometry and the basic techniques behind the test were first described much later in 1952[5]. The purpose of these tests are to provide a measure of the efficacy of ventilation distribution in the lung[6]. Lung clearance index (LCI) is one of the most common measures reported from MBW tests and was first described by Becklake in 1952[7]. However, since its discovery LCI was not immediately implemented so widely into practice. It has recently gained popularity in assessing ventilation distribution in children due the lack of complex respiratory manoeuvres

required to perform the test[6]. As the MBW can be relatively time-consuming, especially in disease, the SBW can be an alternative technique to allow measures of ventilation inhomogeneity to be obtained[8]. Closing volume derived from this test was previously thought to be a sensitive measure of peripheral airways disease, but since then the phase III slope derived from SBW has been measured more widely for this purpose[9]. The evolution of both the SBW and MBW technique was influenced by the invention of fast responding gas analysers and the invention of computers that allowed breath-by-breath analysis to be recorded[9]. Following both these inventions were waves of research enthusiasm, which resulted in the clinical use of inert gas washouts in the paediatric population[9].

1.2. Lung volumes and capacities

The lung can be thought of as having four volumes (tidal volume (TV), inspiratory reserve volume (IRV), expiratory reserve volume (ERV) and residual volume (RV)) and four capacities (total lung capacity (TLC), inspiratory capacity (IC), functional residual capacity (FRC) and VC). The capacities are composed of two or more lung volumes.

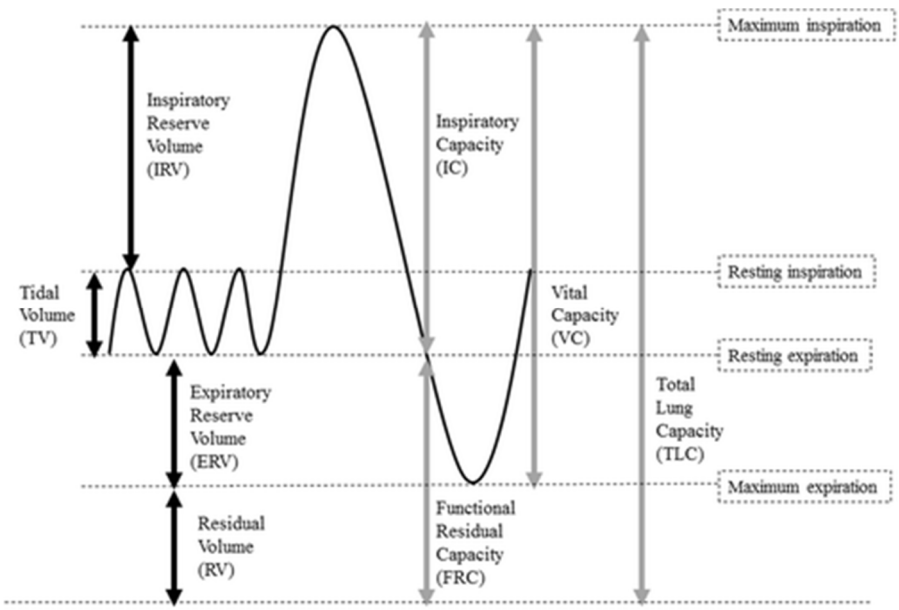


Figure 1:

Spirometer trace illustrating the different lung volumes and capacities. From: Lutfi MF, *Multidisciplinary Respiratory Medicine* (2017) 12:3 [10]. (Creative commons attribution license. Link: http://creativecommons.org/licenses/by/4.0/)

Lung volumes can be thought of being either static or dynamic. Dynamic lung volumes are dependent on the rate of airflow[10] such as FVC (and FEV_1) and can be measured using spirometry. Static lung volumes and capacities (RV, TLC, FRC and VC) are not affected by the rate of air movement in and out the lungs, of which some can be measured directly and some indirectly. RV is the volume of air that remains in the lung after maximal expiration and cannot be measured directly. As it makes up part of the FRC and TLC, these capacities can also not be measured by spirometry alone, and require the aid of plethysmography, gas dilution, nitrogen washout or imaging for assessment[10], whereas VC and its components (IRV and ERV) can be assessed using spirometry alone[11, 12].

1.3. Spirometry

Spirometry "is the measurement of the movement of air into and out of the lungs during various breathing manoeuvres"[13]. Measures of lung volume and flow are obtained through this relatively basic test. Along with simplicity, its advantages include reproducibility[14], a standardised criteria for assessment and its wide availability[15]. The process of obtaining the FVC involves a subject forcibly breathing out from a point of maximal inspiration (the TLC) to the point of maximal expiration (RV). The volume of air expelled in the first second of this forced expiration is the FEV_1. Both these measures can be used together to express the FEV_1/FVC ratio which is used in determining the presence of airways diseases. Slow VC (SVC or sometimes referred to as just VC) is thought to yield the largest measurement of VC as expiration is slow and not forced. FVC is thought to be reduced more when airflow obstruction is present than SVC, therefore the largest available VC measurement is the more preferable one to use[16].The ratio of FEV_1/VC therefore is thought to provide lower values than using FEV_1/FVC, especially in pronounced airflow limitation[17]. Despite this, in both clinical practice and in epidemiological studies, FVC is more commonly used[18]. A Swedish general population study assessed the difference between using FVC and VC for the ratio of FEV_1/VC in the general population[18]. They found that COPD prevalence was significantly higher when the larger of the VC measurements was used (SVC or FVC) compared with using the FVC alone. However, they found that additional subjects identified using this approach tended to have milder COPD, which indicates using the FVC alone may also underdiagnose milder cases of COPD, and as such the FEV_1/SVC can provide a more sensitive definition for COPD[18].

1.3.1. Obstructive and restrictive lung defects

In obstructive lung disease, airway narrowing causes the premature close of airways during expiration and hence an increase in the RV and FRC. The FVC is reduced, however the FEV_1 is reduced to a greater extent as the airway resistance caused by narrowing results in difficulty in exhaling quickly. There is therefore a reduction in the FEV_1/FVC ratio. In restrictive disorders, there is a reduction in the lung compliance due to fibrosis and "stiffening" of the lung and hence inspiration and lung expansion become difficult, resulting in overall smaller lung volumes. However due to the high elastic recoil of the lung, exhaling quickly is not as affected as it is in obstructive disease and therefore FEV_1 is not affected to the same extent as it is in an obstructive disease pattern. As FVC and FEV_1 are both reduced, the FEV_1/FVC ratio can appear normal or even increased. **Figure 2** illustrates the differences in lung volumes associated with both obstructive and restrictive disease compared to normal. The most marked difference that can be seen between obstructive and restrictive lung disease is the difference in the TLC. This difference is firstly due to air trapping in expiration that results in a large RV in obstructive lung disease and secondly to the overall smaller lung volumes in restrictive lung diseases that occur due to a reduction in lung expansion.

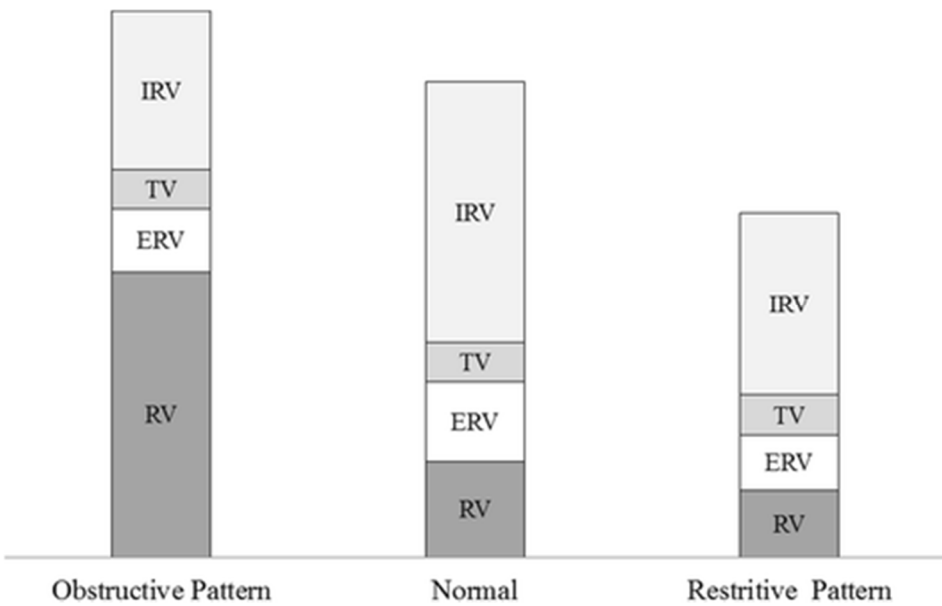


Figure 2:

Changes in the static lung volumes associated with obstructive lung disease and restrictive lung disease compared to normal. From: Lutfi MF, *Multidisciplinary Respiratory Medicine* (2017) 12:3[10]. (Creative commons attribution license. Link: http://creativecommons.org/licenses/by/4.0/)

1.3.2. Volume-time graphs and flow-volume loops using spirometry

The volume-time graphs and flow-volume loops obtained using spirometry can further illustrate differences in obstructive and restrictive lung impairment.

Volume-time curves

Volume-time curves are obtained by asking the subject to fully inhale (to TLC) followed by rapid forced exhalation to RV. The volume forcibly exhaled in the first second is therefore the FEV_1 (as shown in **Figure 3**) and the FVC is shown as the total volume forcibly exhaled over the entirety of the breath (forced expiratory time: FET).

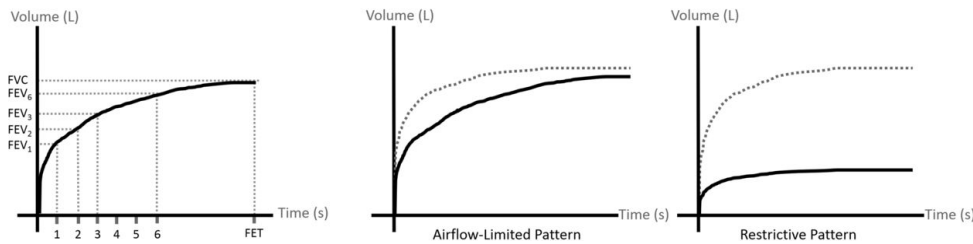


Figure 3:

Volume-time curves; normal spirometry, spirometry in airflow obstruction and spirometry in restrictive lung disease. FET: Forced expiratory time. Reproduced from Interpretation of pulmonary function tests: beyond the basics, Staitieh BS, Ioachimescu OC. J Investig Med 2017;65:301–310[19], Copyright © 2017, with permission from BMJ Publishing Group Ltd

In obstructive lung diseases where a pattern of airflow limitation manifests, the volume-time curve shows a slower rise than normal, resulting in a reduced FEV_1. There is also a lack of a plateau in the curve which is an indication that the subject will continue to exhale beyond the duration of the test. Therefore the FVC obtained during spirometry will under-estimate the true FVC which can lead to a misdiagnosis of restriction or normal airflow[19]. Therefore, the SVC (if larger than original FVC) should be used as part of the ratio for the assessment of airflow limitation. In restrictive lung disease, there is a premature rise and a premature plateau of the curve. As seen from the curve for a restrictive pattern in **Figure 3,** both the FEV_1 and FVC are reduced, such that the ratio can remain unchanged or even increase.

Flow-volume loops

Figure 4 shows normal flow-volume loops obtained using spirometry. The inspiratory phase in indicated on the negative side of the y-axis and expiratory on the positive. The point at total inspiration is the TLC and total expiration is the RV and therefore the distance between the two is the VC.

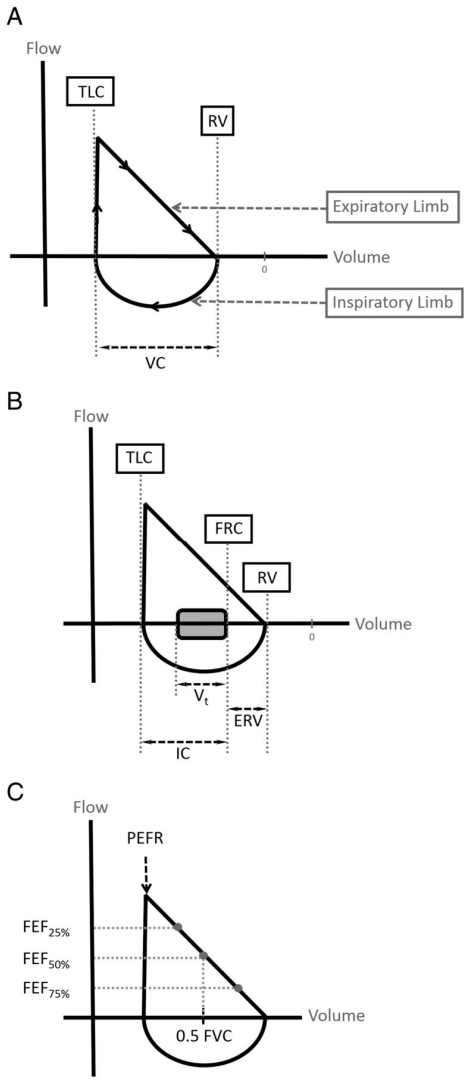


Figure 4:

Normal flow-volume loops, volume on x-axis increasing towards the origin. Total lung capacity (TLC), residual volume (RV) vital capacity (VC) V_t: Tidal volume. FRC: functional residual capacity, IC: inspiratory capacity, ERV: expiratory reserve volume, PEFR: Peak expiratory flow rate, FEF: forced expiratory flow. Reproduced from Interpretation of pulmonary function tests: beyond the basics, Staitieh BS, Ioachimescu OC. J Investig Med 2017;65:301–310[19], Copyright © 2017, with permission from BMJ Publishing Group Ltd.

Forced expiratory flow (FEF) at 25, 50 and 75% can also be illustrated in relation to the FVC on the flow-volume loop (**Figure 4:c**). $FEF_{25\%}$ is the point at which a quarter of the FVC has been exhaled, $FEF_{50\%}$ the point at which half of the FVC has been exhaled and $FEF_{75\%}$ the point at which three-quarters of FVC has been exhaled. A higher value of $FEF_{50\%}$ is therefore indicative of restrictive diseases whereas lower values are more likely seen in obstructive lung diseases[19].

In obstructive and restrictive lung conditions, the shape and position of the flow-volume loop changes (**Figure 5**). In obstructive disease, the loop shifts to the left due to more difficulty in emptying the lungs (larger RV) and has a more concave appearance. The peak expiratory flow rate (PEFR) may be unchanged as the larger airways can still empty normally, whereas the airflow limitation during expiration in the smaller airways becomes more difficult giving the "scooped" appearance in the expiratory phase of the flow-volume loop[19]. In restrictive conditions, airflow is normal such that the shape of the flow-volume curve appears normal. However, as lung volumes are reduced overall due to restriction, the size of the loop is relatively smaller and the flow-volume loop itself shifts to the right[19].

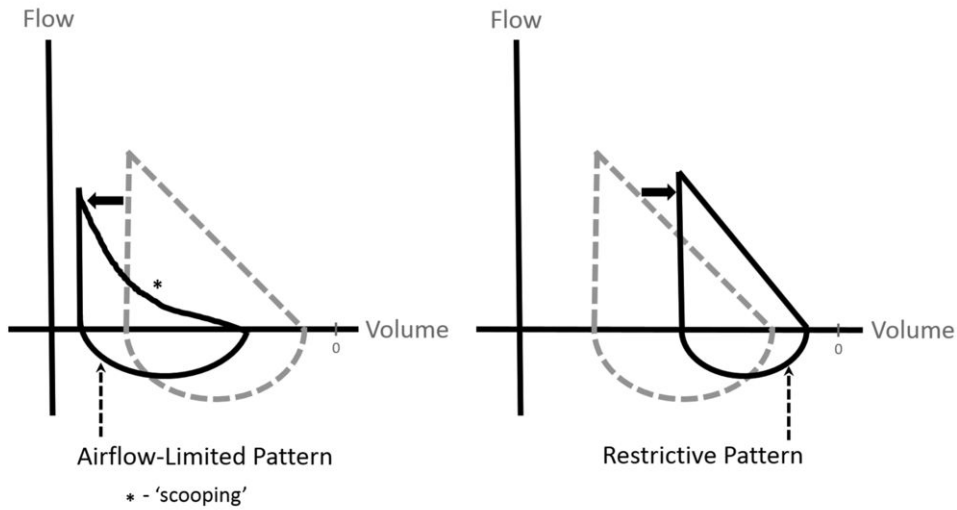


Figure 5:

Flow-volume loops showing an obstructive lung pattern (airflow limitation) and a restrictive lung pattern. Reproduced from Interpretation of pulmonary function tests: beyond the basics, Staitieh BS, Ioachimescu OC. J Investig Med 2017;65:301–310[19], Copyright © 2017, with permission from BMJ Publishing Group Ltd.

1.4. MBW testing

Peripheral airways have been described as the "silent lung zone" as conventional lung function tests such as spirometry are unable to pick up problems in this structural part of the lung in diseases involving the peripheral airways, including chronic obstructive pulmonary disease (COPD)[9]. Pathological changes from respiratory diseases such as COPD causes changes in small airway dimensions, affecting the ventilation in the parallel pathways. This is known as ventilation inhomogeneity and can be measured using the SBW and MBW techniques.

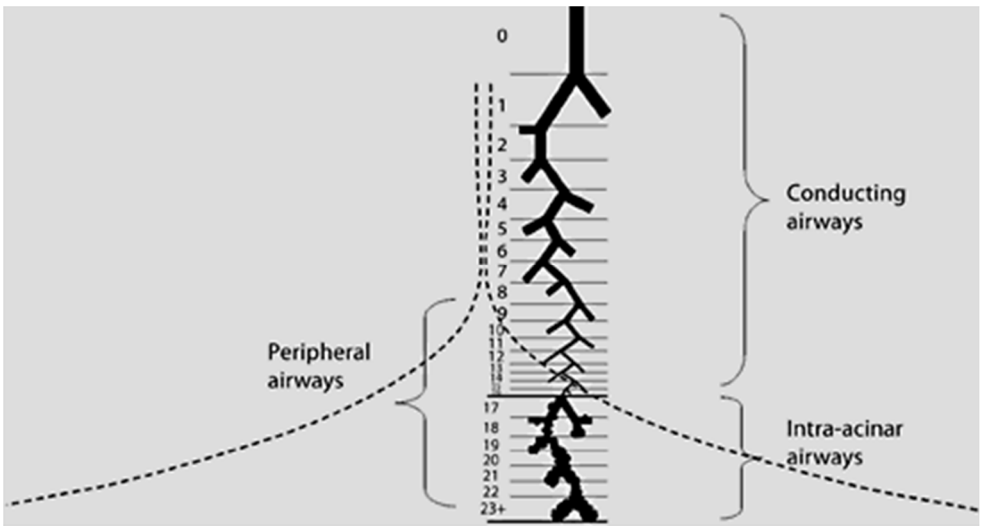


Figure 6:
The airway tree with airway generations. Broken lines corresponds to total airway cross-sectional area. Reproduced from: Inert Gas Washout: Theoretical Background and Clinical Utility in Respiratory Disease, Robinson PD, Goldman MD, Gustafsson PM. Respiration 2009;78:339–355[9]. With permission from Karger Publishers. Copyright © 2009 S. Karger AG, Basel, Switzerland

The MBW gas washout technique assesses how efficiently ventilation is distributed in the lung and relies on a wash-in phase (for extrinsic gases) and washout phase of an inert intrinsic gas (e.g. sulphur hexafluoride or helium) or extrinsic gas(e.g. nitrogen or argon)[20]. As mentioned, the gases must be inert and therefore safe at the concentrations used, they should not be involved in gas exchange and should not be significantly absorbed into the bloodstream and other tissues, or excreted by the body[6]. The process involves the washing out of the inert tracer gas during relaxed tidal breathing[21] and so reflects ventilation inhomogeneity at FRC[22], as the volume of gas in the lungs at the start of the washout is equal to the FRC. For intrinsic gas washouts such as nitrogen washouts, the subject is switched to breathing 100% oxygen, and for extrinsic gases the gas is first washed in and then out of the lungs (by switching off the inert gas supply

and converting to breathing room air). Therefore, there is a fall in the peak concentration of the exhaled gas with each breath of the washout. As the test requires tidal breathing and no complex co-ordination or co-operation, it is easy to perform in all age groups. Ventilation distribution abnormalities found using these techniques are commonly seen in obstructive lung disease as it reflects small airways function where gas mixing occurs[22] and therefore changes in ventilation homogeneity may be seen before ventilatory capacity is affected on spirometry[6]. The washout takes a greater number of breaths to complete in cases of airways disease due to the unevenness of ventilation in small airways disease affecting the gas mixing function of the lung. Both the SBW and MBW can yield measures such as the phase III slope. In SBW the expirogram involves a VC manoeuvre consisting of four phases of which phase III represents the alveolar phase[9]. The slope of this phase reflects the ventilation inhomogeneity over a range of lung volumes. However, the phase III slope from SBW reflects overall ventilation inhomogeneity, and the MBW phase III analysis can add further information with regards to the specific location of pathology in the peripheral airways (S_{con} and S_{acin}, conductive and acinar lung zones respectively)[9, 23].

1.4.1. Lung Clearance Index (LCI)

One of the most common measures derived from MBW is LCI. It is defined as the number of lung volume turnovers that are needed for the inert gas at the start of the MBW (FRC) to reduce to $1/40^{th}$ of its starting concentration. LCI is therefore calculated using the equation below[21]:

$$\text{LCI} = \text{cumulative expired volume (CEV)} / \text{FRC}$$

The LCI value increases as disease severity increases, and therefore can be a simple but sensitive measure of small airways disease that is easily interpreted and can be carried out with relative ease. It is widely used in the paediatric population due to its methodological simplicity (from the subjects perspective this is normal tidal breathing throughout the test), notably in young children with cystic fibrosis (CF)[24, 25]. Its value in detecting early destructive changes in children as young as preschool age has been found, where LCI has been found to be raised in preschool children with CF when spirometry values may still be within the normal range[24]. The sensitivity of LCI as a marker of small airway dysfunction has been found to be of value over spirometry, where considerable structural damage needs to occur before FEV_1 becomes impaired[21]. This has been demonstrated on computerised tomography (CT) scanning, where damage has been found on imaging but FEV_1 has remained in the normal range[26]. Although the value of LCI in CF has been well established, it has since been suggested that the value of LCI can extend to all pulmonary conditions that initially involve the small airways, such as asthma and COPD, particularly in the development of such conditions[27].

1.5. Other methods of testing lung function

Body plethysmography and imaging are alternative ways to measure static lung volumes. Static lung volumes that can be assessed using body plethysmography include FRC, RV and TLC[28]. CT-detected emphysema has been thought to be associated with lung function decline and development of airflow obstruction in current and former male smokers[29]. It has been suggested that CT-detected emphysema can identify early signs of airflow obstruction in those who may have normal lung function on spirometry and therefore identify those at risk of developing airflow obstruction later in life[29].

The single breath diffusion capacity (transfer factor of carbon monoxide) is a measure of the ability of the lung to transfer gas from inhalation into the pulmonary capillary blood (diffusion from the alveoli to pulmonary capillaries) and has use in distinguishing between the phenotypes of COPD (emphysematous or bronchial- see section 1.7.1).

The forced oscillation technique (FOT) uses small-amplitude pressure oscillations on normal breathing to measure respiratory mechanics[30]. It has been shown that FOT is as sensitive as spirometry in detecting smoking or occupational hazard related lung function impairment, with an added advantage being that no respiratory manoeuvre is needed[30]. Furthermore, it has also been suggested that FOT has the ability to detect small airway abnormalities and correlates to respiratory symptoms when spirometry appears normal and as such has a potential role in early diagnosis[31]. However, an alternative view is that the value of FOT in disease prediction and progression has not yet been proven to the extent is has been with spirometry and as such spirometry does continue to have some benefit over FOT, especially in longitudinal studies assessing long term outcomes[32].

1.6. The use of lung function testing

The current use of lung function tests are primarily to assess, to aid in the diagnosis of and the management of pulmonary diseases. An individual's value from various lung function tests are compared to what would be the predicted value for their age, height, gender and ethnicity. Values obtained are commonly presented as percent of the individuals predicted value (i.e. normal FEV_1 and FVC are ≥80% of their respective predicted values)

1.6.1. Reference equations for FEV_1 and FVC %predicted

The choice of a reference population to obtain predicted values for subjects is therefore key. Various reference equations exist, and the selection should ideally be made from a representative sample of "healthy" subjects from the general population that have the same anthropometric measures (age, height, gender and ethnicity) as the population under study[33]. Additionally, for the meaningful use of a reference equation to interpret the lung function measure in question, the measurement itself should be reliable with its sources of variation known[34]. The techniques and conditions used to obtain the measure in both sources (reference study population to obtain the predicted value and the lung function measure being expressed as a percent of the predicted value) should also be comparable[34].

1.6.2. The FEV_1/FVC ratio threshold

The ratio of FEV_1/FVC as previously mentioned, can give important information regarding the type of lung disease that could be present and its subsequent management. There are two cut-off points that are commonly used to express the "normal" ratio, and there is currently no general consensus on which should be used to aid the diagnosis of obstructive conditions that affect the ratio such as COPD. The Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD) criteria for the diagnosis of airflow limitation remains at a fixed post-bronchodilator value of $FEV_1/FVC < 0.70$[17]. However the use of the lower limit of normal (LLN) – a statistically defined cut-off point of the lower 5th percentile of a healthy reference population for the ratio has also been advocated[33, 35].

As FEV_1 declines faster with age than FVC, the GOLD fixed-ratio may result in the over-diagnosis of COPD in the elderly, along with the under-diagnosis of COPD in those under the age of 45 years[17]. The threshold of $FEV_1/FVC <$ LLN has been thought to provide lower prevalence estimates of COPD in the elderly compared to the fixed-ratio of 0.70[36]. Whether over-diagnosis (false positives) and hence over-treatment of individuals using the fixed-ratio or "missing" cases (false negatives)- (especially milder cases of COPD)[36] that do not meet the LLN threshold[37] is more critical remains debatable. **Figure 7** illustrates the misdiagnosis in both directions that can occur with these thresholds.

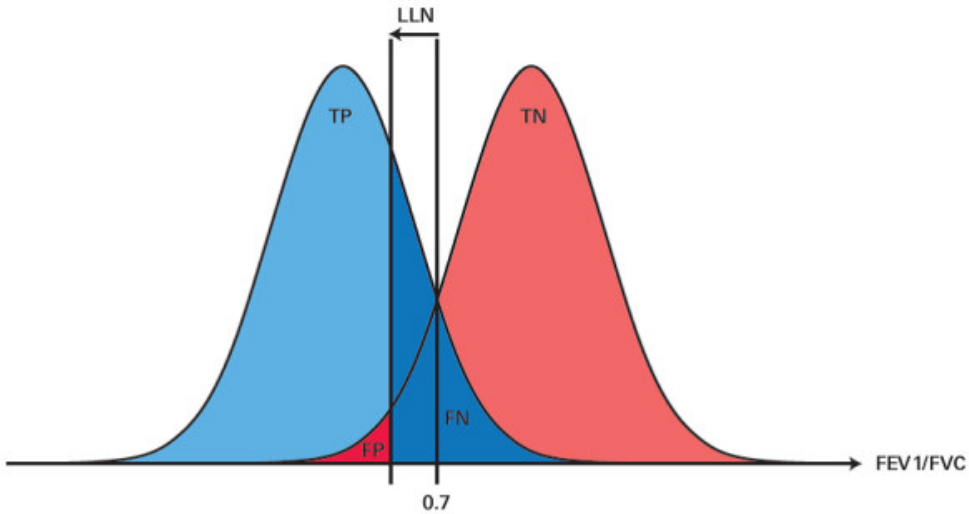


Figure 7:
Misdiagnosis related to the changing thresholds of the FEV_1/FVC ratio in the elderly. LLN (lower limit of normal), TP (true positive), TN (true negative), FP (false positive), FN (false negative). From Güder et al. *Respiratory Research*. (2012)[36] 13(1): 13. (Creative commons attribution license. Link: http://creativecommons.org/licenses/by/2.0)

1.7. The history of COPD

1.7.1. Pathogenesis

Although its earliest references can be traced back to 1679[38], COPD continues to constitute a current global public health problem[39]. It was not until landmark meetings in 1959 and 1962 that the definitions of what COPD constitutes were formally established[38]. A susceptible lung that is exposed to some form of noxious stimuli, which in the western world is mainly cigarette smoking but can also include air pollution (especially household air pollution) in other parts of the world, is what forms the basic pathogenesis of COPD. Characterised by chronic bronchitis and emphysema, COPD is a progressive disease which in many cases is defined clinically by increasing dyspnoea, a chronic cough and sputum production. Loss of lung parenchyma (emphysema) and changes in the bronchial epithelium of the small airways are characteristic structural changes in the lung during COPD, which leads to airway obstruction and hypersecretion[40], causing the clinical features associated with COPD. FEV_1 decline is thought to be related to thickening of the walls of small conducting airways along with mucous exudates, causing airway obstruction[40].

There are two phenotypes that are commonly described as being associated with COPD. Burrows et al first described these[41]; type-A (emphysematous) patients are typically thin and elderly with progressive dyspnoea and are thought to mainly exhibit emphysematous features with late-onset mild bronchitis[41], and type-B patients (bronchial) typically have a more stocky body-build, with a chronic history of a productive cough but minor or no emphysematous changes in the lung parenchyma[41]. Imaging may show the evidence of inflammatory changes in the lung of type-B patients, and by middle-age severe disability and heart-failure can occur[41]. A reduction in the diffusion capacity is caused by parenchymal diseases such as emphysema, whereas it can appear normal in predominantly "bronchial" COPD. Therefore, the diffusion capacity has been thought to be an easily producible measure that can help distinguish between these two specific phenotypes of COPD. However, it has been found that rather than using clusters of clinical features to define mutually exclusive COPD subtypes or phenotypes, the heterogeneity associated with COPD could be better defined by using continuous traits such as airflow limitation (using spirometric measures) and quantitative emphysema (using CT) that may co-exist to different extents within the same individual[42].

1.7.2. COPD progression

Biochemical and cellular changes can occur early before any clinical features may be present or changes on spirometry (**Figure 8**). Often when clinical signs are present, COPD may have progressed to a moderate-advanced stage[43]. It has been found that low lung function is found in more than 10% of the population aged over 45 years, but is not associated with reported current obstructive lung disease 63.3% of the time[43]. This is thought to represent unrecognised obstructive lung disease which if identified early when subjects are less symptomatic or asymptomatic, targeted interventions could subsequently alter its course [43].

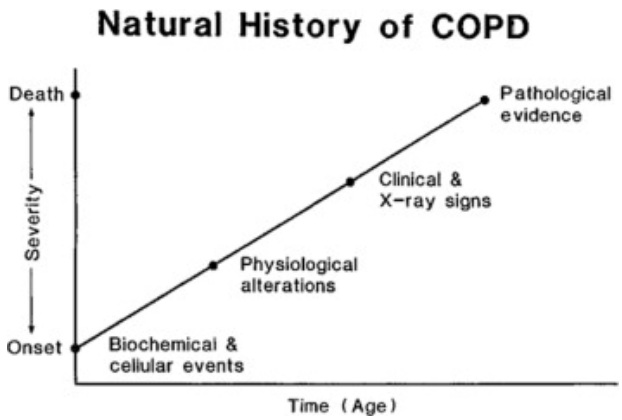


Figure 8:
The natural history of COPD. Reprinted from COPD in perspective, TL Petty Chest, 121 Suppl 116S-120S, 2002[44]. Copyright © 2002 The American College of Chest Physicians, with permission from Elsevier Inc.

1.7.3. Exacerbations

A COPD exacerbation is defined as "an acute worsening of respiratory symptoms requiring a change in the treatment"[45], triggered for example by smoking/air pollution or respiratory infections[45]. COPD exacerbations are a frequent cause for patients to present to secondary care, leading to COPD-related hospitalisations. Severe acute exacerbations are associated with an increased risk of death (especially if these are frequent or result in hospitalisations)[46] and a deterioration in the health status of an individual. The airflow obstruction, dyspnoea and impaired exercise tolerance that occurs after an exacerbation can also result in struggles in performing activities of daily living[40].

1.8. Lung function: Age and smoking effects

1.8.1. Age and lung function

During the first two decades of life the lungs grow and mature to achieve maximal lung function around age 20 for women and 25 for men [47, 48]. After the age of 35 years, there is a progressive decline in lung function. The three physiological processes thought to underline the functional changes seen with increasing age are; a decrease in the elastic recoil of the lung, a decrease in chest wall compliance, and a decrease in respiratory muscle strength[48]. These changes of normal ageing result in airflow limitation and increased air trapping, leading to a decline in FEV_1 of up to 30mL/ year, a decline in the FEV_1/FVC ratio, and an increase in RV by 50% from the ages 20-70 years[49]. FVC

declines at a later age than FEV_1 and at a slower pace, hence the suggestion that the use of FEV_1/FVC ratio alone can lead to the over-diagnosis of COPD in the elderly [50]. Concerning LCI, a study assessing the measures of small airway function and the effect on them from normal ageing found that LCI (along with other MBW indices) increased with age, which was represented by a worsening of ventilation heterogeneity in the age range 25-65 years[51]. A 0.22 unit increase in LCI per decade was found, which was concluded as being a small but important factor that needs to be considered when assessing LCI in adults[51].

1.8.2. Smoking and lung function

COPD and its relationship with "susceptible smoking" was brought to light by an 8 year follow-up study of British male post-workers by Fletcher and Peto in 1977[52]. Their findings still provide the basis for the management and advice given to smokers in the earlier stages of their life who could be susceptible to premature disability and death due to the effects of smoking. The study demonstrated the relationship between smoking and airflow limitation as measured by FEV_1 and explained the relationship in smokers in terms of their "susceptibility" to the effects of smoking on airways function. They found that FEV_1 gradually declines over a lifetime, and the rate of loss accelerates to a certain degree with age. This age-related decline is additionally accelerated in susceptible smokers. In those who have never smoked, or in those who smoke but are not susceptible to the effects of it, clinically significant airway obstruction does not develop over the course of life. In smokers who are susceptible to the effects of smoking, irreversible obstructive changes can occur. If smoking cessation then occurs in a susceptible smoker, the damage cannot be reversed, however further loss of FEV_1 over the remaining life-span can revert to normal[52].

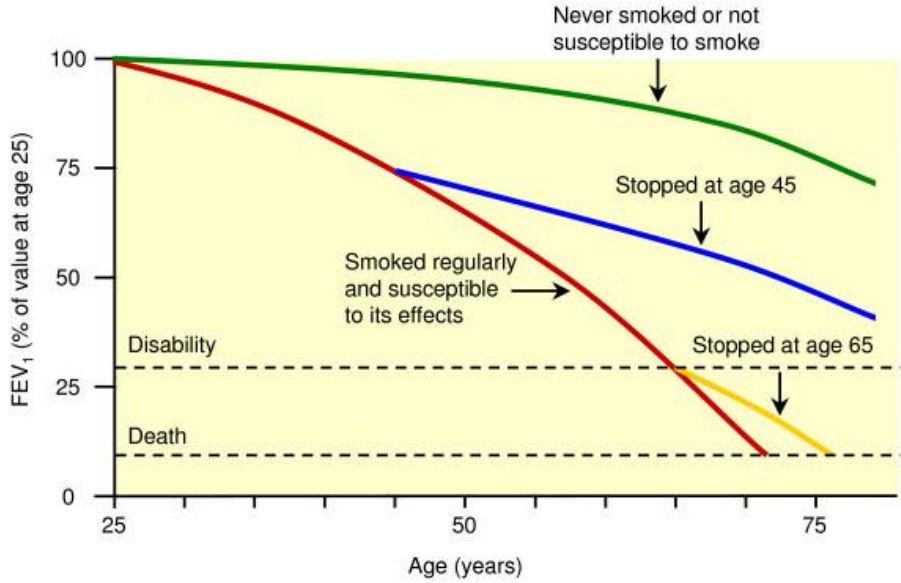


Figure 9:

"The fletcher curve". From: Kotz et al. BMC Public Health. 2007; 7: 332[53]. (Creative Commons Attribution License. Link http://creativecommons.org/licenses/by/2.0), Adapted from Fletcher & Peto (1977): "The natural history of chronic airflow obstruction"[52].

Severity of airflow obstruction as measured by FEV_1 is therefore the main determinant of prognosis in COPD[52]. Based on these findings, Fletcher and Peto (1977) advocated the screening of lung function in smokers in early life in order to prevent severe obstructive airways disease in those with identified low lung function[52]. It was also advised to take measures to help with smoking cessation in those with identified poor lung function[52]; principles which are still utilised today. Studies since have also found that the rate of COPD development in smokers can be reduced when those at risk of developing it stop smoking, which forms the basis of the smoking cessation advice given in practice and still remains even in those with advanced COPD[54, 55]. However, smoking has since been thought to affect more than the rate of decline in lung function. Smoking in early life has be found to affect three determinants of the FEV_1 at any given time in adult life[34]. Firstly, it affects the peak lung function that is achieved during early adulthood. In individuals who start actively smoking in adolescence, the level and rate of growth in lung function is affected[34]. Secondly, the early adulthood plateau phase of FEV_1 (between ages 18-25 years) is shortened in smokers[34], i.e. the onset of decline is earlier in smokers compared to non-smokers. Thirdly, smoking affects the rate of decline after the plateau (as described by Fletcher et al). Therefore it has been suggested that a single low measurement of FEV_1 in an adult cannot indicate which of these reasons (or a indeed a combination of) is responsible for the resultant low value observed[34].

1.8.3. The "horse-racing effect"

This concept first defined in 1981 by Peto[56] and then termed in 1987 by Burrows et al[57] describes the effect early life lung function can have on later lung function and disease processes. The principles behind the phenomenon can be applied to FEV_1 decline (or other similar measures such as blood pressure increase). An individual who for whatever reason, has an FEV_1 that is declining faster than the average rate in early life, will have a lower than average FEV_1 later in life, and will find that FEV_1 continues to decline faster than average in later life too[56]. Therefore there is a correlation between the true absolute value and true rate of change[56]. The "horse-racing" analogy to this is that in a horse race where one horse is faster than another, the faster horse would be leading at the half-way point of the race. Similarly, for those that have a faster rate of decline in lung function during early and mid-life are more likely to develop chronic lung disease later (i.e. "win the race")[58]. Therefore, a low initial level of FEV_1 is thought to predict subsequent rapid decline in FEV_1 and development of COPD.

However, it has since been suggested that not all patients with COPD will experience this rapid decline in FEV_1 and some individuals will "start out" with a low level of lung function and subsequently develop airways obstruction[59] (**Figure 10**). The focus should therefore be on attaining maximal lung function in early life, through various general lung health promotion measures starting as early as childhood and adolescence[60]. The concept of early life events such as childhood respiratory infections and adult lung function was supported by the early work of Burrows et al [61] and then the widely known work on foetal-programming[62].

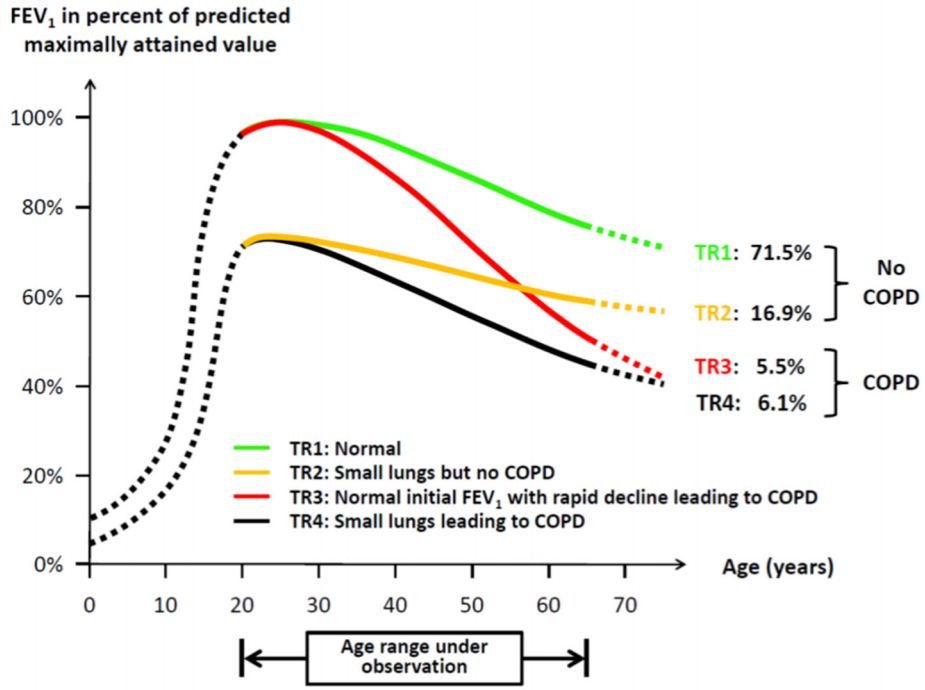


Figure 10:
Distribution of the participants into four trajectories TR1 to TR4 (defined according to baseline level of FEV_1 (above or below 80% predicted value) and presence or absence of COPD (GOLD grade >2) at final examination. NB: broken lines represent hypothetical trajectories. Reproduced with permission from Lange et al. N Engl J Med. 2015 (Supplement)[59], 373(2):111-22. Copyright Massachusetts Medical Society.

1.9. Lung function and health

1.9.1. Low lung function and future cardiovascular disease

Low lung function has been found to be associated with fatal and non-fatal cardiovascular disease (CVD) outcomes including myocardial infarction (MI), ischaemic stroke, heart failure and atrial fibrillation along with all-cause mortality, beyond the effect of smoking[63-75]. There is a known co-morbidity between COPD and cardiac disease, and indeed both diseases share many risk factors making COPD a plausible explanation for the link between low pulmonary function and cardiac diseases. However, the relationship between low lung function and cardiac disease exists in life-long never smokers, and beyond the effect of systemic inflammation and physical inactivity, therefore the exact mechanism behind this relationship remains largely

unknown[76]. Prospective cohort studies as early as the mid 1970's found that low levels of lung function could actually predict the onset of cardiac diseases[63, 70] and this relationship has since been repeated in studies from the general population[71]. Low levels of lung function in early life even in never-smokers can increase the future risk of coronary heart disease (CHD), stroke and heart failure, all of which can lead to premature mortality. Low levels of lung function in early life can also in addition lead to premature all-cause mortality in smokers and in life-long never smokers. FEV_1 has been found to be of the same magnitude as cholesterol concentration and social class for ischaemic heart disease (IHD), and second in importance to smoking for all-cause mortality in terms of the population attributable risk[73]. As CVD itself constitutes a large morbidity and mortality burden, knowledge of any potential mechanisms involved in its aetiology and subsequent disease prevention can be of immense value to the individual and society. The relationship between lung function and other incident diseases that in turn increase the risk of CVD and mortality could in part explain the strong association known to exist between lung function and cardiac diseases and death.

1.9.2. Low lung function and future COPD

There have not been many studies that assess the risk of incident COPD in relation to early life lung function[59], which again is known to be a risk factor for the development of CVD. If low levels of lung function predict the onset of incident COPD events, this could also be in part the explanation that links early life lung function to CVD risk later in life. Lange et al[59] used three cohorts to find that the classic trajectory of accelerated FEV_1 decline from a normal level is not always the trajectory in COPD, and in many people, a low maximally attained FEV_1 in early life with a subsequent normal rate of decline can also be the trajectory to COPD. Although this illustrates the different trajectories and decline patterns associated with baseline FEV_1 level, quantifying the risk of COPD associated with low levels of lung function in early life in relative terms could also be of immense value.

1.9.3. Low lung function and future diabetes mellitus

Diabetes mellitus (DM) is known to have a cross-sectional correlation with poor lung function in that individuals with DM tend to have lower levels of FEV_1 or FVC than individuals without DM[77-80]. However, the temporality of this association remains unclear. The systemic effects of DM resulting from high levels of glucose have included inflammation, autonomic neuropathy that can involve the lung along with various other organs, microangiopathy of the lung vasculature, and glycosylation of the lung parenchyma leading to a loss of elastic recoil[81], all of which can lead to a decline in lung function[78, 82]. However, other factors may influence this association in the opposite direction i.e. low levels of lung function leading to DM later in life. Early life factors

that result in low maximal levels of adult lung function such as foetal-programming, and low birth weight, or prematurity could potentially also increase the risk of DM. Metabolic syndrome consists of a cluster of insulin resistance, hypertension, abdominal obesity, and deranged blood lipids and is associated with increased cardiovascular risk. The reduction in physical activity associated with poor lung function may result in a more sedentary lifestyle, which in turn can increase the risk of abdominal obesity and subsequent metabolic syndrome, predisposing to future DM [83, 84]. Associations between low levels of lung function and other factors associated with metabolic syndrome such as blood pressure, lipids and visceral obesity have additionally been explored. Studies have shown that a relationship exists between poor lung function and abdominal obesity, which has been suggested as a potential mechanism that links low lung function to DM and metabolic syndrome and may partly explain the association between CVD and poor lung function[85-87].

Studies have also shown an inverse relationship between hypertension and lung function[88, 89]. However, it has been suggested that hypertension in combination with the use of anti-hypertensive medication rather than hypertension itself may be the reason for the relationship between lung function and blood pressure[90]. Although the relationship between high serum cholesterol levels and CVD is well known, there is thought to be a "U-shaped" curve associated with all all-cause mortality, due to low levels of cholesterol associated with non-cardiac mortality such as malignancy and respiratory diseases[91]. Lower levels of low-density lipoprotein and total cholesterol have been thought to be associated with better lung function, and lower levels of high-density lipoprotein cholesterol associated with poorer lung function[91].

Therefore, there may be various mechanisms which may link poor lung function and the development of metabolic syndrome and DM. Lower levels of lung function preceding and predicting the onset of DM could be a potential mechanism linking lung function to the development of future CVD and other adverse outcomes related to DM.

2. Aims

The general aim of the current thesis was to investigate the association of lung function at baseline and the risk of future adverse health outcomes in an urban population. The specific aims of the papers included in this thesis are as follows:

I. To assess the incidence of COPD hospitalisations, all-cause mortality and future lung function decline in relation to baseline spirometry.

II. To assess the role of baseline LCI in the development of future pulmonary obstruction and COPD hospitalisations, including the added value of LCI to conventional spirometry when assessing this risk.

III. To add clarity to the temporal relationship between low lung function and DM by establishing if low lung function precedes the development of DM and if so, how long after baseline lung function measurement this risk is observed.

IV. To assess how decrements in different measures of lung function at baseline are associated with the future risks of COPD and coronary events, including the significance of any differences or similarities in risks observed.

3. Methods

3.1. Study populations

3.1.1. The Men Born in 1914 cohort

The "Men Born in 1914" cohort was a prospective population-based study in Malmö, Sweden[92]. The purpose of the study was to perform a health examination on a selected number of men with an interest mainly on cardiac, vascular and lung diseases. All men born in even-numbered months in the year 1914 and living in Malmö in 1968 were invited in 1968-1969 to take part in a health examination when the men were 55 years old (most examinations were conducted between 1969-1970, close to participants 55th birthday). The examination included lung function testing, electrocardiography (ECG) examination, laboratory tests (urine and blood testing), blood flow examination in the lower limbs, x-ray of the heart and lungs, and a general physical examination. It also included a health questionnaire with questions related to existing health problems (e.g. DM, hypertension, cancer, asthma, myocardial infarction, stroke), symptoms of cardiac, lung or vascular diseases (e.g. dyspnoea, chest pain, cough, claudication). Questions on lifestyle habits included smoking/tobacco use, physical activity, coffee consumption, stress, and employment status. The questionnaire also included questions on medication use, and family history of health conditions.

Out of an eligible population of 809 individuals, 703 men attended the examinations in 1969 (participation rate 87%). Between 1982-1983 subjects from the study who were alive and still residing in Malmö were invited to take part in a re-examination which included lung function testing. Non-participants in the1969 examination and men who moved to the city after the first examination were also invited.

The cohort was used for analyses in **Papers I, II** and **IV.** Study flow of participants for these studies are shown in **Figure 11**.

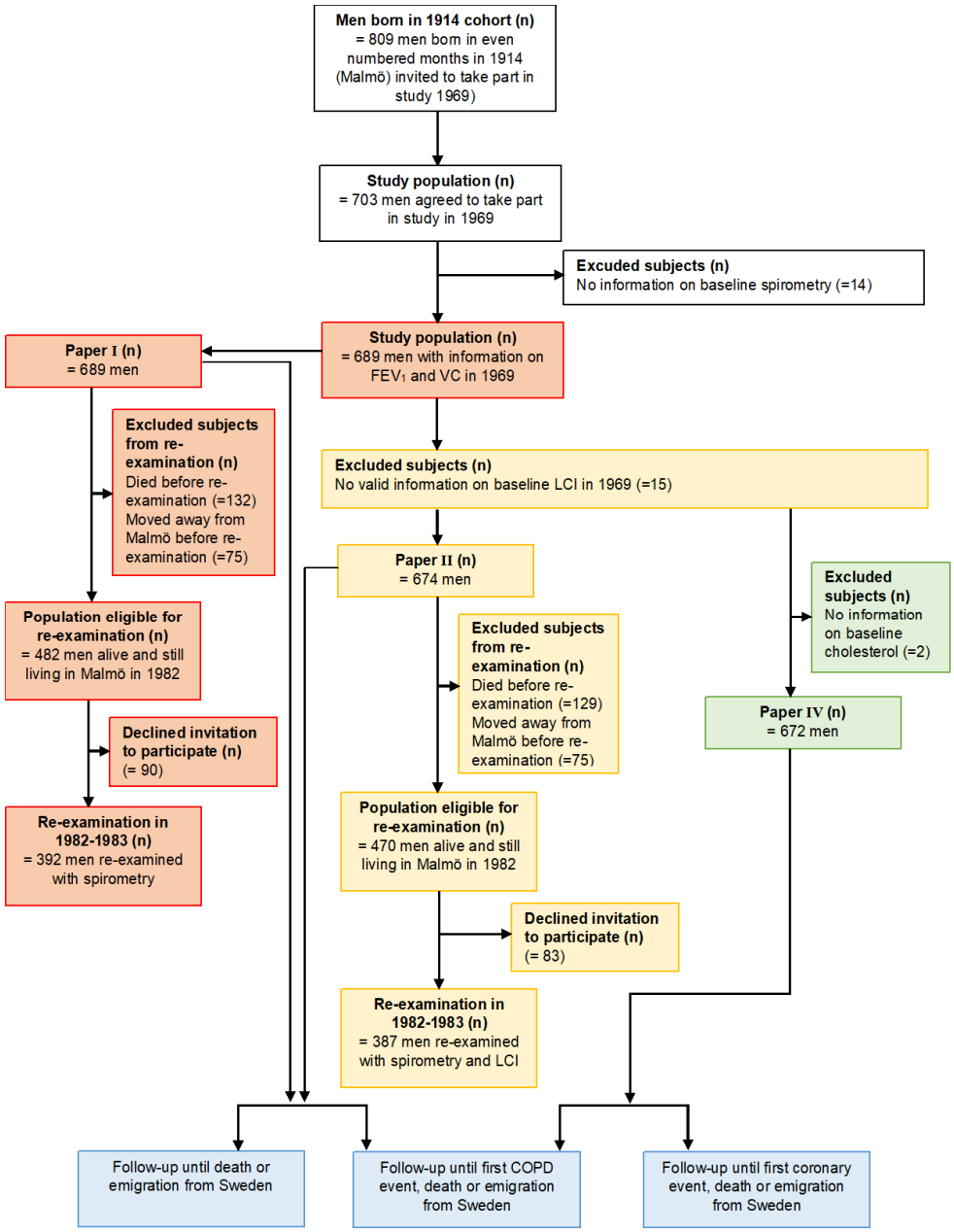


Figure 11:
Flow of participants in Papers I, II and IV

3.1.2. The Malmö Preventive Project

Between 1974 and 1992 a health screening project known as the Malmö Preventive Project (MPP) was undertaken. The aim of this project was to screen a large population of middle-aged adults with an aim to identify high-risk subjects and offer preventative treatment. Mail invitations were sent to complete birth cohorts born between 1921 and 1949 (men born in 1921, 1926-1942, 1944, 1946, or 1948-1949 and women born in 1926, 1928, 1930, 1932-1936, 1938, 1941-1942, or 1949). A total of 33,346 subjects participated in the screening, 22,444 men and 10,902 women between 1974 and 1992. From 1974-1981 mainly men were examined and from 1982-1991 mainly women were examined. Attendance rate was over 70% and the mean age at baseline was 44 years for men (range 27-61 years) and 50 years for women (range 28-58 years). As part of the project, subjects underwent a physical examination, laboratory tests and questionnaires. Data from the MPP was used for analyses in **Paper III**.

3.1.3. The Malmö Diet and Cancer study-cardiovascular cohort

The Malmo diet and cancer study- Cardiovascular cohort (MDC-CC) is a sub-cohort of the Malmö diet and cancer study (MDCS). The MDCS is a population-based cohort study which aims to assess the relationship between dietary and lifestyle factors on malignancy and mortality outcomes. Baseline examinations were taken from 1991 to 1996. In 1991, men and women born between 1926-1945 were invited (n=53,325), and in 1994 the invitation was extended to include women born between 1923-1950 and men born between 1923-1945 (n=74,138). Eligible subjects (n=68,905) were invited to take part in baseline examinations of which 28,098 individuals completed components of the examinations (questionnaire, dietary assessments, and anthropometric measurements) and included 11,063 men and 17,035 women. Between 1991-1994 a random 50% (every other subject) screened in the MDCS were invited to take part in the cardiovascular sub-cohort (MDC-CC) of the MDCS (n=12,445), the aim of which was to study carotid disease and its epidemiology. Of these, 6,103 subjects responded to the invitation for carotid ultrasound examination and of these 5,540 took part in further laboratory anaylyses[93]. In **Paper III**, a sub-analysis using the MDC-CC was carried out, which included subjects from the MPP who also took part in the MDC-CC.

Figure 12 shows the flow of participants in the MPP and MDC-CC for **Paper III**.

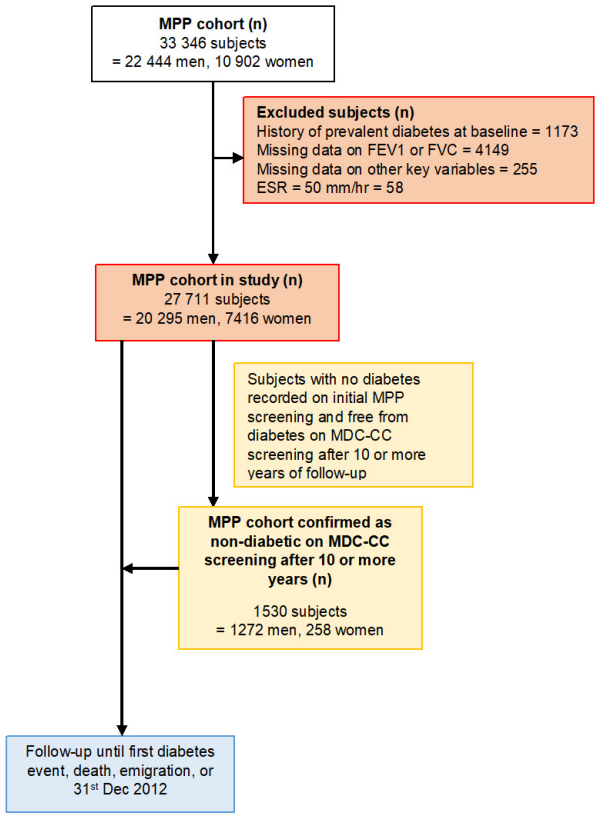


Figure 12:
Flow of participants in Paper III

3.2. Assessment of exposure

3.2.1. Paper I

All measurements were carried out at the Department of Clinical Physiology (Malmö University Hospital). Baseline spirometry was assessed using a Bernstein-type spirometer to obtain measurements of FEV_1 and VC without prior bronchodilation, corrected for body temperature, atmospheric pressure and water saturation. Two acceptable manoeuvres were required. The curves were inspected to ensure performance and co-operation were satisfactory, and repeated until two acceptable measures were

taken. The cohort was then divided into three groups based on the fixed ratio (FR) cut off for COPD ($FEV_1/VC \geq$ or < 0.70) and the LLN cut off point for COPD. European reference values were used in the calculation for the cut-off point of the LLN[94]. The three exposure groups were therefore: $FEV_1/VC \geq 0.70$ (normal), $FEV_1/VC < 0.70$ but $\geq$ LLN (FR+LLN- intermediary group) and $FEV_1/VC < 0.70$ and <LLN (FR+LLN+). FEV_1 and VC were also expressed as a percent of their respective predicted values, using European reference equations and the cohort was then divided into quartiles for both measurements (Q4: reference group for both). Restrictive lung function was defined as VC ≤ 80% of the predicted value but normal or above normal FEV_1/VC ratio (≥ 0.70). Subjects who were re-examined in 1982 were also divided into the three groups using the FR and LLN cut-off points. FEV_1 decline was then assessed between the two time points along with re-classification of groups from 1969-1982.

3.2.2. Paper II

Spirometry measurements (FEV_1 and VC) were carried out as described in **Paper I** at the Department of Clinical Physiology (Malmö University Hospital). In addition to spirometry, LCI was also measured using nitrogen MBW (Ohio 700 nitrogen meter-Biomedical Products, Houston, Texas, USA). The technique involved the individual breathing normally (room air) through a mouth-piece to obtain a stable breathing level, after which a stopcock was turned at FRC and the individual was then switched to breathing 100% oxygen (whilst continuing normal tidal breathing). This continued until the end-expiratory concentration of nitrogen reduced to 2%. A diffusion tight aluminium-plastic bag was used to collect the washout volume, and the exhaled volume then was measured with a wet gas meter which has an accuracy of <1%[95]. The LCI was calculated according to Bouhuys et al[96]. European reference values were used to express FEV_1 as %predicted values, and for the calculation of the LLN for FEV_1 and the FEV_1/VC ratio[94]. The upper limit of normal (ULN) was calculated for LCI using the present cohort (distribution in 104 never-smokers). Categories of lung function exposure groups therefore included quartiles of LCI at baseline (Q1: reference), LCI > or < ULN, and division of cohort into groups based on both FEV_1 or FEV_1/VC and LCI (e.g. normal FEV_1 and LCI, normal FEV_1 but LCI>ULN, FEV_1<LLN but normal LCI, and FEV_1<LLN, LCI >ULN).

3.2.3. Paper III

Spirometry (FEV_1 and FVC) was measured by experienced nursing staff using a Spirotron apparatus at baseline. Only one acceptable manoeuvre was required. Linear regression of never-smokers in the present MPP cohort was used to derive the reference equations needed to express FEV_1 and FVC as %predicted values[97-99]. However, European reference equations were used as part of a sensitivity analysis to compare the

results to that obtained from the MPP cohort reference equations. Sex-specific quartiles were created for FEV_1 and FVC%predicted.

3.2.4. Paper IV

Baseline exposure (spirometry and LCI) was assessed as described in **Paper I** and **Paper II**. Baseline lung function groups were expressed in their original forms (VC and FEV_1 in litres) and FEV_1/VC as a ratio. European reference equations were used to calculate the LLN for the FEV_1/VC ratio, and for %predicted values of VC and FEV_1 (used for comparing results per 1 standard deviation (SD) decrement in FEV_1 and VC (L) to 1 SD change in %predicted values of FEV_1 and VC).

3.3. Assessment of covariates

3.3.1. Paper I

Adjustments were made for well-known potential confounding factors between the exposure (low baseline lung function) and the outcomes (incident COPD events, and all-cause mortality). Smoking, physical activity and diabetes history were assessed using the health questionnaire in 1969. Smoking history was also re-assessed in 1982 but as not all subjects took part in re-examination (57% of initial cohort took part in re-examination in 1982), smoking data at follow-up was not available for all subjects initially recruited to the study in 1969. Smoking history was divided into three groups (never smokers, ex-smokers and current smokers), where men who has stopped smoking at least a month before the examination were considered ex-smokers. Physical activity was also divided into three groups (low, moderate and high physical activity) based on questions about leisure time activity. Low physical activity was equal to being almost completely inactive (i.e. reading or watching television), moderate physical activity was equal to some physical activity at least 4 hours a week (e.g. riding a bicycle, walking to work or light gardening). High physical activity was equal to either regular physical training at least 2-3 hours a week (e.g. heavy gardening, tennis, swimming, going for a run) or regular hard physical training several times a week (e.g. soccer or competitive running/racing)[100]. A history of DM was determined by taking a medical history and by screening for diabetes at baseline using urine tests, followed up by an oral glucose tolerance test for those with a positive urine test. Although %predicted FEV_1 or VC takes into account the height of an individual, to be sure the effect of height and weight on lung function was fully accounted for, the analysis was also adjusted for body mass index (BMI) and height. Weight was measured using a lever balance to the nearest 0.1kg, and height (without shoes) to the nearest 0.5cm. Systolic

blood pressure (mmHg) was measured using a mercury sphygmomanometer and 12 x 16 rubber cuff in the morning after 10-15 minutes of rest in the sitting position. Plasma cholesterol (mmol/L) was measured after an overnight fast at the Department of Clinical Chemistry at Malmö University Hospital.

3.3.2. Paper II

Adjustments were made for potential confounding factors between the exposure (poor baseline LCI) and the outcomes (incident COPD events and all-cause mortality) as for **Paper I**. Both outcomes were adjusted for smoking, diabetes, physical activity, height and BMI. Adding plasma cholesterol and systolic blood pressure to the model for all-cause mortality did not affect the outcome and so was not included in the adjustments for all-cause mortality in this paper. The outcome of incident COPD events was additionally adjusted for baseline FEV_1 when assessing the effect of baseline LCI, as it was deemed a significant confounder in the relationship between the baseline exposure and outcome.

3.3.3. Paper III

Adjustments were made for potential confounding factors between the exposure (low baseline lung function) and the outcome (incident DM). As males and females were analysed separately, gender was not adjusted for. Age of participants, height, BMI, smoking status, baseline erythrocyte sedimentation rate (ESR), baseline glucose, baseline cholesterol, physical activity, blood pressure medication, social class, family history of diabetes, and alcohol abuse were all adjusted for when assessing the relationship between low lung function at baseline and incident DM. Blood samples for the examination of baseline ESR, glucose and cholesterol were taken after an overnight fast and examined at the Department of Clinical Chemistry, Malmö University Hospital (ESR was determined by the Westergren method). For the subgroup analysis, further inflammatory markers were used in the adjustments (Complement 3 (C3), fibrinogen, ceruloplasmin, haptoglobin, orosomucoid and alpha-1 antitrypsin) and were analysed using electroimmunoassay. Subjects were divided into never, former or current smokers based on answers to smoking habits on a questionnaire. Alcohol use was assessed using nine questions on the Malmö modification of the brief Michigan Alcoholism Screening Test (Mm-Mast) questions)[101] and more than two positive responses to the questions was considered problematic alcohol use. Physical activity was assessed using different questions in men and women as some questions were changed during the screening period. In men physical activity was assessed using the question "Are you mostly engaged in sedentary activity in your spare time?" In women it was assessed using the two questions "Are you engaged in physical activity (e.g. swimming, gymnastics, badminton, tennis, folk dance,

running etc.) 1-2 hours a week?" or "do you get to do light exercise like walking or cycling (or other activities with similar effort) on a regular weekly basis?" Low socio-economic status was defined as per the Statistics Sweden socioeconomic index (SEI) group 11-36 (i.e. unskilled or skilled manual workers or low-level non-manual workers). Prevalent cases of DM were excluded from the analysis, and were determined by fasting whole blood glucose ≥ 6.1 mmol/L at baseline (= plasma glucose ≥ 7.0mmol/L), or by self-reported DM, questionnaire reporting of DM medication or any prior diagnosis of DM according to the follow-up registers used for determining incident cases.

3.3.4. Paper IV

In order to compare the risks of both outcomes in this paper, adjustments were kept identical for the analysis of COPD events and CE. These included height, BMI, DM, cholesterol, smoking, systolic blood pressure, physical activity and IHD at baseline. All covariates were assessed as described for **Paper I**. IHD at baseline was determined by ECG examination (Q waves on baseline ECG) and questions related to past myocardial infarction (MI) or angina pectoris on the baseline questionnaire.

3.4. Ascertainment of outcomes

3.4.1. Paper I

An incident COPD event was the primary outcome, which referred to the first ever diagnosis of COPD on the Swedish inpatient register (incident COPD hospitalisation) or mortality related to COPD. Of those with poor lung function at baseline (FEV_1/VC <0.70 or <LLN) an overwhelming majority reported no dyspnoea. Incident COPD events were determined from hospital discharge summaries (primary or secondary diagnosis) and outpatient data from Swedish hospitals. Information from death certification was used to obtain information on mortality related to COPD and all-cause mortality. The International Classification of Diseases (ICD) codes used were: ICD-8; 490-492, ICD-9; 490-492, 496 and ICD-10; J40-J44 to define incident COPD events (both hospitalisations and mortality related to COPD). The Swedish patient register and Swedish cause of death register were used for case retrieval. The diagnoses in the Swedish patient register were taken from hospital discharge summaries set by board-certified physicians in all Swedish hospitals. This registry was in operation in the south of Sweden throughout the follow-up period. FEV_1 decline between the ages 55 to 68 years was assessed as an additional outcome which was adjusted for initial FEV_1 and smoking.

3.4.2. Paper II

The outcomes of incident COPD events (hospitalisations and mortality) and all-cause mortality were assessed and ascertained as described for **Paper I**. New cases of pulmonary obstruction at age 68 years were assessed as an additional outcome (FEV_1/VC < LLN) according to categories of baseline LCI among those with no obstruction at age 55 years (baseline).

3.4.3. Paper III

Incident DM was defined using various registers. The Malmö HbA_{1c} Register (MHR) includes HbA_{1c} measurements analysed at the Department of Clinical Chemistry, Malmö University hospital that have been collected from both institutional and non-institutional care in the greater Malmö area from 1988 onwards. The Swedish National Diabetes Register (NDR) includes a physician diagnosis of DM; fasting plasma glucose ≥7.0 mmol/L on two separate occasions or two HbA1c values ≥6.0% as per the Swedish Mono-S standardization system. The Swedish hospital discharge register (operating since 1970 and nationwide since 1987), the Swedish outpatient register, the nationwide Swedish drug prescription register (operating since 2005), and the regional diabetes 2000 register of the Scania region (requiring a physician diagnosis of DM - fasting plasma glucose ≥7.0 mmol/L on two separate occasions) were also used to retrieve incident cases. Incident cases of DM were also retrieved from re-examination of the MPP cohort.

3.4.4. Paper IV

The two primary outcomes included in this paper which were compared and contrasted were; incident COPD events (COPD related hospitalisations and COPD related mortality) and incident coronary events (fatal or non-fatal MI and mortality related to IHD). The main or first secondary diagnosis of MI was considered a non-fatal MI event. Incident COPD cases were ascertained as described for **Paper I.** Data linkage between the National Cause of Death Registry, the Swedish hospital discharge register and the Malmö Myocardial Infarction Register was used to retrieve incident cases of coronary events. The ICD codes used for diagnosis included: ICD-9; 410, and ICD-10; I21 for fatal and non-fatal MI, and ICD-9; 410-414 and ICD-10; I20-I25 for IHD mortality as the underlying cause of death.

3.5. Study design and statistical analysis

Statistical analyses were carried out using IBM SPSS (Windows) version 22.0 for **Papers I-III** and version 24.0 for **Paper IV**, and Stata (Windows) version 12.0. P-values < 0.05 were considered significant (two-sided). Baseline characteristics were compared between the exposure groups using one-way analysis of variance (ANOVA) for continuous variables and Pearson's chi-square test or Fisher's exact test for categorical variables in **Papers I-III.**

3.5.1. Cohort studies

A cohort study is a form of observational study where no interventions by the investigator take place and any relationships between an exposure and outcome are determined by observation over time. A cohort is a group of people who are followed up over a period of time; more specifically this group will not have the disease or outcome of interest at baseline, but will contain subjects who are "exposed" and "non-exposed" depending on whether they have a certain baseline characteristic of interest. The differences in the risk of disease/outcome over time between the exposed group and the unexposed group is what is fundamental about the nature of cohort studies. Cohort studies can however be prospective or retrospective in design. Prospective cohort studies (or longitudinal studies) "look ahead" to determine causal associations whereas retrospective cohort studies (or historical cohort studies) "look back" in time to determine this association[102]. In prospective cohort studies one begins with a defined population and the cohort is divided based on an exposure of interest. The cohort is then followed-up over a period of time into the future where the outcome status is then determined (disease of interest or not). In a retrospective study design, some subjects may in the present time have developed the outcome of interest and the investigators look back in time to a point when the outcome had not yet developed to determine the exposure status and then subsequent outcome status. This type of study requires the use of pre-existing historical records and as such the purpose of the cohort study would not have been pre-planned as it would be for a prospective cohort study. However, both types of cohort studies share the same principles; comparing exposed and non-exposed subjects to their subsequent outcome status.

Methodological issues of cohort studies

Selection bias

This type of bias takes place when the association between exposure and outcome in the sample population under study does not truly reflect the association between exposure and outcome in the target population for the study. In cohort studies, this can occur mainly due to non-respondents and loss to follow-up in the study population.

Concerning non-response bias, if those who agreed to take part in study differ from those who declined to participate this will result in a bias in the association observed between the exposure and outcome. There is also a high risk of attrition bias (loss to follow-up) due to the extensive time-periods used for follow-up in cohort studies. This leads to issues with missing data, and therefore in situations where large numbers of subjects have been lost to follow-up, any systematic differences in the baseline characteristics or outcome status of those lost to follow-up and those that remained in the study, should be made apparent. This ensures transparency between the type of population who dropped out of the study and those that remained the study for the final analysis. External sources can sometimes be used to obtain this information if necessary.

Information bias

Also known as misclassification bias, refers to the misclassification of disease or exposure status to an incorrect group, rather than that to which is should be assigned in reality.

"The means for obtaining information about the subjects in the study are inadequate so that as a result some of the information gathered regarding exposures and/or disease outcome is incorrect"[103].

Misclassification bias can be differential or non-differential but essentially refers to participants being assigned to a group or category that is incorrect and therefore incorrect associations are made. If the probability of misclassification is similar across all groups this is known as non-differential misclassification bias, which is usually a data collection issue not related to exposure or outcome status. This can lead to a dilution in the risks observed and as such we are less likely to find an association even if it does exist in reality[103]. However, if the probability of misclassification differs across different groups in the study this is known as differential misclassification bias, which can lead to an apparent association between the exposure and outcome when in reality it does not exist, or a lack of association when in reality it does exist[103].

3.5.2. Survival analysis

Cox regression

When modelling survival data, the most commonly used method is a type of multivariate regression analysis known as Cox regression. A major assumption behind this method is that there is a constant relationship between the explanatory variables and the dependent variable. In other words, the proportionality assumption in this type of analysis is that the hazards for persons with different patterns of covariates are constant over time[104]. Cox regression allows us to test simultaneously the effect of several factors (or covariates) on the rate of an event occurring (known as the "failure"-outcome of interest or death). The hazard function is the probability of an event

occurring in a subject in a time interval, given that the subject has survived up until the start of this interval, therefore it is also the dependent variable in this method of multiple regression. It is equal to the risk of dying (or failure event) at a certain time point which is determined by the covariates in the model (explanatory variables). The probability of a failure event/death when all explanatory variables are zero is known as the baseline hazard (equivalent to the intercept in an ordinary regression equation). As there are no assumptions about the shape of this baseline hazard, the model can be thought of as semi-parametric. The regression coefficients give us the hazard ratios (HR) for the explanatory or exposure variables in question, and is a type of risk ratio that allows us to estimate the relative survival (or failure) in one group compared to another.

Cox regression analysis was the method used to analyse survival data in **Papers I-IV**. Time to event was calculated as the time between the date of entry to the study (baseline measurements) until either date of the first outcome of interest (e.g. COPD event, coronary event or DM), mortality or emigration from Sweden (whichever came first). In **Paper III** follow-up time was divided into four 10 year time intervals: 0-10, 10-20, 20-30 and >30 years, and only the first DM event was counted (each subject could only be a case in one of the time intervals). For a time interval (e.g. 10-20 years follow-up) all subjects with more than 10 years of follow-up were included, however only cases of DM between 10-20 years of follow-up were counted and those with follow-up time over 20 years were restricted to the maximum follow-up time for that time interval i.e. 20 years.

Kaplan Meier curve

The Kaplan Meier curve (also known as the survival curve), is a method used to plot the cumulative probability of survival (or proportion free of events) over time. Data from life-tables are used in the calculation of this probability, in which 1-minus the probability of death/event is used to determine the probability of surviving or being event-free at each time interval. When plotting the Kaplan Meier curve, there is a step-down every time an event/death occurs. Therefore, at the end of the study period, the cumulative probability of being event-free or surviving is given on the y-axis. As the mean survival time is difficult to calculate with most survival data due to studies ending prematurely in many situations, (i.e. subjects have survived beyond the follow-up time of the study and hence we cannot know their outcome beyond the study time) the median survival time is often used. This is the time at which half the study subjects are expected to be alive/event-free and therefore the probability of surviving past the median survival time (which can be in months or years for example) is 50%. When the survival curve of two or more exposure groups is shown on the Kaplan Meier plot, a non-parametric statistical test known as the log-rank test should be used to formally test the survival of both groups. The null hypothesis of the log-rank test is that the probability of an event occurring at any time point is the same for each population[105].

A significant p-value indicates a significant difference between the population survival curves, however it does not allow for other explanatory variables to be taken into account, which is why the method of choice for assessing the "survival" times of different groups is Cox regression, where this difference can be tested while taking into account other explanatory variables[105]. Both the log-rank test and the Cox regression model assume that the HR is constant over time (the proportional hazards assumption), which is something that should be formally tested before continuing forward with the Cox regression models.

Proportional hazards assumptions

This is the assumption that the HRs of the two (or more) groups being compared is constant over time. There are some ways to formally test this assumption; 1) graphically using log-log plots 2) the use of time-dependent variables in an extended cox model and 3) the goodness of fit test[106]. Graphically the log-log plot can be used, where the assumption is that the difference between hazards for two (or more) groups does not change over time[105]. Therefore, parallel survival curves that do not cross-over for any time period are suggestive of the proportional hazards assumption being met. The time-dependent covariate analysis is another method that can be used to test the proportionality assumption. The time-dependent covariate is constructed as a function of time and the covariate or exposure one would like to test. The significance level for the time-dependent covariate coefficient in the Cox regression model gives an indication as to whether the proportional hazards assumption has been met. If the coefficient of the product term (time and covariate being tested) is non-significant we can conclude that the proportional hazards assumptions have been met[106] and that the value of the covariate is constant over time. In cases where this assumption is not met, the extended cox model where time-dependent variables are included in the model or a stratified cox model are alternative ways to proceed. The third way to test the proportional hazards assumption is using a global goodness of fit test such as the Schoenfeld residual test. The Schoenfeld residual is defined as the covariate value for an individual that experienced a failure event, minus its expected value. If the plot of the residuals against time shows a non-random pattern (the slope of scaled residuals should be zero), the proportional hazards assumption has not been met. Therefore a "pattern" to the residuals indicates that the covariate effect is changing with time, and hence is time- dependent, which violates the proportional hazards assumption.

Proportional hazards assumptions were formally tested for all Cox models included in **Papers I-IV**. In **Papers I and II**, Kaplan Meier plots and log-log plots were constructed and time-dependent covariate analysis for all cox models in the respective papers was performed. In **Paper III,** Kaplan Meier and log-log plots were used to test proportional hazards assumptions and in **Paper IV** time-dependent covariate analyses were used to check that the assumptions were fulfilled.

Competing risks regression

Competing risks are an important consideration in survival analysis, as the event of interest is not the only outcome that can occur; other events such as death from other causes can compete with the outcome of interest[107]. In the presence of competing risks, individuals are followed up until the event of interest, the competing event or censoring[108]. This competing event subsequently alters the chance that the event of interest occurs. In the case of censoring, the event of interest has not occurred during the duration of the study (e.g. end of study period, drop-out of study) but we do not know when or if it would have otherwise. However, in the case of a competing risk event occurring, the event of interest is prevented from occurring altogether. In the presence of competing events, cumulative incidence function (CIF) for competing risks can be used. The CIF depends on both the HR for the event of interest and the competing event[109]. Competing events are therefore not handled as censored events without influence on the CIF of the event of interest[107]. The CIF therefore gives the proportion of subjects who have had the event of interest before a certain time, accounting for the fact that the competing event (e.g. death from another cause) could prevent the event of interest from occurring. The probability of failure before a certain time is lowered by the presence of the competing event, as those that experience the competing event are not longer at risk for the failure (event of interest)[107].

In the presence of competing risks, two families of models can be used for regression purposes 1) modelling the effect of covariates on the cause-specific hazard of the outcome, or 2) modelling the effect of covariates on the CIF[110]. The choice of either method ultimately depends on the type of research being carried out. For studying disease aetiology (causal relationships between the exposure and outcome) the cause-specific hazards are deemed more useful, whereas in prognostic research (predicting an individual's risk or probability of an outcome) modelling the effects of covariates on the CIF (sub-distribution hazards) are seen to be more appropriate[107, 108]. The fundamental principles behind each approach is how the "risk set" (group who have not experienced outcome of interest and are still at risk of outcome of interest at time *t*) [108] is altered by the presence of the competing event.

Briefly, in the cause-specific hazards regression, subjects who have a competing event are removed from later risk sets for the outcome of interest[108]. Therefore, subjects who experience the competing events are treated as censored observations. In contrast, in the sub-distribution hazards approach the risk set includes both subjects who have not yet experienced the event and those who have experienced the competing event[108]. The subject that has had the competing event is still considered in the risk set for developing the outcome of interest[111]. Therefore, a new hazard function (the sub-distribution hazard) is defined, which is the probability of the event of interest occurring given that the subject has survived up until time *t* without the event of interest or has experienced the competing event. The Fine and Gray competing risks regression model[112] is the

way a CIF covariate analysis can be performed, where a sub-hazard ratio (SHR) is obtained instead of hazard ratio. The model allows us to assess the effect of covariates on the CIF.

In **Papers I and III** the Fine and Gray competing risks regression model was performed. In **Paper I** SHRs were obtained for the outcome of incident COPD events taking into account the competing risk of death from any cause (without prior COPD event). In **Paper III**, SHRs were obtained for the outcome of incident DM taking into account the competing risk of death from any cause (without prior DM event).

Lunn and McNeil competing risks method

In this method of competing risks, a data duplication method is used to treat both types of event that are competing as "failures". In this method a Cox regression model with censored data can be used to analyse competing risks in a survival setting where two failure types can exist in addition to censoring[113]. This method involves duplicating data for each subject such that each subject has two rows in the dataset. Strata are created on these two rows and each type of failure is used per stratum. All covariates are also duplicated and one duplicate is coded as 0 in stratum 1 and the other duplicate coded as 0 in stratum 2. A Cox model stratified by these two strata can then be performed which allows for different results for the association between the covariate and the two failure outcomes. To obtain a p-value for the difference in outcomes in relation to a covariate of interest, the same model is re-run with just one variable for the covariate (or exposure) of interest. This method allows us to run Cox regression stratified by the type of failure.

In **Paper IV** a modification of the Lunn and McNeil competing risks method was used. In this modified method, subjects could have both events (CE or COPD events), and such a subject with both events was coded as failures in both strata. This could then allow the HR obtained from this method for each failure (in relation to baseline lung function) to be identical to if they were run as separate Cox regression models. The p-value obtained from this approach can therefore provide a significance value to the difference in risk of both failures in relation to the exposure of interest.

Harrell's c-statistic

Also known as the concordance index, is a measure of goodness of fit of a model; usually logistic regression models with binary outcomes. It is often referred to as being equivalent to the area under the Receiver Operating Characteristic (ROC) curve (sensitivity vs 1-specificity). Its main use has been in risk algorithms where the performance of a risk prediction model is assessed using the algorithms ability to distinguish between cases and controls[114]. The ability of the models to discriminate between those who do and do not have the outcome of interest is assessed using the C-statistic. This represents the proportion of pairs of subjects (where one is a subject that

experienced the outcome and one is a subject that did not experience the outcome) in which the subject who experienced the outcome had a higher predicted probability of experiencing the outcome than the subject in which the outcome did not occur[115].

The value of C-statistic can be extended to survival models and the predictive ability of such models, in which case the C-statistic is measure of the probability that in a randomly selected pair of subjects, the subject with a shorter survival time (time to event) had a higher predictive risk of the event[116]. In **Paper II** survival models with conventional spirometry (FEV_1 and FEV_1/VC) were compared to models with added LCI. An increase in the C-statistic after adding LCI would indicate some value in having LCI in a survival model in predicting the outcomes of interest (provided there is statistical significance of such a difference).

4. Results

4.1. Paper I

Baseline characteristics of 689 subjects by lung function group at baseline are shown in **Table 1**.

Table 1:
Baseline characteristics by group of lung function classification method (n=689)

Group	Normal	FR+LLN-	FR+LLN+	p-value
Number (%)	545 (79.1)	56 (8.1)	88 (12.8)	-
Height, m	1.75 ± 0.06	1.75 ± 0.07	1.76 ± 0.07	0.115
BMI, kg/m^2	24.6 ± 3.0	24.6 ±3.3	23.7 ± 3.2	0.025
Current smoker, %	58.5	75.0	75.0	0.001
Ever-smoker %	83.0	87.5	92.0	0.089
Diabetes %	1.7	1.8	3.4	0.700
Cholesterol mmol/L*	6.39 ± 1.14	6.48 ± 1.03	6.21 ± 1.10	0.297
SBP (mmHg)	139 ± 22	141 ± 23	139 ± 21	0.772
DBP (mmHg)	85 ± 12	84 ± 14	84 ± 12	0.771
VC, L	4.4 ± 0.7	4.2 ± 0.6	4.2 ± 0.8	0.016
FEV_1, L	3.5 ± 0.5	2.9 ± 0.4	2.5 ± 0.6	<0.001

Data are presented as mean ± SD unless otherwise stated. FR: fixed ratio, LLN: lower limit of normal; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; VC: vital capacity; FEV_1: forced expiratory volume in 1 second * Data from 687 participants

The three groups were classified according to the two different criteria used to define COPD diagnosis (FR or LLN), using baseline lung function: 1) FR-LLN- (normal baseline lung function) 2) FR+LLN- (intermediate group) and 3) FR+LLN+ (lowest baseline lung function group). The purpose for creating these groups was to be able to assess future outcomes in relation to these groups at baseline as opposed to assessing prevalence of COPD. Subjects were relatively asymptomatic at baseline (only 2.8% of men reported dyspnoea grade 2 or above, as per the Medical Research Council Breathlessness scale). As expected, the proportion of current smokers was higher in the groups with lower lung function at baseline, along with a lower mean BMI.

There were 88 cases of incident COPD events (COPD related hospitalisations or COPD related mortality) over 44 years of follow-up, the majority of which were diagnosed from hospital inpatient admissions (only 6 of the 88 cases of COPD were

diagnosed through death certification data, of which 5 had autopsy confirmation of the cause of death). Of the 689 men that took part in baseline examinations, three were lost to follow-up due to emigration, and the remaining 686 died during the follow-up period.

4.1.1. Incident COPD events

Table 2 shows the incident rates and HR for incident COPD events classified according to the three groups of lung function (Normal, FR+LLN- and FR+LLN+)

Even after adjustment for potential confounding factors, the risk of incident COPD events increased almost 4-fold in the FR+LLN- group and over 8-fold in the FR+LLN+ group, relative to the reference group (normal). Additionally, when taking into account the competing risks of death from non-COPD related mortality using the Fine and Gray method, the risks reduced in both the FR+LLN- and FR+LLN+ groups, but still remained significantly increased relative to the reference group (adjusted HR 3.31 (confidence interval(CI):1.80-6.09) and 5.53 (CI:3.34-9.15) respectively.

Figure 13 shows a Kaplan Meier survival curve of the 689 men in the study. The proportion free of incident COPD events is represented in the three groups: Normal, FR+LLN- and FR+LLN+. The curves show a smaller proportion of subjects free from incident COPD events as lung function decreases (from the normal to the poorest lung function group) at any given time.

Table 2
Incidence and hazard ratios of incident COPD events per group of lung function classification method for 689 participants at 55 years of age

	Normal	FR+LLN-	FR+LLN+	p-value
Number	545	56	88	
COPD events n (/1000 person years)	42 (3.3)	14 (12.7)	32 (22.1)	-
COPD events, unadjusted hazard ratio (95% CI)	1.00	4.22 (2.30-7.74)	7.87 (4.94-12.54)	<0.001
COPD events, adjusted hazard ratio (95% CI)†	1.00	4.15 (2.24-7.69)	7.88 (4.82-12.87)	<0.001
COPD events with competing risks regression (95% CI) †*	1.00	3.31 (1.80-6.09)	5.53 (3.34-9.15)	<0.001
COPD events, adjusted hazard ratio (95% CI)†** (excluding 30 restrictive) n=659	1.00	4.50 (2.40-8.45)	8.80 (5.31-14.58)	<0.001

†Adjusted for smoking status (3 groups: never, ex and current smokers), diabetes, BMI, height, and physical activity (3 groups: high, moderate and low physical activity). *Competing risks regression; 88 incident COPD cases as failure event and 598 deaths without COPD as competing risk. **30 restrictive subjects (FEV_1/VC ≥70% VC ≤ 80%) excluded from the normal group.

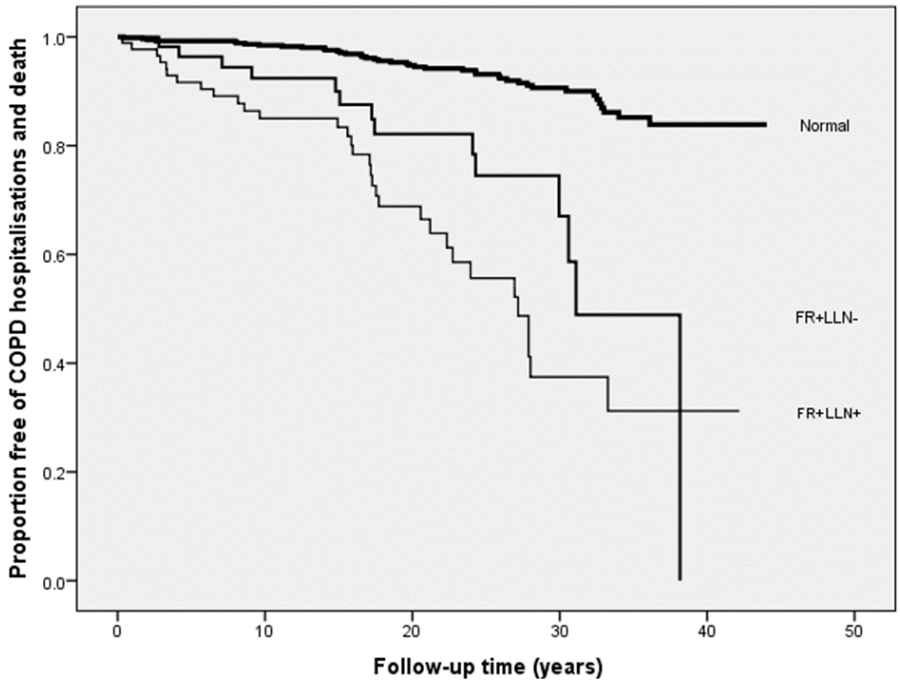


Figure 13:
Kaplan Meier survival curve of incident COPD events by 3 groups: $FEV_1/VC \geq 70\%$, (Normal) $FEV_1/VC < 70\% > LLN$ (FR+LLN-) and $FEV_1/VC < 70\% < LLN$ (FR+LLN+). Incident COPD was defined as hospitalisation due to COPD or a COPD related death.

There was an increased risk of incident COPD events in Q1 of FEV_1% predicted relative to the reference (Q4) (Q1 range: 42-86% FEV_1%predicted) even after adjustment for potential confounding factors (HR 3.84 (CI: 2.03-7.27) p-value for trend across quartiles <0.001). This association was not however observed in quartiles of VC%predicted (Q1 HR: 1.31 (CI: 0.74-2.32) p-value for trend across quartiles 0.183).

4.1.2. All-cause mortality

There was an increased risk of death from all-causes in both the FR+LLN- group and the FR+LLN+ group (unadjusted HR 1.40 (CI:1.04-1.84) and 1.63 (CI:1.30-2.05) respectively. However after adjustment for potential confounders, the risk in the FR+LLN- group was no longer significant but remained significant in the FR+LLN+ group (adjusted HR 1.30 (CI:0.98-1.72) and 1.58 (CI:1.25-2.00) respectively). The adjusted risk of death from all causes was also significant in Q1 for FEV_1%predicted

relative to the reference (Q4) (HR 1.67 (CI:1.34-2.10) p-value for trend across quartiles <0.001). The adjusted risk of death from all causes was also significant after adjustment in Q1 of VC%predicted (HR 1.26 (CI:1.01-1.57), p-value for trend across quartiles 0.02).

4.1.3. FEV_1 decline

Of the 689 55-year-old men who initially took part in the study in 1969, 392 were able to participate in the re-examination, which took place in 1982 when subjects were 68 years old. FEV_1 values recorded in 1969 and again in 1982 were used to determine the decline according to their initial baseline groups (normal, FR+LLN- and FR+LLN+). Univariate linear regression was used to adjust the decline in FEV_1 for initial FEV_1 and smoking status at baseline (1969). **Table 3** shows the FEV_1 decline from 55 to 68 years according to the three categories of lung function at baseline.

Table 3:
FEV_1 decline from 55 to 68 years: information from baseline and re-examination in 392 subjects

Lung function group at 55 years	Normal	FR+LLN-	FR+LLN+	p-value
Number	317	32	43	
Initial FEV_1, L (55 years)	3.52 (±0.54)	2.81 (±0.39)	2.55 (±0.53)	<0.001
FEV_1 decline, L (55 to 68 years)	0.44 (±0.31)	0.50 (±0.36)	0.49 (±0.66)	0.568
Adjusted FEV_1 decline (95% CI) (55 to 68 years)†	0.42 (0.38-0.46)	0.58 (0.45-0.71)	0.60 (0.48-0.72)	0.009
Lung function group at 68 years				
Normal	227	4	8	-
FR+LLN-	72	11	2	
FR+LLN+	18	17	33	

Data are presented as mean ± SD unless otherwise stated. FR: fixed ratio; LLN: lower limit of normal; VC: vital capacity; FEV_1: forced expiratory volume in 1 s; † Linear regression models used to adjust FEV_1 decline. Adjusted for initial FEV_1 (at 55 years) and current smokers at age 55 years.

There was a larger adjusted decline in FEV_1 in the FR+LLN- and FR+LLN+ groups with a significant difference between the normal vs FR+LLN- groups and normal vs FR+LLN+ groups. (p-values for adjusted FEV_1 decline: 0.025 (N vs FR+LLN-) and 0.009 (N vs FR+LLN+) but no significant difference between the FR+LLN- and FR+LLN+ groups (p-value 0.847). In terms of changes in the lung function category between 1969 to 1982, 28.4% of those initially in the normal group at baseline were in a poorer lung category group in 1982 (FR+LLN- or FR+LLN+), and 53.1% of those in the FR+LLN- at baseline were in a poorer lung function category group in 1982 (FR+LLN+). Of those in the FR+LLN+ group at baseline, 76.7% were still in the FR+LLN+ group in 1982.

4.2. Paper II

Baseline characteristics of 674 subjects by lung function group at baseline are shown in **Table 4**.

Table 4:
Baseline characteristics by quartiles of LCI (n=674)

	Q1 (best) (4.4-6.9)	Q2 (7.0-7.9)	Q3 (8.0-8.9)	Q4 (worst) (9.0-12.6)	p-value
Number (=674)	162	171	173	168	-
Height (m)	1.74 (±0.06)	1.75 (±0.07)	1.75 (±0.07)	1.75 (±0.07)	0.334
BMI (kg/m^2)	25.1 (±2.6)	24.2 (±3.0)	24.6 (±3.2)	24.2 (±3.2)	0.013
Current smoker (%)	47.5	58.5	63.0	76.8	<0.001
Ever smoker (%)	82.1	80.1	86.1	89.9	0.062
Tobacco per day (%)					<0.001
Non-smoker	52.5	41.5	37.0	23.2	
1-14g/day	29.0	36.8	38.2	38.7	
15-24g/day	15.4	16.4	20.8	28.6	
≥25g/day	3.1	5.3	4.0	9.5	
Diabetes (%)	1.9	3.5	1.2	1.2	0.420
P-cholesterol mmol/L *	6.38 (±1.13)	6.39 (±1.13)	6.42 (±1.19)	6.31 (±1.10)	0.861
SBP (mmHg	142 (±21)	139 (±22)	138 (±24)	137 (±21)	0.123
DBP (mmHg)	88 (±12)	85 (±12)	83 (±13)	82 (±12)	<0.001
LCI	6.1 (±0.7)	7.5 (±0.3)	8.4 (±0.3)	10.0 (±0.8)	<0.001
FEV_1 (L)	3.45 (±0.55)	3.45 (±0.61)	3.30 (±0.59)	3.03 (±0.73)	<0.001
$FEV_{1\%pred}$	101.2 (±14.8)	99.6 (±15.7)	96.3 (±15.4)	87.8 (±20.0)	<0.001

Data are presented as mean ± SD unless otherwise stated. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; LCI: Lung clearance index; FEV_1: forced expiratory volume in 1 s. *Data from 672 participants.

As expected, the proportion of current and ex-smokers was highest in the quartile with the highest LCI (Q4: LCI range 9.0-12.6), along with lower values of FEV_1 in Q4 relative to Q1-3. Pearson's correlation coefficient between LCI and FEV_1 was -0.265 ($p < 0.001$).

4.2.1. Incident COPD events

Over 44 years of follow-up, there were 85 incident COPD events of which 79 were either hospitalisations for COPD or outpatient visits (n=2).

Table 5:
Incidence and HR of COPD events by quartiles of LCI and LCI>ULN (n=674)

	Q1 4.4-6.9 reference	Q2 7.0-7.9	Q3 8.0-8.9	Q4 9.0-12.6	p-value trend	LCI<ULN (n=589) reference	LCI>ULN (n=85)
COPD events n (/1000 person years)	12 (3.1)	16 (4.0)	22 (5.9)	35 (10.9)	-	69 (5.2)	16 (10.6)
COPD events, unadjusted (95% CI)	1.00	1.30 (0.61-2.74)	1.97 (0.97-3.98)	3.99 (2.06-7.71)	<0.001	1.00	2.37 (1.36-4.10)
COPD events, adjusted (95% CI)[a]	1.00	1.21 (0.56-2.59)	1.73 (0.85-3.52)	3.40 (1.74-6.67)	<0.001	1.00	2.24 (1.29-3.92)
COPD events, adjusted (95% CI)[ab]	1.00	1.18 (0.55-2.53)	1.63 (0.80-3.32)	2.34 (1.17-4.69)	0.006	1.00	1.85 (1.05-3.27 [ac]

SD: Standard deviation. [a] Adjusted for smoking status (3 groups: never, ex and current smokers), diabetes, BMI, height, and physical activity (3 groups: high, moderate and low physical activity). [b] Additionally adjusted for FEV_1 or [c] FEV1<LLN

Table 6:
Hazard ratios of incident COPD events by categories of LCI and FEV_1 or FEV_1/VC

	Normal FEV_1 Normal LCI (Reference)	Normal FEV_1 LCI>ULN	FEV_1<LLN Normal LCI	FEV_1<LLN, LCI>ULN (Highest risk category)	p-value*
N (=674)	535	60	54	25	
COPD events, unadjusted (95% CI)	1.00	1.48 (0.67-3.26)	3.05 (1.59-5.83)	7.36 (3.61-15.0)	<0.001
COPD events, adjusted (95% CI)[a]	1.00	1.36 (0.61-3.00)	2.63 (1.35-5.12)	7.81 (3.78-16.1)	<0.001
	Normal FEV_1/VC Normal LCI (Reference)	**Normal FEV_1/VC LCI>ULN**	**FEV_1/VC<LLN Normal LCI**	**FEV_1/VC<LLN LCI>ULN (Highest risk category)**	**p-value**
N (=674)	530	63	59	22	
COPD events, unadjusted (95% CI)	1.00	1.45 (0.62-3.38)	5.38 (3.16-9.15)	10.32 (5.16-20.6)	<0.001
COPD events, adjusted (95% CI)[a]	1.00	1.34 (0.58-3.19)	5.15 (2.95-9.01)	11.75 (5.79-23.8)	<0.001

[a] Adjusted for smoking status (3 groups: never, ex and current smokers), diabetes, BMI, height, and physical activity (3 groups: high, moderate and low physical activity). *p-value: 3 degrees of freedom

After adjustment for potential confounding factors there was over a 3-fold increase in the risk of incident COPD events in subjects with a baseline LCI from 9.0-12.6 (Q4: highest LCI group) relative to the reference group (Q1: LCI 4.4-6.9). Even after further adjusting for baseline FEV_1, there remained an almost 2.5-fold increase in risk of COPD events in those with the highest LCI relative to those in the low LCI group (p-value for trend across quartiles, 0.006). When comparing LCI above and below the ULN, those with LCI>ULN at baseline had an almost 2-fold increase in risk of COPD events even after adjusting for baseline FEV_1, relative to those with LCI<ULN (**Table 5**)

There was a significant difference in the risks of incident COPD events between the category of subjects with poor spirometry but normal LCI and the category with poor spirometry and poor LCI. (For the difference between FEV_1<LLN, normal LCI category and FEV_1<LLN, LCI>ULN category: p-value 0.019 and for the difference between FEV_1/VC<LLN, normal LCI category and FEV_1/VC<LLN, LCI>ULN category: p-value 0.041) (**Table 6**) The HR per 1 SD reduction in FEV_1 %predicted was 2.04 (CI:1.63-2.56) and for 1 SD increase in the LCI was 1.62 (CI:1.29-2.03).The likelihood ratio test (LR test) and Harrell's C statistic showed an improved goodness of fit after adding LCI to a model already containing conventional spirometry in the prediction of COPD events. The increase in the C-statistic was not found to be significant (p-value 0.192 and 0.174 after adding LCI to a survival model with FEV_1 and FEV_1/VC respectively). However, there was a significantly improved goodness of fit with the LR test after adding LCI to a model with FEV_1 (p-value 0.009) and to a model with FEV_1/VC (p-value 0.015)

4.2.2. All-cause mortality

The adjusted risk of mortality from any cause was higher in the quartile of highest LCI (Q4) relative to the reference group (p-value for trend <0.001), along with the risk being higher in subjects with LCI >ULN relative to LCI<ULN (reference), (adjusted HR: 1.78 (CI:1.41-2.24)).

4.2.3. FEV_1 decline and pulmonary obstruction at 68 years

There was a significant difference in FEV_1 reduction (L) from 55 to 68 years between those with poor LCI and those with normal values of LCI at baseline. (FEV_1 decline (L) after adjustment for baseline FEV_1 and smoking: Q4 (0.56 (0.49-0.64) vs Q1 (0.42 (0.34-0.49), p-value for trend across quartiles, 0.011), and for LCI>ULN (0.61 (0.50-0.72) vs LCI<ULN (0.43 (0.40-0.47)), p-value for difference <0.01).

The proportion of new cases of pulmonary obstruction at 68 years was higher for those with poorer LCI at baseline. Even after adjustment for smoking and FEV_1 at baseline,

the odds ratio (OR) for pulmonary obstruction (FEV_1/VC <LLN) at age 68 years was significantly higher in those with poorer LCI at baseline relative to those with normal LCI at baseline.

Table 7:
Pulmonary obstruction at 68 years in relation to LCI at 55 years, among men with normal FEV_1/VC at baseline.

	Q1 (best) 4.5-6.9	**Q2 7.0-7.9**	**Q3 8.0-8.9**	**Q4 (worst) 9.0-12.6**	**p-value (trend)**
N (=347)	95	95	86	71	
FEV_1/VC<LLN at 68 years n (%)	2 (2.1)	7 (7.4)	13 (15.1)	13 (18.3)	<0.001
OR	1.00	3.65 (0.74-18.1)	7.93 (1.73-36.4)	9.80 (2.12-45.3)	<0.001
OR[a]	1.00	4.11 (0.80-21.1)	7.24 (1.52-34.4)	8.07 (1.67-39.1)	0.004

OR adjusted for smoking. [a] OR adjusted for smoking and FEV_1 baseline

4.3. Paper III

Baseline characteristics for 20,295 men are shown in **Table 8** by quartiles of FEV_1%predicted, and in **Table 9** for 7416 women by quartiles of FEV_1%predicted. The proportion of current smokers was significantly higher in quartiles with lower values of FEV_1%predicted. Subjects in quartiles of lower FEV_1 %predicted were overall more disadvantaged in terms of general health characteristics. There was a higher proportion of reported physical inactivity in quartiles of lower FEV_1%predicted in both men and women, along with higher alcohol consumption, use of anti-hypertensive medication, and higher ESR and cholesterol levels.

Table 8:
Baseline characteristics for quartiles of FEV_1 %predicted in males (n=20,295)

	Overall	Q4	Q3	Q2	Q1	p-value for trend
FEV_1 %predicted	95.4 (±17.7)	117.0 (±9.9)	100.7 (±3.1)	90.3 (±3.2)	73.3 (±11.3)	-
Number (n)	20,295	5074	5074	5074	5073	-
Age (years)	43.4 (±6.6)	43.9 (±6.5)	42.9 (±6.6)	42.8 (±6.6)	44.0 (±6.7)	0.350
Height (m)	1.77 (±0.07)	1.77 (±0.07)	1.77 (±0.07)	1.77 (±0.07)	1.77 (±0.07)	0.820
Current-smokers (%)	49.1	35.8	43.4	53.5	63.8	<0.001
BMI (kg/m^2)	24.6 (±3.2)	24.4 (±2.9)	24.5 (±3.1)	24.5 (±3.2)	24.8 (±3.6)	<0.001
Physical inactivity (%)	52.4	45.9	50.4	54.6	58.6	<0.001
Anti-hypertensive medication (%)	3.7	3.0	3.5	3.5	4.7	<0.001
High alcohol consumption (%)	17.8	15.6	16.8	18.7	20.1	<0.001
ESR (mm/hr)*	4.05	3.86	3.90	4.01	4.46	<0.001
Baseline glucose (mmol/L)	4.93 (±0.50)	4.89 (±0.49)	4.94 (±0.51)	4.94 (±0.50)	4.94 (±0.52)	<0.001
Cholesterol (mmol/L)	5.59 (±1.05)	5.52 (±1.01)	5.56 (±1.03)	5.60 (±1.06)	5.66 (±1.08)	<0.001
Family history of diabetes (%)	11.0	11.4	10.1	10.8	11.8	0.371
Social class (%)						0.125
- Low skilled	44.9	42.5	42.6	45.8	48.8	
- High skilled	43.9	47.1	47.3	42.3	38.8	
- Self-employed	8.3	8.0	8.0	9.0	8.0	
- Other	3.0	2.5	2.5	2.9	4.5	

Data consist of mean (±standard deviation) unless otherwise stated. *Geometric mean presented for ESR. Linear by linear association for chi square tests used for p-value for categorical variables, ANOVA test for linearity used for p-value for continuous variables.

Table 9:
Baseline characteristics for quartiles of FEV_1%predicted in females (n=7416)

	Overall	Q4	Q3	Q2	Q1	p-value for trend
FEV_1 %predicted	95.4 (±18.1)	116.9 (±10.2)	101.3(±3.0)	90.9 (±3.2)	72.5 (±11.9)	-
Number (n)	7416	1854	1854	1854	1854	-
Age (years)	47.6 (±7.8)	48.0 (±6.6)	46.9 (±7.8)	46.6 (±8.6)	48.8 (±8.1)	0.023
Height (m)	1.64 (±0.06)	1.64 (±0.06)	1.64 (±0.06)	1.64 (±0.06)	1.64 (±0.06)	0.868
Current-smokers (%)	45.2	27.0	38.8	48.4	66.6	<0.001
BMI (kg/m2)	23.8 (±3.9)	23.8 (±3.5)	23.7 (±3.7)	23.7 (±3.9)	24.0 (±4.4)	0.135
Physical inactivity (%)	43.3	36.6	40.6	45.0	51.0	<0.001
Missing data (%)	12.2	11.2	13.9	14.5	9.2	
Anti-hypertensive medication (%)	6.6	5.0	6.5	6.3	8.6	<0.001
High alcohol consumption (%)	2.7	2.5	2.4	2.9	3.1	0.150
ESR (mm/hr)*	7.47	6.99	7.13	7.48	8.36	<0.001
Baseline glucose (mmol/L)	4.74 (±0.52)	4.70 (±0.52)	4.74 (±0.51)	4.75 (±0.53)	4.79 (±0.53)	<0.001
Cholesterol (mmol/L)	5.67 (±1.10)	5.64 (±1.07)	5.62 (±1.08)	5.62 (±1.11)	5.79 (±1.12)	<0.001
Family history of diabetes (%)	16.0	16.0	16.1	15.2	16.7	0.788
Social class (%)						0.785
- Low skilled	44.9	42.2	43.8	45.0	48.5	
- High skilled	45.4	48.5	48.2	45.4	39.5	
- Self-employed	2.9	3.1	2.3	3.2	3.0	
- Other	6.8	6.3	5.8	6.4	8.9	

Data consist of mean (±standard deviation) unless otherwise stated. *Geometric mean presented for ESR. Linear by linear association for chi square tests used for p-value for categorical variables, ANOVA test for linearity used for p-value for continuous variables.

4.3.1. Incidence of diabetes

The mean follow-up time for men was 27 years and for women was 26 years, during which there were 3753 incident DM events in men, and 993 incident DM events in women.

Adjusted risks for incident DM events for men and women by quartiles of FEV_1%predicted are shown in **Table 10** and illustrated graphically for Q1 HRs in **Figure 14**. After adjustment for potential confounding factors, the risk for DM remained significant in Q1 (FEV_1%predicted ≤84.65) relative to Q4 (reference; FEV_1%predicted ≥106.34) throughout all time periods. Even after more than 30 years of follow-up, there was an almost 50% increase in the risk of DM in Q1 relative to Q4 of FEV_1%predicted in men. A broadly similar pattern was seen in women, however as numbers of events in each time interval were smaller, the confidence intervals were larger and HR for certain intervals were no longer significant after adjustments (HR: 20-30 years follow-up; unadjusted Q1: 1.73 (CI:1.32-2.28), after adjustment for confounding factors Q1: 1.32 (CI:0.99-1.76), relative to the reference (Q4). The adjusted HR for incident DM events in the overall follow-up time per 10% decrease in FEV_1%predicted was 1.09 (CI:1.07-1.11) and 1.07 (CI:1.03-1.11) in men and women respectively.

Table 10:
HR for incident DM for males and females by quartiles of FEV_1% predicted

	Follow-up time (years) (n=Incident DM events)	Q4 (reference)	Q3	Q2	Q1	p-value trend
Males		≥106.34	95.57-106.34	84.65-95.57	≤84.65	
Females		≥106.67	96.13-106.67	85.14-96.12	≤85.13	
Males	Overall follow-up time (n=3753)	1.00	1.06 (0.97-1.17)	1.15 (1.05-1.27)	1.48 (1.35-1.63)	<0.001
Females	Overall follow-up time (n=993)	1.00	1.26 (1.04-1.53)	1.26 (1.04-1.53)	1.45 (1.20-1.75)	<0.001
Males	0-10 (n= 365)	1.00	0.99 (0.70-1.38)	1.06 (0.76-1.47)	1.64 (1.21-2.22)	<0.001
Females	0-10 (n=135)	1.00	2.00 (1.12-3.58)	1.72 (0.95-3.11)	1.68 (0.94-3.01)	0.275
Males	10-20 (n= 1059)	1.00	0.98 (0.81-1.19)	1.12 (0.93-1.35)	1.52 (1.27-1.81)	<0.001
Females	10-20 (n=395)	1.00	1.33 (0.98-1.81)	1.37 (1.01-1.87)	1.51 (1.11-2.05)	0.012
Males	20-30 (n=1984)	1.00	1.12 (0.98-1.27)	1.20 (1.05-1.36)	1.39 (1.22-1.59)	<0.001
Females	20-30 (n=419)	1.00	1.04 (0.78-1.39)	1.16 (0.87-1.54)	1.32 (0.99-1.76)	0.043
Males	>30 (n= 345)	1.00	1.06 (0.79-1.44)	1.06 (0.77-1.44)	1.46 (1.08-1.97)	0.023
Females	>30 (n=44)	1.00	1.58 (0.63-3.94)	0.79(0.28-2.20)	2.35 (0.93-5.90)	0.186

All HR presented are adjusted for age, height, BMI, smoking status, ESR (log transformed), baseline glucose, cholesterol, physical activity, BP medication, social class, family history of diabetes, and alcohol abuse.

Inflammation

In a sub-cohort of 5133 men with information on inflammatory markers, it was found that even after further adjustment for ESR, white cell count, fibrinogen, complement C3, haptoglobin, ceruloplasmin, alpha-1 antitrypsin and orosomucoid levels, the HR in Q1 FEV_1%predicted remained significant (HR 1.29 (1.07-1.54) for the overall follow-up time, relative to the reference Q4 (n=1025 incident DM events).

Smoking

The relationship between low FEV_1%predicted and incident DM remained significant even in non-smokers (never and former smokers) in Q1 for men for all time intervals except >30 years follow-up. There was no significant interaction between smoking status and FEV_1%predicted for both men and women (p-value 0.49 and 0.11, respectively).

Competing risks

A competing risks analysis was carried out to take into account the competing risk of deaths (all-cause mortality) in those with no recorded DM events (n=6609 deaths in men and 1746 deaths in women without prior DM). SHR remained significant even after adjustment for confounding factors in Q1 and Q2 of FEV_1%predicted relative to the reference Q4 in both men and women (SHR in Q1 men: 1.33 (CI1.21-1.47), SHR in Q1 women: 1.31 (CI1.08-1.60).

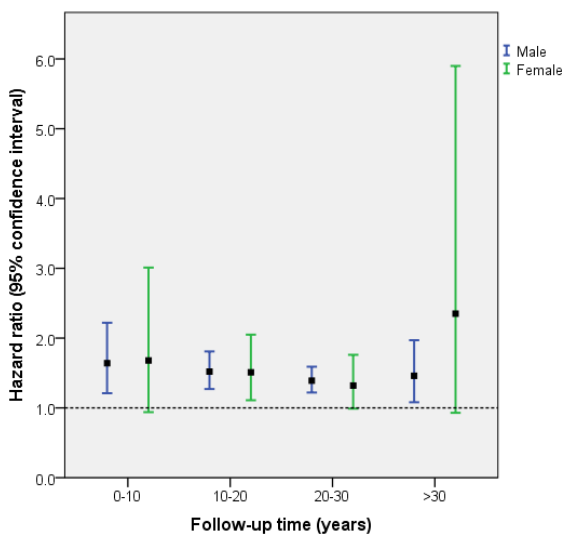


Figure 14:
Hazard ratios for incident DM in Q1 of FEV_1%predicted (relative to the reference Q4) for different follow-up time intervals in males and females.

Some subjects in the study who were still free from DM after more than 10 years from initial screening in the MPP study were re-screened with fasting blood glucose as part of the MDC-CC study cohort (n=1530; 1272 men and 258 women). A total of 214 subjects developed DM during 18.4 years of follow-up after this examination (mean follow-up time 14.1 years (range 10-18.4 years). FEV_1 at the initial examination in MPP was associated with incidence of DM in this sub-group (HR per 10% decrease in FEV_1%predicted: 1.11 (1.02-1.21).

4.4. Paper IV

Baseline characteristics for the 672 men examined in 1969 are presented **in Table 11.**

Table 11:
Baseline characteristics of 672 men examined in 1969

Demographic characteristics	
Age (years)	55
Height (m)	1.75 (±0.07)
BMI (kg/m^2)	24.5 (±3.0)
Smoking status (%) Current smokers Non-smokers (never/ex-smokers)	 61.6 38.4
Diabetes (%)	1.9
Cholesterol (mmol/L)	6.38 (±1.14)
Systolic BP (mmHg)	139 (±22)
Diastolic BP (mmHg)	84 (±12)
Lung function measurements	
VC (L)	4.3 (±0.7)
FEV_1 (L)	3.3 (±0.6)
LCI	8.0 (±1.5)
FEV_1/VC (%)	76.2 (±8.8)

Data are presented as mean ± SD, or %.

4.4.1. Incident COPD events

During 44 years of follow-up, there were 85 incident COPD events. The rate of incident COPD events was highest in quartiles of the poorest lung function (**Table 12**) represented by Q1. The HR for COPD events per 1 SD change in lung function measures and by FEV_1/VC categories is shown in **Table 14**. There was a significant increase in the adjusted risk of COPD per 1 SD decrease in VC, FEV_1, and FEV_1/VC and per 1 SD increase in LCI. Additionally a FEV_1/VC ratio below both cut-off points (<0.70 and <LLN) was associated with an increased risk of COPD events.

Table 12:
Incident rates of COPD events by quartiles of lung function measures

	Q1	Q2	Q3	Q4
VC (L)	1.57-3.88	3.89-4.33	4.34-4.78	4.80-6.53
COPD events n (rate)*	25 (7.6)	19 (5.0)	26 (6.9)	15 (3.7)
FEV_1 (L)	1.19-2.89	2.91-3.32	3.33-3.73	3.74-4.97
COPD events n (rate)*	36 (11.7)	23 (6.2)	17 (4.3)	9 (2.2)
FEV_1/VC (%)	41.57-71.71	71.72-77.44	77.46-81.90	81.93-98.96
COPD events n (rate)*	48 (15.8)	5 (1.3)	17 (4.4)	15 (3.6)
LCI	9.0-12-6	8.0-8.9	7.0-7.9	4.4-6.9
COPD events n (rate)*	35 (10.9)	22 (5.9)	16 (4.0)	12 (3.1)

Q1 represents poorest lung function for all lung function markers. *incidence rate: n per 1000 person-years.

4.4.2. Incident coronary events

During 44 years of follow-up there were 266 incident CE. The rate of incident CE was highest in Q1 for VC, FEV_1/VC and LCI, whereas for FEV_1 high rates were found in both Q1 and Q2 (**Table 13**) The HR for CE per 1 SD change in lung function measures and by FEV_1/VC categories is shown in **Table 14**. There was a significant adjusted risk of CE per 1 SD decrease in VC and FEV_1. After adjustment however, the risk of CE per 1 SD increase in LCI and by a decrease in the FEV_1/VC ratio (both per 1 SD decrease and by cut-off criteria <0.70 or <LLN) was no longer significant.

Table 13:
Incident rate of CE by quartiles of lung function measures

	Q1	Q2	Q3	Q4
VC (L)	1.57-3.88	3.89-4.33	4.34-4.78	4.80-6.53
Coronary events n (rate)	73 (22.5)	69 (19.1)	74 (20.3)	50 (12.7)
FEV_1 (L)	1.19-2.89	2.91-3.32	3.33-3.73	3.74-4.97
Coronary events n (rate)	70 (22.8)	87 (24.1)	59 (15.6)	50 (12.5)
FEV_1/VC (%)	41.57-71.71	71.72-77.44	77.46-81.90	81.93-98.96
Coronary events n (rate)	64 (20.7)	69 (18.8)	69 (18.5)	64 (16.0)
LCI	9.0-12-6	8.0-8.9	7.0-7.9	4.4-6.9
Coronary events n (rate)	67 (21.4)	62 (17.0)	67 (17.1)	70 (18.4)

Q1 represents poorest lung function for all lung function markers. *incidence rate: n per 1000 person-years.

4.4.3. Comparison of the risks of incident COPD events and CE

Results from the modified version of the Lunn McNeil competing risks analysis can be seen in **Table 14**. HR presented are identical to those when cox models were run separately for the two different outcomes. The p-value represents the significance for equal associations for the baseline lung function measure with the two outcomes. After adjustment, a low FEV_1 and high LCI showed significantly stronger relationships with incident COPD events than CE (p-value for equal associations: <0.001 and 0.015

respectively). A 1 SD reduction in the FEV_1/VC ratio, FEV_1/VC <0.70 and FEV_1/VC<LLN all had significantly stronger relationships with incident COPD events than incident CE (p-value for equal associations: <0.0001 for all three FEV_1/VC baseline measures).

Low VC was significantly associated with both COPD and CE, however the HR between the two outcomes was not found to be significantly different (p-value for equal associations: 0.706).

Table 14:
HR for COPD events and CE per 1 SD change in lung function measures and by FEV_1/VC categories (n=672)

		COPD events (n=85)	Coronary events (n=266)	p-value**
VC	Unadjusted	1.42 (1.14-1.77)*	1.34 (1.18-1.52)*	0.662
	Adjusted	1.38 (1.05-1.81)*	1.30 (1.11-1.52)*	0.706
FEV_1	Unadjusted	2.14 (1.73-2.66)*	1.38 (1.22-1.56)*	<0.001
	Adjusted	2.11 (1.66-2.68)*	1.30 (1.13-1.49)*	<0.001
LCI †	Unadjusted	1.71 (1.37-2.13)*	1.19 (1.05-1.36)*	0.006
	Adjusted	1.58 (1.26-1.98)*	1.14 (1.00-1.31)	0.015
FEV_1/VC	Unadjusted	2.03 (1.70-2.44)*	1.15 (1.02-1.31)*	<0.0001
	Adjusted	1.95 (1.60-2.36)*	1.11 (0.98-1.26)	<0.0001
FEV_1/VC <0.70 §	Unadjusted	6.07 (3.95-9.32)*	1.23 (0.90-1.68)	<0.0001
	Adjusted	5.89 (3.74-9.26)*	1.13 (0.82-1.55)	<0.0001
FEV_1/VC <LLN §	Unadjusted	6.17 (3.92-9.73)*	1.08 (0.71-1.63)	<0.0001
	Adjusted	5.77 (3.55-9.39)*	1.02 (0.67-1.55)	<0.0001

Adjustments: height, BMI, diabetes, cholesterol, smoking, systolic blood pressure, physical activity, IHD at baseline. Hazard ratios are per SD decrease in lung function variable unless otherwise stated †per SD *increase* in lung function variable. § COPD events and CE for exposed (n): COPD: FEV_1/VC<0.70: 43, FEV_1/VC<LLN: 29. CE: FEV_1/VC<0.70: 49, FEV_1/VC<LLN: 25. *p value <0.05. ** Null hypothesis for this p-value is that the lung function variable has the same association with incident COPD events and CE (1 degree of freedom)

5. Discussion

5.1. General discussion

There is a known relationship between low levels of lung function and adverse outcomes later in life. The association has been strong for low levels of lung function and the future risk of poor cardiovascular outcomes [63-68, 72, 73] and for all-cause mortality [71, 117-121], even after many years of follow-up from baseline lung function. The relationship between spirometry and longevity has been known since the time of John Hutchinson (1846), when the term VC was introduced, indicating its prognostic value in predicting the capacity to live[122, 123]. Although well known, the specific explanations for the association between low lung function and CVD and mortality from any cause remain unclear. Along with baseline lung function, respiratory decline has also been associated with increased cardiovascular and mortality risk, independently of baseline spirometry and smoking[119, 124]. Furthermore, the presence of CVD has been also been found to be related to lower levels of lung function. Elderly subjects with CHD, hypertension or congestive heart failure have been found to have lower levels of FEV_1 and FVC than those without these conditions[125]. In a study assessing the cross-sectional association between the presence of heart disease and reduced lung function, Enright et al[125] found lower levels of FEV_1 and FVC were associated with the presence of IHD and hypertension, however unless heart failure also occurred, the effects were found to be small[125].

It has previously been thought that the increased cardiovascular morbidity and mortality experienced by individuals with COPD could be present due to the high prevalence of cardiovascular risk factors in this population[40, 126]. Although the apparent co-morbidity between COPD and atherosclerosis has been suggested to be due to common risk factors such as smoking[127], the prevalence of CHD and heart failure has been found to be high among those with pulmonary disease, even after taking into account common risk factors such as smoking[75]. Additionally, it has been suggested that COPD is associated with systemic inflammation, endothelial dysfunction and impaired vascular reactivity[128], however the relationship between airflow limitation and atherosclerosis has not been found to be mediated via endothelial dysfunction, and both airflow limitation and endothelial dysfunction have been found to be mutually exclusive predictors of atherosclerosis[129].

If poor lung function earlier on life influences the development of adverse incident COPD events in older age, this may potentially also aid in identifying those who may be at additional risk of adverse health outcomes in the form of CVD morbidity and mortality, along with premature mortality from other causes. Furthermore, understanding the relationship between poor lung function and other cardiovascular risk factors such as DM may also help in understanding the complex relationships between poor lung function and poor health outcomes.

Figure 15 demonstrates the pathways that are well established in the literature (black) and the pathways that were additionally explored in more detail in this thesis (blue), which were either unclear or not widely explored previously.

"Poor lung function" has usually been defined using spirometry measures alone, especially for purposes of disease prediction, namely FEV_1, FVC and FEV_1/FVC. However, in this thesis an additional marker of poor lung function- the LCI, was also assessed, which has not been widely explored in adults as a predictive marker of disease. In this thesis, we have shown that poor lung function as defined not only by spirometry, but also by measures of ventilation heterogeneity to be an important predictor of adverse incident COPD outcomes, beyond the effect of conventional cardiovascular risk factors. Patterns of baseline spirometry and LCI in middle-aged men are also important in determining the risk of COPD events compared to future CE. In this thesis, the temporal relationship between poor lung function and DM was established in a large cohort of subjects, which had previously been unclear. Poor lung function as measured by spirometry preceded and predicted the development of incident DM; an association which was present many years after baseline spirometry was measured and after adjustment for baseline glucose levels, BMI, smoking, and even systemic inflammation. Additionally, we established there is a relationship between poor lung function at baseline in otherwise healthy middle-aged men and future COPD events such as hospitalisation and deaths due to COPD, whether the FR criteria is used or the LLN criteria for the FEV_1/VC ratio, and for poor values of LCI. The FR or LLN criteria therefore also have use in the prediction of future adverse COPD outcomes in subjects who are asymptomatic at baseline. In this thesis, the different lung profiles at baseline that can affect the risk of a coronary outcome or a COPD outcome were explored, which can help us to further identify subjects who are at potential risk of future CE or COPD events, and specifically target preventive strategies to reduce these risks.

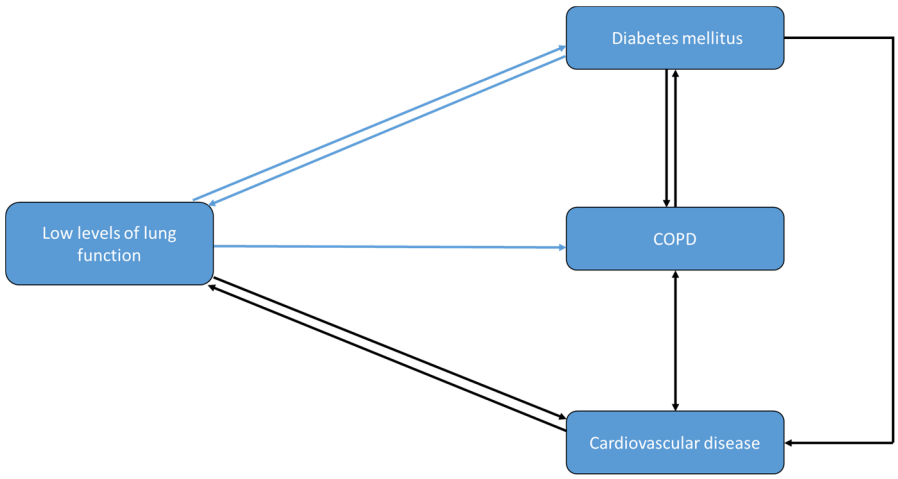


Figure 15:
The complex relationship between poor lung function and three chronic disease outcomes. Black arrows indicate associations that have been explored in the past and blue arrows indicate associations explored to a lesser extent in the past and were explored more in this thesis.

5.2. Methodological considerations

5.2.1. Associations in epidemiological studies

In epidemiological studies, we explore associations between various exposures and outcomes, which in turn can later be further explored to determine if a causal relationship exists. An association in an epidemiological study can be a measure of relative effects (a ratio; a risk, a rate or odds) or absolute effects (risk differences). If an association is found in an epidemiological study, the four possible explanations are:

1. It is a true finding (what we hope for)
2. It is a chance finding
3. The association is observed due to the exposure and outcome being linked to a third factor – a confounder
4. The association is observed due to a systematic error in the study i.e. some bias in the study design, conduct of study or analysis.

Therefore, during the study design stage we aim to reduce the possibility of the association being observed due to chance, bias or confounding.

5.2.2. Study design and external validity

All four papers included in this thesis were based on prospective cohort studies. The study populations included was a sample of the total population of interest. As such, the aim is for the study population to be as representative as possible of the population of interest to which study findings would be applied. An advantage of the prospective cohort study design is ensuring the temporal sequence of exposures and outcomes.

Men Born in 1914

Due to the nature of cohort study designs with long follow-up times, certain baseline characteristics are likely to change over the follow-up period. Smoking rates have known to be declining in Sweden over time[130, 131], therefore we would expect the rates to have declined even further after the re-examination in 1982 and be quite substantially lower than the initial prevalence estimates by the end of the follow-up period. For subjects included in **Paper I**, there was a substantial reduction in the proportion of current smokers (62.0% in the initial baseline assessment in 1969 and 33.5% in the re-examination in 1982). The proportion of prevalent DM increased from to 2.2% in 1969 to 5.1% in 1982, and the mean BMI increased from 24.5 kg/m^2 in 1969 to 25.0 kg/m^2 in 1982. The smoking prevalence in a study assessing the change in cardiovascular risk factors over time in a Swedish population found a reduction in smoking prevalence from 56.1% in 1963 to 11.9% in 2013[130]. Although the mean BMI and prevalence of DM had also increased in the study population over time, they found that in general the total cardiovascular risk factor burden has decreased in 50 year old men living in Sweden over the past 50 years (years 1963-2013)[130]. As the baseline values from 1963 in the study are consistent with that has been found in the "Men born in 1914" cohort measured in 1969, we also expect that by the end of follow-up in 2013, the changes in risk factor prevalence would be mirrored in our cohort for **Papers I, II and IV.**

Additionally other factors over the course of follow-up could also have influenced the association observed in **Papers I, II and IV**. Inhaled corticosteroids did not gain popularity for the management of COPD until the 1990's along with the importance of smoking cessation around this time[38]. This along with other developments in the management of COPD and reduction in risk factors over time, would have reduced the likelihood of severe incident COPD events occurring such as hospitalisations or mortality, and reduced the incidence of coronary events in this cohort (**Paper IV**). Therefore, if anything we would expect such changes to have biased any associations seen towards the null.

In the "Men Born in 1914" cohort, a birth cohort of men born in the same year was used as the study population. Although by doing so the cohort allowed us to assess the relationships of exposures on various outcomes assuming all subjects share common life experiences (living in the same city, born in the same year, and of the same gender), a

disadvantage is that findings from such studies are generalised to other populations with caution. The external validity of a study refers to this concept. For a study to exhibit high external validity, the findings from the study population must be generalizable to the reference population. The results from such a cohort may not be so readily generalizable to women for example, or to subjects who are not living in an urban population. However, a study by Luoto et al[132] assessing the incidence of airflow limitation in subjects aged 65-100 years, found female sex to be a risk factor for developing airflow obstruction and COPD in a 6 year follow-up study (baseline examinations 2001-2004). Therefore, it is possible that major risk differences between gender may not have existed, even if the results from our study cannot be directly applied to women. There may also be specific unmeasured birth cohort effects that may affect directly or indirectly the associations observed. Additionally, the change in risks and exposures even within the same population over time is another factor that can affect the external validity of a study. Middle-aged men living in Malmö today may exhibit different risk profiles from that which were measured in 1969 or even 1982. The changing prevalence of risk factors within the same population that was studied (e.g. smoking, physical activity, medication use, diabetes) at a different time point can also influence how generalizable the results are today, and the conclusions we can draw from them for the current population.

Malmö Preventive Project

The issues related to the prospective cohort study design and changes of risk factors during follow-up, also apply to **Paper III** using the MPP cohort for the outcome of incident DM. As previously mentioned, the use of corticosteroids may have taken place for some subjects during follow-up, which in this study cohort, could have increased the risk of DM. However, while this is likely with oral corticosteroid treatment, a study evaluating data from large randomised controlled trials found that the risk of new onset DM or hyperglycaemia was not increased in COPD or asthma patients treated with inhaled corticosteroids (budesonide) compared to those not treated with it[133]. Additionally the use of statins for the treatment of high cholesterol has been implicated in the development of new onset DM[134], which would again increase the risk of DM in some individuals. Although cholesterol and BMI were adjusted for in the analyses with DM as an outcome, there would be residual effects expected due to these changes in the cohort over time.

5.2.3. Internal validity

The internal validity of study refers to how well a study was conducted (i.e. does the study examine what was intended, without the presence of confounding or systematic errors).

"An experiment with a high degree of internal validity has reduced the potential influence of extraneous variables to such an extent that the independent variable is the most likely cause of the observed change in the dependent variable"[135].

Therefore, we aim for an epidemiological study to have sound internal validity in order for us to make valid conclusions from the findings. A study can have internal validity without external validity, but in order for there to be external validity, internal validity is necessary.

Bias: Selection and information bias

Bias in epidemiology refers to any systematic error in the design, conduct or analysis of a study that results in a mistaken estimate of an exposure effect on the risk of disease[136]. In contrast to random error, which refers more to the precision of measurements, a systematic error can occur at the level of both the investigator and the level of the study design, and the absence of systematic errors improves the internal validity of a study. The two main types of biases in the cohort studies to be aware of in this thesis are selection bias and information bias.

Selection bias

Non-response bias can cause a serious form of selection bias in cohort studies. This was demonstrated well by a Swedish study which characterised non-responders of a mailed questionnaire in a prevalence study on asthma, chronic bronchitis and respiratory symptoms[137]. The response rate was 85%, and of those who did not respond, a small proportion were contacted by phone to answer the same questionnaire. They found that the non-responders had higher proportions of current smoking and manual labourers as their occupation than the responders, along with a higher prevalence of respiratory symptoms compared to responders. Subsequently, the prevalence of respiratory symptoms was underestimated by the postal questionnaire. It is of high importance to keep the proportion of non-responders as low as possible and if there are any non-responders, to attempt to characterise them as much as possible[103].

Participation rate in the "Men Born in 1914 cohort" was relatively high with 703 men attending the baseline examination out of an eligible population of 809 men (87%). However, participation rate was significantly lower at the re-examination stage in 1982-1983 among those invited (participation rate 80.5%, p-value<0.01)[138]. A study was carried out to determine factors influencing participation in the re-examination stage in 1982-1983 of the "Men born in 1914" cohort[138]. The aim was to establish ways to increase participation rate in population surveys by investigating factors that lead to low participation. A total of 621 men were invited to the re-examination, the majority of which were from the first examination in 1969, but also included non-participants from the 1969 examination and men who moved to the city after the first examination. Of those invited, 80.5% attended the re-examination (n=500) and of the 121 that did not attend, 94 agreed to take part in a phone interview instead. Of those who did not

agree for a phone interview (n=27), 11 had died and 16 refused a phone interview or could not be reached. These 27 subjects were more likely to be single, and dependent on social welfare compared to the examination or phone-interview attendees. In the phone-interview, for the initial non-responders, the two reasons for initial non-response included illness/regular contact with a doctor and a negative attitude towards health surveys (due to a fear of the examination or result of the examinations).

In the second examination stage (re-examination in 1982) for **Paper I**, the participation rate for re-examination was 57% of the initial cohort and 81% for the cohort eligible for re-examination (**Paper I**: initial baseline examination in 1969 n=689, subjects eligible to take part in re-examination (alive and still living in Malmö) n=482, subjects participating in re-examination n=392). Similar participation rates for re-examination were found in **Paper II.** In **Paper I,** those who moved away from Malmö (so could no longer take part in re-examination n=75) or declined to take part in the re-examination (n=90) had similar baseline prevalence of smoking, similar baseline FEV_1 and VC. A significant advantage of this cohort was that almost all subjects were followed up until the event of interest, death or the end of the follow-up period (n=3 lost for follow-up due to emigration from Sweden).

In a study assessing the long term outcomes of the MPP (mortality and cardiovascular morbidity)[139], invited subjects were compared to non-invited subjects and it was found that risk factor screening for those who were invited overall did not result in a reduction in the total mortality when compared to non-invited subjects. Interestingly, invited participants were also compared to invited non-participants (subjects who declined to take part). It was found that the invited non-participants had a higher total and cause-specific mortality than participants. It was also found that social and demographic characteristics differed significantly between invited non-participants and invited participants, where non-participants had a less favourable socio-demographic profile than participants (less likely to be Swedish born, more likely to have lower education level, more likely to be living alone, and more likely to be from a lower socio-economic group)[139]. Findings such as these stress the difference that can exist between subjects who are invited and accept an invitation to take part in the study, and those that decline to take part in the study. This can lead to a form of selection bias, in that those taking part in the study are thought to be "healthier". However, the high participation rate in the MPP (mean participation in invited cohorts was 71% (range 64-78%)[139] and the prevalence of risk factors in the MPP cohort (49.1% of men and 45.2% of women current smokers, 52.4% of men and 43.3% of women reporting physical inactivity) it is likely the MPP population was not much healthier in terms of risk factors than the general population.

In **Paper III**, the proportion of subjects who were loss to follow-up due to emigration was low. In men 2.3% of subjects emigrated by 2013 (n=470), and in women 1.4% emigrated by 2013 (n=104). In men, those who were lost to follow-up were similar in

terms of baseline characteristics to those who were not (proportion of current smokers at baseline: 50.2 vs 49.1%, mean BMI: 24.6 vs 24.6kg/m^2, mean FEV_1: 3.56 vs 3.52 L and mean baseline glucose 4.96 vs 4.92 mmol/L, respectively). In women, the prevalence of smoking was slightly lower in those who were lost to follow-up compared to those who were not (41.3 vs 45.2% respectively) and had slightly higher BMI than those who were not (24.2 vs 23.7 kg/m^2). Therefore, the likelihood of any selection bias influencing the associations observed is low; follow-up of subjects was almost complete and those who were lost to follow-up did not differ greatly from those that remained in the study.

Information bias

Misclassification of exposures: All measurements of spirometry were carried out by experienced staff from the Department of Clinical Physiology at Malmö University Hospital for the "Men Born in the 1914" cohort and trained nursing staff in the MPP. The first American Thoracic Society (ATS) statement on the standardisation of spirometry was formed in 1979[140] and a similar European initiative in 1983. Guidelines for spirometry were not implemented at the time of baseline examinations for the "Men Born in 1914" cohort (no prior bronchodilation, but two acceptable manoeuvres required) and the MPP cohort (no nose clips used and only one acceptable manoeuvre required). Therefore, it is understood that performance in many subjects would not have been standardised to the guidelines which are used currently[141]. In the MPP cohort, 94% of men and 71% of women in the study underwent spirometry as not all birth cohorts were screened with spirometry (not all women screened after 1985 were offered spirometry). However, subjects were not selected for spirometry based on symptoms or disease status and the validity of the spirometry measurements in the MPP has been acceptable for predicting long-term outcomes in previous studies[142, 143]. No prior bronchodilation was used when spirometry was assessed in the "Men born in 1914" cohort. In the current guidelines, post-bronchodilation measurements are required for the diagnosis and classification of COPD[17]. However, many longitudinal studies that assess the effect of low lung function on mortality and hospitalisations use pre-bronchodilator values alone[144-146]. In a study assessing the use of pre and post-bronchodilator values of spirometry for the purposes of prediction of mortality, it was found that both pre and post-bronchodilator values of spirometry predict mortality with similar accuracy and it was therefore concluded that post-bronchodilator readings may not be necessary in population based studies that predict long-term outcomes[144]. Additionally, in the "Men Born in 1914 cohort, VC was measured as opposed to FVC. The use of FEV_1/VC instead of FEV_1/FVC can lead to lower values of the ratio, however the cut off of 0.70 is still recommended[147]. If FVC had been used instead of SVC for baseline lung function measurement in **Papers I, II** and **IV**, we anticipate that fewer subjects would have been classified as having poor lung function at baseline according to the FEV_1/FVC ratio, as the FVC is likely to yield smaller estimates of the VC than SVC. In **Paper I** the risk of COPD hospitalisations was increased even in the

group with $FEV_1/VC<0.70$ but > LLN at baseline. By using the SVC, this intermediate group contained relatively milder cases of airflow limitation on spirometry and the risk of COPD hospitalisations was nevertheless still increased relative to the reference group. Had FVC been used instead of SVC for the ratio, some of these subjects in the intermediate group would have been reclassified as "normal" (reference group). However, as the larger measurement of VC is more likely to be achieved using SVC than FVC, it can be thought that the SVC was an acceptable measure for doing analyses using VC as part of the FEV_1/VC ratio.

In the measurement of LCI, there is known to be good intra and inter-visit reproducibility of using the MBW (with sulphur hexafluoride) in both children and adults with CF[27]. For LCI measurements, until more recently, there were no universal standards for performing these tests[21]. Therefore we also have to assume the MBW technique performed in 1969 (using nitrogen) in **Papers II** and **IV** would be somewhat altered from the techniques that would be advised today[6]. These factors are likely, if anything, to effect the precision of the spirometry measurements in both cohorts and for LCI in the "Men Born in 1914" cohort. Therefore, any misclassification of subjects for the baseline exposure would not be related to the outcomes status, and so we would expect this to be non-differential misclassification of the exposure. This type of misclassification is likely to have re-classified those with high lung function in reality to a lower lung function category due to imprecisions in measurements. This would have therefore caused the "exposed" group to contain "unexposed" subjects, thereby biasing the results towards the null. This, along with fluctuations of measurements within an individual over time, could potentially result in regression dilution bias[148].

Misclassification of outcomes: The outcomes for **Paper I, II** and **IV** were one or more of either COPD hospitalisations, COPD-related mortality, all-cause mortality or coronary events (which included fatal or non-fatal MI and mortality related to IHD). These all represent the more severe end of the disease spectrum, where subjects had to seek secondary care. Therefore, any "milder cases" of community managed COPD or CHD would not be included in the case definitions. Primary or secondary diagnosis for COPD hospital admissions were taken from hospital discharge summaries where board-certified physicians settled the diagnoses. This would ensure either that the main reason for admission was COPD (such as a COPD exacerbation) or that even if admitted to hospital for a different reason, COPD was part of the patients admission. For **Paper I**, there were 88 incident cases of COPD events of which 80 were diagnosed from hospital admissions. For **Paper II** and **IV** there were 85 cases of incident COPD events of which 77 were diagnosed from hospital admissions. The Swedish inpatient registry has been validated as acceptable for epidemiological research, including for the diagnosis of COPD[149]. The register was established in 1964 and complete coverage was established in 1987 when the registry became nationwide. In 2001 data from outpatient visits was added to the registry, which therefore would include both in and outpatient data. Importantly, the register was in operation in the south of Sweden throughout the

follow-up period. General validation studies of this register have shown that 85-95% of diagnoses reported by the registry are correct[150]. Additionally, a specific validation study for the diagnosis of COPD was performed which showed that over half of patients with COPD on discharge summary had proven or clinically probably COPD, a third had possible COPD and only a small proportion (under 10%) had an uncertain diagnosis or were misclassified[149]. A limitation to this register however is that there was no register coverage outside of the south of Sweden (Skåne) before 1970. Therefore, we cannot exclude the possibility that some of these men could have been diagnosed with some form of airflow limitation/emphysema before 1970 that the registry would not have recorded. However, as these men were 55 years old at baseline in 1969 and the risk of first-time hospitalisations for COPD is thought it be low before the age of 50-59 years[151], it is not expected that many subjects would have presented to secondary care for COPD (or equivalent) before baseline examinations. For COPD-related mortality (**Papers I, II** and **IV**), almost all cases had autopsy confirmation of the cause of death, which adds validity to the outcome ascertainment.

For the retrieval of incident CE, data linkage between the Swedish inpatient registry, the Malmö myocardial infarction register and the national cause of death register was used. Morbidity and mortality from IHD had been continuously monitored by the Malmö myocardial infarction register in the earlier years[152 153] which was then taken over by the Swedish inpatient registry for the remainder of the follow-up time. For non-fatal MI, the main or first secondary diagnosis of MI were counted as events, which would ensure that the MI was a significant reason for the hospital admission, or it occurred during the hospital admission if it was not the reason for admission. The Swedish inpatient registry has also been found to have high validity for the diagnosis of MI, where a positive predictive value of approximately 98-100% has been found[150].

In terms of all-cause mortality as an outcome in the "Men Born in 1914 cohort", the most common cause of death was deaths due to cardiovascular diseases (**Paper I** n=323 and **Paper II** n= 315 of reported deaths had cardiovascular disease as an underlying cause of death). Information from death certification was used to establish the main cause of death. The Swedish cause of death register is known to be a complete register of all deaths in Sweden since 1952, and is thought to be of high quality and an important source of data for medical research[154].

The likelihood of misclassification of the outcomes for **Papers I, II** and **IV** is therefore low, as the robustness of the patient registers used for case retrieval have been proven to have high validity in ascertaining the outcomes of interest.

In **Paper III**, the outcome of interest (DM) was ascertained using various registers along with data from re-examination of the MPP cohort. For a condition such as DM (type 2), the exact time of onset is often impossible to know as it can remain undetected for long periods of time. However, a subgroup analysis was performed in **Paper III** on subjects who were examined in the MPP and later again in the MDC-CC and who

were still free from DM more than 10 years after baseline measurement in the MPP. These subjects were found to have an 11% increase in risk of DM for every 10% decrease in FEV_1%predicted (HR 1.11 (1.02-1.21) (included both men and women). This supports the conclusion that poor lung function is predictive of DM even after many years of follow-up, and the confirmation of these subjects being non-diabetic at enrolment in the MDC-CC challenges the possibility that the cases were just "late" in being ascertained due to being undetected over time. During the course of follow-up of the MPP cohort, the case definition of DM as per the World Health Organization (WHO) changed[155]. Before 1998, a plasma glucose of ≥7.8 mmol/L was commonly used for diagnosis, whereas after the WHO recommendations in 1998, the current cut-off of 7.0 mmol/L was used. Therefore case ascertainment during the later years of follow-up would be higher relative to the initial follow-up times, especially for men who were followed up from 1974-1981 onwards. Furthermore, the awareness of DM and its clinical symptoms has increased over the years, both in the general population and within health-care. This would result in an increase in the case detection rates over time. Additionally, the coverage of registers also improved over time, which would again increase the likelihood of better case detection as the follow-up time increased. If anything these factors would have biased the results in the earlier parts of the follow-up time towards the null due to an underestimation of the true incidence of DM in the earlier follow-up periods, and increased incidence of diabetes in the last years of follow-up. It is less likely, though, that any differences in case ascertainment rates over time would be associated with the spirometry results. It is important however to be aware of the fact that there has also been an actual increase in the prevalence in DM over time in Swedish populations, consequently an increase in cases towards the later parts of the follow-up periods would actually in part represent a true increase in the incidence of DM[156].

Confounding

In observational studies, an important consideration when an association is found between an exposure and an outcome is the issue of confounding. Confounding is:

"the distortion of the effect estimate of an exposure on an outcome caused by the presence of an extraneous factor associated with both the exposure and outcome"[157].

Confounders are present in "real life" and so when interpreting an observed association, the issue of confounding and whether all possible confounders were controlled for must be addressed. In an association between an exposure and outcome, a third factor is a confounder if it is:

1) Associated with the outcome (e.g. a risk factor for the outcome)
2) Associated with the exposure (but not a result of it, i.e. independently associated with the exposure).
3) Is not on the causal pathway of the association between the exposure and outcome.

To control for the effects of confounding in cohort studies, one must first identify potential cofounding factors. In the association between poor lung function at baseline and future incident COPD events, incident CE, all-cause mortality or incident DM many confounders may distort the effect estimate if not controlled for. The selection of confounders to be included in the regression models was based on factors in the literature that are known to affect the association between poor lung function and health outcomes. Information from other similar studies using similar methodologies was used, such that adjusting for similar confounding variables would allow results to be compared more readily to existing literature. However, there are other techniques available to identify possible confounders in the relationship between the exposure and outcome that can be used to help identify which variables should be adjusted for in regression models.

In **Papers I, II** and **IV** the main associations assessed were between poor lung function and incident COPD events, CE or all-cause mortality. As poor lung function has been known to be associated with CHD which in turn has been associated with COPD, controlling for known cardiovascular risk factors was deemed important (such as BMI, DM, physical activity and smoking). Systolic blood pressure and cholesterol was additionally adjusted for when considering all-cause mortality in **Paper I** and in **Paper IV** as both incident COPD events and CE events were being compared in a single model. In **Paper II**, adding cholesterol and systolic BP to the model for all-cause mortality as the outcome did not affect the HR, and so were not included in the final adjusted model. In **Paper III,** variables included in the model were again based on previous literature and known cardiovascular and metabolic risk factors that could affect the exposure-outcome relationship. Although in many instances in **Papers I-IV**, the lung function variable being used as the exposure was expressed as a percent of the predicted value (which would take into account the height of an individual) and additionally BMI was included in the model adjustments (which also takes into account the height), height was still added to the final adjusted models. The purpose of the prediction equation is to adjust as well as possible the effects of age and height on the lung function values. However, any residual effects can be corrected by adding these covariates to the model adjustment to ensure that subsequent results obtained are independent of a subject's age, height and body mass.

Forwards or backwards elimination is an alternative way to identify variables to be included in the model for adjustment. This is based on defining a p-value to keep a variable in the model (e.g. <0.05 - forwards elimination), or to remove a variable from

the model (e.g. >0.1- backwards elimination). However, it is thought that this method can be potentially problematic and should never replace the process of thinking about the issue[158] (e.g. other variables that have a plausible association between the exposure and outcome, or variables known to be associated with exposure and outcome from previous work)[158]. The number of variables included in the model is an additional factor to be considered alongside the size of the study population. There has been a suggestion that a minimum of 10 outcome events should be present per predictor variable in cox regression models[159, 160]. However, it has since been thought that in Cox regression models this "rule of ten" can be relaxed, especially when performing sensitivity analysis to show adequate control of confounding[161].

An alternative way to identify possible confounders is using a diagrammatic representation of the connections between different variables and the exposure and outcome. This is done using Directed Acyclic Graphs (DAGS). The DAG allows us to identify if the associations we expect between the different variables and exposure/outcome relationship mean that a certain variable is a confounder, a collider or an intermediate variable. A collider (both the exposure and outcome lead to the variable- a common effect) should not be adjusted for, and an intermediate variable is thought to be on the causal pathway between the exposure and outcome and therefore also should not be adjusted for.

A visual representation of all the connections between different variables and the exposure-outcome relationship is shown for **Paper I** in **Figure 16** [162]:

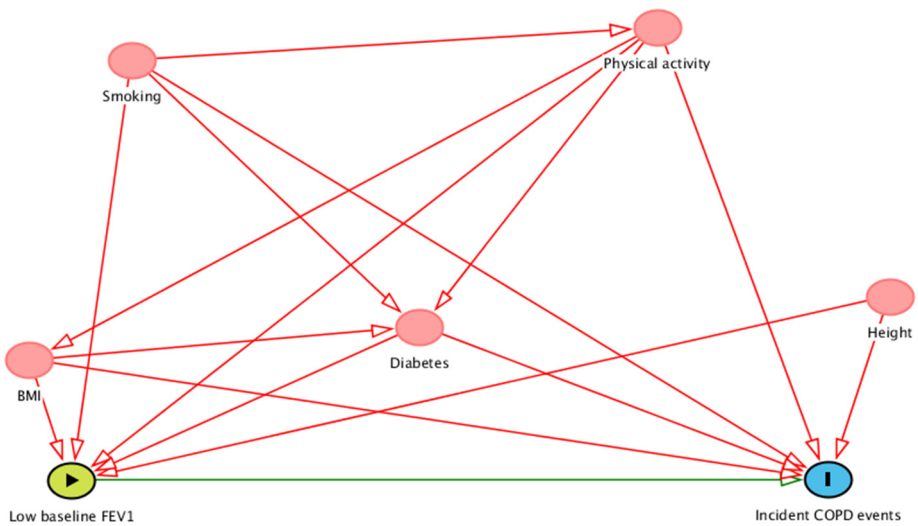


Figure 16:
DAG representation of the confounders included in a model for **Paper I** and its complex associations with each other.

Due to the complex interaction between CVD and both poor lung function at baseline and COPD co-morbidity, risk factors associated with the development of CVD were thought to be important to control for when assessing the association between poor lung function at baseline and future incident COPD events. Further adjustment for prevalent IHD or CVD in 1969 did not affect the results to any significant degree. DM was also adjusted for in the analysis and although its association with poor lung function became clearer in **Paper III**, as it was known that subjects with DM tend to have poorer lung function than non-diabetics[77] and DM is known to be associated with COPD[84], it was important that DM was included in the model.

The role of residual confounding should always be considered when associations between exposures and outcomes are found in observational studies. Residual confounding can be due to either unmeasured confounders, confounders that have been inaccurately measured or inappropriate statistical models. Socio-economic status (SES) has an important association with levels of lung function and with various outcomes including mortality[163]. It has been found that there is a strong negative correlation between lung function and SES which exists even after adjustment for smoking, occupational exposures and race[164]. SES is thought to be made up of measures including income, education, occupation/employment status, residence location and housing[164] In **Papers I, II** and **IV** where the "Men Born in 1914" cohort was used, there was insufficient data recorded on these measures to allow for adjustment for SES, therefore there may be some residual confounding due to this. Additionally, occupational exposure is associated with both low levels of lung function and future occupational COPD[165], and again there could be some residual confounding resulting from the lack of data recorded on this in the "Men born in 1914" cohort at baseline. In **Paper III**, information on employment was used as a surrogate for SES in the MPP, where subjects were classified into groups according to their type of employment/employment status at baseline. For **Paper III**, obesity- particularly abdominal obesity is thought to be an important factor in the association between poor lung function and diabetes. Although BMI was adjusted for, waist circumference is thought to be a stronger predictor for type 2 DM compared to BMI[166], where it has been found the combination of using BMI with measures of abdominal obesity is more strongly associated with DM than using BMI alone[167].

If a confounding variable is identified, there are a few ways of effectively dealing with it such that it does not affect the association one is trying to assess. At the analysis stage of observational studies, variables that have been identified as possible confounders can be added to the regression models and their effects on the exposure –outcome relationship can be therefore adjusted for. Additionally at the analysis stage, data can be stratified on the confounding variable. We performed a stratified analysis in **Paper III**, where subjects were stratified on smoking status. Smoking would be a potential

confounder in such an association and by stratifying the analysis by smoking status we were able to assess the risks in both smokers and non-smokers, allowing us to make firmer conclusions about the association and if it is present in the non-smoking groups as well as the smoking group. Restriction is another method of controlling for confounding where you restrict the analysis to a group of subjects that do not exhibit the potential confounder. However this is not possible when many confounders could be present, and due to the reduction in the size of the study population that would result from this, the subsequent effect could be loss of power in the study and a loss of external validity[168].

Chance

An alternative explanation for the observed association between the exposure and outcome is that the results occurred due to chance. The null hypothesis referring to the observational studies conducted as part of this thesis would be that there is no association between the exposure and outcome, and sample data is used to either accept or reject this hypothesis.

The two types of errors that can occur in this situation are a type I error (α) or a type II error (β). Type I errors occur when based on the sample data one rejects the null hypothesis when it is actually true. A pre-defined significance level for this must be stated. In **Papers I-IV** a significance level of <0.05 was thought to be adequate for exposure-outcomes situations. This means less than 5 % of the time we would reject the null hypothesis when it is actually true. There was an *a priori* for the associations in all **Papers I-IV** which were based on previous knowledge for potential conceivable mechanisms linking the exposure and outcome, however two-tailed hypotheses were used so that an association in either direction could be detected. A type II error refers to accepting the null hypothesis when is it not true based on sample data. Therefore, the probability of accepting the alternative hypothesis when it is true is $1-\beta$, which is equivalent to the power of study. The power of a study refers to the probability of finding a difference when it does actually exist (i.e. the alternative hypothesis is true) and depends on:

- The size of difference that is hoped to be detected
- The sample size of the study population
- The p-value (type I error)
- The variation in the sample (standard deviation of the study population)

We are aware that the power in the "Men born in 1914" cohort may be limited due mainly to the small sample size. This could have affected for example the C-statistic in **Paper II**, where a non-significant increase in the C-statistic was found after adding LCI to a model with conventional spirometry, in the prediction of COPD events. However, the good precision of baseline measurements carried out by trained professionals and the robustness of the registers used for outcome ascertainment, along with the extensive

follow-up time for this cohort allows us to be confident about the internal validity of the studies and their respective findings.

5.3. Summary of main findings

5.3.1. Baseline FEV_1/VC <0.70 >LLN and future disease risk

In **Paper I** a significant association was found between low baseline FEV_1/VC ratio and future COPD hospitalisations and COPD related mortality, regardless of whether the FR or LLN criteria for a low FEV_1/VC ratio was used. This risk was significantly increased even after taking into account the competing risk of death from all other causes. It was also found that future FEV_1 decline in 55 year old men was also related to their baseline FEV_1/VC, and both the FR+LLN- groups and FR+LLN+ groups had greater FEV_1 decline from age 55-68 years, compared to those in the normal FEV_1/VC group at baseline. All-cause mortality was however more strongly related to FEV_1/VC <0.70 and <LLN (FR+LLN+) at baseline. Airflow obstruction at baseline was therefore found to be a powerful predictor for future COPD hospitalisations, and the intermediate group of FR+LLN- at baseline should also be a target for preventive strategies to reduce future severe COPD events and FEV_1 decline. When the predictive value of both cut-off criteria for the FEV_1/VC ratio at baseline was compared for incident COPD events and incident CE (**Paper IV**), it was found that both the $FEV_1/VC<0.70$ and FEV_1/VC <LLN are more strongly associated with future incident COPD events than future CE (p-for equal associations <0.0001). An $FEV_1/FVC < 0.70$ was not found to be associated with future incident DM (**Paper III**). Therefore, these findings when taken together further reinforces the importance of preventive strategies to be implemented early to reduce the risks of COPD hospitalisations and future lung function decline in particular in those with a poor FEV_1/VC ratio at baseline, defined by either criteria.

A low FEV_1/VC (FEV_1/VC <LLN) in combination with a high LCI at baseline was associated with a significantly higher risk of COPD hospitalisations than $FEV_1/VC<LLN$ in combination with a normal LCI (p-value for difference between both groups 0.041) (**Paper II**). This indicates the value of additionally measuring markers of ventilation inhomogeneity in those with an obstructive lung profile at baseline to further clarify this risk (LR test Chi-square 5.89, p-value 0.015 after adding LCI to a model containing baseline FEV_1/VC for the prediction of incident COPD hospitalisations, **Paper II**).

5.3.2. Low FEV_1 and FVC or VC at baseline and future disease risk

The incidence of COPD hospitalisations was strongly related to poor baseline FEV_1 (%predicted values and per 1 SD decrease in FEV_1) (**Paper I** and **IV** respectively) as was the risk of all-cause mortality (**Paper I**). Low FEV_1 at baseline was also associated with incident CE (**Paper IV**), however it was found that a low FEV_1 at baseline is more strongly associated with future COPD hospitalisations than with future CE (p-value for equal associations <0.001). A low FEV_1 (FEV_1<LLN) at baseline in combination with a high LCI also was more strongly associated with incident COPD hospitalisation than a low FEV_1 at baseline with a normal LCI (p-value for difference between groups 0.019) (**Paper II**). This also adds value to using baseline spirometry such as FEV_1 measurement to identify individuals at risk of developing adverse COPD events later in life, which if used in combination with LCI can be a powerful predictor for the development of such future events. Low FEV_1 (%predicted value) was found to be predictive of incident DM even after many years of follow-up (**Paper III**). This is an important finding that can help in determining the temporal relationship between poor lung function and DM, where consensus has not been reached.

Low VC (%predicted) was no longer associated with incident COPD hospitalisations after adjusting for confounding factors when comparing the lowest quartile of VC (48-93%predicted) with the highest (reference, 109-139%predicted) (**Paper I**). However when analysing the data without the division into quartiles (per 1 SD decrease in VC) (**Paper IV**), there was found to be an association between VC and future incident COPD events (HR 1.38 (1.05-1.81). This difference is likely due to the loss of power that may occur after dividing data into quartiles or groups. A low VC at baseline was found to be associated with future CE, however the difference between the two outcomes (incident CE and incident COPD events) in relations to baseline VC was not found to be significant (p-value for equal associations 0.706). We conclude therefore that a low VC has a similar association with developing COPD events or CE later in life (**Paper IV**). Low FVC at baseline was found also be associated with incident DM and the association was broadly similar to that reported for FEV_1 and incident DM (**Paper III**). However, the risks were significant up to 30 years from baseline in men with poor FVC, and up to 20 years from baseline in women.

5.3.3. Ventilation inhomogeneity at baseline and future disease risks

Incident COPD events and all-cause mortality were strongly associated with baseline LCI. Incident COPD events were associated with baseline LCI even after adjusting for baseline FEV_1. Future FEV_1 decline and pulmonary obstruction in those free from obstruction at age 55 years was also associated with baseline LCI (**Paper II**). When assessing the relationship between LCI and future CE (**Paper IV**) the association became non-significant after adjustment for confounding factors. Therefore based on

these findings a high LCI at baseline is more strongly associated with future pulmonary events such as adverse COPD outcome, lung function decline and new pulmonary obstruction than with incident CE (**Papers II and IV**). Using LCI in combination with conventional spirometry provided higher risk estimates of incident COPD than when using LCI alone or spirometry alone (**Paper II**). Although a non-significant HR for incident COPD events was found in the group with high LCI but normal FEV_1 (or normal FEV_1/VC), when dividing the overall cohort into quartiles of LCI there was found to be a significant risk of COPD events in the highest LCI quartile relative to the lowest, even after adjustment for baseline FEV_1. The former findings were likely different due to a lack of power affected by the smaller numbers of subjects in each category (aside from the reference) (**Paper II**). Therefore, LCI can be thought of as being associated with incident COPD events such as hospitalisations, independently from conventional spirometry measures such as baseline FEV_1.

6. Conclusions

This thesis examined the relationship between low levels of lung function and the prediction of future health outcomes in an urban population. Based on the findings from the studies included in this thesis, the following conclusions were made:

1. Airflow obstruction at age 55 years is a strong risk factor for future COPD hospitalisations in otherwise healthy men, independently of smoking.

2. A FEV_1/VC ratio <0.70 but over the LLN should still be considered a strong predictor for future incident COPD events, CE and future FEV_1 decline in healthy middle-aged men.

3. High LCI is a strong predictor of incident COPD events independently of baseline FEV_1 in healthy middle-aged men. High LCI is additionally a predictor of all-cause mortality, future pulmonary obstruction and FEV_1 decline in healthy middle-aged men.

4. Poor lung function precedes and significantly predicts the development of future incident DM in middle-aged men and women, even after adjustment for smoking, obesity and inflammation. This relationship is present many years after baseline measurements, particularly in men.

5. A low FEV_1, an obstructive lung profile and ventilation inhomogeneity at age 55 years are more strongly predictive of future incident COPD events than CE among middle-aged men. A low VC however is similarly associated with incident COPD and CE in middle-aged men. These are important considerations when assessing the risk of future health outcomes associated with poor measures of lung function earlier in life.

7. Future perspectives

The use of spirometry as a general health screening tool has been controversial and currently not advocated by GOLD (2018)[17]. However, the rationale for this has been that the diagnostic yield of incident COPD cases is higher when spirometry is used in subjects who have symptoms or a risk factor such as smoking, compared to asymptomatic non-smokers. Additionally it addresses the issue of active case finding of new cases of COPD rather than identifying those at risk of severe COPD events such as hospitalisations and deaths due to COPD. In this thesis, it has been found that asymptomatic subjects with low levels of lung function in middle-age are at future risk of COPD hospitalisations and mortality due to COPD, beyond the effect of smoking. The identification of such individuals using spirometry through general health screening has two main advantages: 1) using early intervention to alter the disease course and therefore potentially reduce the risk of developing outcomes at the more severe end of the obstructive lung disease spectrum, and 2) to reduce the economic burden of COPD morbidity such as prolonged hospitalisations and living with advanced COPD in the community. COPD remains a major public health problem and is thought to make up two-thirds of the disability-adjusted life years (DALY) for chronic respiratory diseases[169]. As such, there is a substantial economic and societal cost of living with severe COPD (years of life lost due to premature mortality from COPD and years of life lost due to disability for those living with COPD). It has been found that the most important factors that determine economic and societal costs of COPD are disease severity, exacerbation frequency and the presence of co-morbid conditions[169]. This thesis shows that low levels of lung function at baseline can therefore potentially identify high-risk subjects where early identification using screening could allow early intervention strategies to put be in place and subsequently potentially alter the disease course and prognosis. The use of spirometry in general health screening can therefore potentially be of major individual and societal benefit. MBW measures such as LCI can be used in addition to spirometry to find those at highest risk of developing severe COPD outcomes later in life. Therefore, the use of LCI beyond CF could provide new insights into screening for future COPD hospitalisations.

The findings from this thesis go beyond establishing the use of spirometry as a screening tool for potential severe cases of future COPD; the use of spirometry in identifying those who are risk of DM is of major importance in not only reducing the risk of DM for the individual but its complications and future associated co-morbidities.

FEV_1 is thought to be second in importance to smoking as a predictor for all-cause mortality, ranked above blood pressure, social class and cholesterol[71, 73]. Therefore its use in reducing premature mortality and cardiovascular risk should not be over-looked. Lung function screening should have a firm place as part of the general health assessment in the population. Population-wide screening of not only current smokers can potentially provide vital information to guide disease prevention strategies.

Financial support

Studies included in this thesis were supported by the Swedish Heart-Lung Foundation (2012-0269, 2013-0249, 2016-0315).

Acknowledgments

I would firstly like to thank all the study participants in the "Men Born in 1914" cohort and the Malmö Preventive Project cohort for giving their time to be part of these studies and for their contribution to scientific research. Thank you to everyone involved in the planning of these studies and in the data collection and management stages. A special thank-you to **Sven-Olof Isacsson** and **Sven-Eric Lindell** for all their efforts in the initiation and establishment of the "Men Born in 1914" cohort and for creating such a rich data source for use in scientific research.

I would like to thank everyone who has been part of my journey as a PhD student. I am so grateful to have had such a supportive network of people around me, both professionally and personally.

In particular, I would like to thank:

My main supervisor, **Gunnar Engström**. I will always be grateful to you for giving me this amazing opportunity. Your constant support throughout the last few years has been invaluable. Thank you for accommodating my role as a working mum and supporting me in balancing my work life with having a young family. Thank you for always being available for advice, feedback, or to discuss whatever is on my mind. Above all- thank you for believing in me.

My co-supervisor, **Per Wollmer**. Thank you for coming on board and always being available for feedback on all the numerous versions of manuscripts I have sent you over the past few years! Thank you for all your advice on planning this thesis and all your wonderful words of encouragement at every stage.

Peter Nilsson, thank you for your invaluable feedback as a co-author

Yan Borné, thank you for being a friendly face from day one! Thank you for all the work and life advice you have given me over the last few years. Thank you for re-assuring me that there is always a way!

Iram Faqir Muhammad, thank you for being such a caring colleague and friend. It has been lovely getting to know you over the past year or so, and I will always be grateful to you for helping me stay calm in times of panic or stress.

Linda Johnson, thank you for your input as a co-author and for always being an email away, even when you have been away taking care of your little one. I have reached out to you on many occasions for advice and you have always been so helpful and kind in your responses.

Bo Hedbland, thank you for letting me take a permanent seat in your office.

Martin Söderholm, thank you for your advice in helping me prepare for this defence, and for all the discussions about statistics over the past few years!

John Berntsson thank you for all the discussions about various statistical methods and for always sharing useful knowledge.

Margaretha Persson, Xue Bao and all other colleagues in the department, past and present, thank you for making this department such a wonderful place to work!

Viktor Hamrefors and **Ulf Nihlén**, thank you for your constructive feedback at my half-time review.

Sophia Frantz, thank you for all the lovely discussions about work and life.

Joana Alves Dias, thank you for all your advice throughout the last few years (and your help with printing in the early days!)

Sophie Hellstrand thank you for all your advice and kind words of encouragement.

Thank you to the staff at Human Resources and the Faculty office, especially **Claes Moreau** and **Anette Saltin** for your quick responses to my endless queries.

I would also like to thank the staff at CRC who have also been a part of my daily life at work:

Thank you to **Fredrik, Julio, Helena, Lotta** and all the rest of the **Mötesplats CRC staff** for being so kind and friendly every morning! Thank you for also helping me practice speaking Swedish!

Julia, thank you for always stopping by to say hello and ask how I am doing. It has been a pleasure getting to know you over the past few years.

Thank you to my wonderful network of support:

Mum and **Dad,** thank you for your prayers and for always being proud of me. Thank you for making me believe I can achieve anything I put my mind to. I hope I continue to make you proud.

My siblings, **Sameera, Noreen** and **Yousuf** and their respective better halves - **Imran, Ejaz** and **Fareen.** Thank you for being refreshingly unaware of what I have actually been doing at work over the last few years. Thank you for thinking whatever it is I am doing makes me clever.

My parents in-law, thank you for your prayers, your generosity and your continuous support.

My brother in-law **Hassan** and sister in-law **Mehreen**, thank you for always having words of encouragement and for making me believe that I can do this!

Thank to **Majda** and **Uzma** for being such kind, thoughtful and supportive friends.

Last but not at all the least, thank you to the three people who are the meaning to my life:

My beautiful children **Zainab** and **Mustafa**. You are my inspiration. Whatever I do, I do for you. Thank you for being my biggest fans. Thank you for making me smile from my heart. Thank you for making me the best version of myself and for your unconditional love. Thank you for understanding when Mama had to do her "lungs work" instead of playing Lego. I hope one day when you read this book it will make you proud.

And finally, my wonderful husband, **Bilal**. None of this would have been possible without your love, support and encouragement. You have always made me believe I can do anything and you will be there right beside me through it all. You are my rock. You always find the good in everything and everyone, which inspires me everyday to do the same. Thank you for always making it possible for me to pursue my dreams. Thank you for being my partner in life and thank you for being my best friend.

8. References

1. Petty TL. John Hutchinson's mysterious machine. Transactions of the American Clinical and Climatological Association. 1987;98:11-20.
2. Petty TL. John Hutchinson's mysterious machine revisited. Chest. 2002;121(5 Suppl):219s-23s.
3. Milic-Emili J, Marazzini L, #x, Angelo E. 150 Years of Blowing: Since John Hutchinson. Canadian Respiratory Journal. 1997;4(5).
4. Yernault JC. The birth and development of the forced expiratory manoeuvre: a tribute to Robert Tiffeneau (1910-1961). The European respiratory journal. 1997;10(12):2704-10.
5. Fowler WS, Cornish ER, Jr., Kety SS. Lung function studies. VIII. Analysis of alveolar ventilation by pulmonary N2 clearance curves. The Journal of clinical investigation. 1952;31(1):40-50.
6. Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, et al. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. The European respiratory journal. 2013;41(3):507-22.
7. Becklake MR. A new index of the intrapulmonary mixture of inspired air. Thorax. 1952;7(1):111-6.
8. Mikamo M, Shirai T, Mori K, Shishido Y, Akita T, Morita S, et al. Predictors of phase III slope of nitrogen single-breath washout in COPD. Respiratory physiology & neurobiology. 2013;189(1):42-6.
9. Robinson PD, Goldman MD, Gustafsson PM. Inert gas washout: Theoretical background and clinical utility in respiratory disease. Respiration; international review of thoracic diseases. 2009;78(3):339-55.
10. Lutfi MF. The physiological basis and clinical significance of lung volume measurements. Multidisciplinary Respiratory Medicine. 2017;12(1):3.
11. Ruppel GL. What is the clinical value of lung volumes? Respiratory care. 2012;57(1):26-35; discussion -8.
12. Evans SE, Scanlon PD. Current practice in pulmonary function testing. Mayo Clinic proceedings. 2003;78(6):758-63; quiz 63.
13. Crapo RO. Pulmonary-function testing. The New England journal of medicine. 1994;331(1):25-30.

14. Moore VC. Spirometry: step by step. Breathe. 2012;8(3):232-40.
15. McNulty W, Usmani OS. Techniques of assessing small airways dysfunction. European clinical respiratory journal. 2014;1:10.3402/ecrj.v1.25898.
16. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. The European respiratory journal. 2005;26(5):948-68.
17. Global strategy for the diagnosis, management and prevention of Chronic Obstructive Pulmonary Disease, 2018 Report. Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2018 [Available from: http://goldcopd.org/wp-content/uploads/2017/11/GOLD-2018-v6.0-FINAL-revised-20-Nov_WMS.pdf.
18. Torén K, Olin A-C, Lindberg A, Vikgren J, Schiöler L, Brandberg J, et al. Vital capacity and COPD: the Swedish CArdioPulmonary bioImage Study (SCAPIS). International journal of chronic obstructive pulmonary disease. 2016;11:927-33.
19. Staitieh BS, Ioachimescu OC. Interpretation of pulmonary function tests: beyond the basics. Journal of Investigative Medicine. 2017;65(2):301-10.
20. Subbarao P, Milla C, Aurora P, Davies JC, Davis SD, Hall GL, et al. Multiple-Breath Washout as a Lung Function Test in Cystic Fibrosis. A Cystic Fibrosis Foundation Workshop Report. Annals of the American Thoracic Society. 2015;12(6):932-9.
21. Horsley A. Lung clearance index in the assessment of airways disease. Respiratory medicine. 2009;103(6):793-9.
22. Robinson P, Latzin P, Gustafsson PM. Multiple-breath washout: European Respiratory Monograph.; 2010.
23. Verbanck S, Schuermans D, Van Muylem A, Paiva M, Noppen M, Vincken W. Ventilation distribution during histamine provocation. Journal of applied physiology (Bethesda, Md : 1985). 1997;83(6):1907-16.
24. Aurora P, Bush A, Gustafsson P, Oliver C, Wallis C, Price J, et al. Multiple-breath washout as a marker of lung disease in preschool children with cystic fibrosis. American journal of respiratory and critical care medicine. 2005;171(3):249-56.
25. Stanojevic S, Davis SD, Retsch-Bogart G, Webster H, Davis M, Johnson RC, et al. Progression of Lung Disease in Preschool Patients with Cystic Fibrosis. American journal of respiratory and critical care medicine. 2017;195(9):1216-25.
26. Brody AS, Klein JS, Molina PL, Quan J, Bean JA, Wilmott RW. High-resolution computed tomography in young patients with cystic fibrosis: distribution of abnormalities and correlation with pulmonary function tests. The Journal of pediatrics. 2004;145(1):32-8.
27. Horsley AR, Gustafsson PM, Macleod KA, Saunders C, Greening AP, Porteous DJ, et al. Lung clearance index is a sensitive, repeatable and practical measure of airways disease in adults with cystic fibrosis. Thorax. 2008;63(2):135-40.

28. Criée CP, Sorichter S, Smith HJ, Kardos P, Merget R, Heise D, et al. Body plethysmography – Its principles and clinical use. Respiratory medicine. 2011;105(7):959-71.
29. Mohamed Hoesein FA, de Hoop B, Zanen P, Gietema H, Kruitwagen CL, van Ginneken B, et al. CT-quantified emphysema in male heavy smokers: association with lung function decline. Thorax. 2011;66(9):782-7.
30. Oostveen E, MacLeod D, Lorino H, Farré R, Hantos Z, Desager K, et al. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. European Respiratory Journal. 2003;22(6):1026-41.
31. Berger KI, Goldring RM, Oppenheimer BW. POINT: Should Oscillometry Be Used to Screen for Airway Disease? Yes. Chest. 2015;148(5):1131-5.
32. Enright PL. COUNTERPOINT: Should Oscillometry Be Used to Screen for Airway Disease? No. Chest. 2015;148(5):1135-6.
33. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. European Respiratory Journal. 2005;26(5):948-68.
34. Kerstjens HA, Rijcken B, Schouten JP, Postma DS. Decline of FEV1 by age and smoking status: facts, figures, and fallacies. Thorax. 1997;52(9):820-7.
35. Swanney MP, Ruppel G, Enright PL, Pedersen OF, Crapo RO, Miller MR, et al. Using the lower limit of normal for the FEV1/FVC ratio reduces the misclassification of airway obstruction. Thorax. 2008;63(12):1046-51.
36. Güder G, Brenner S, Angermann CE, Ertl G, Held M, Sachs AP, et al. "GOLD or lower limit of normal definition? a comparison with expert-based diagnosis of chronic obstructive pulmonary disease in a prospective cohort-study". Respiratory research. 2012;13(1):13.
37. Bhatt SP, Sieren JC, Dransfield MT, Washko GR, Newell JD, Jr., Stinson DS, et al. Comparison of spirometric thresholds in diagnosing smoking-related airflow obstruction. Thorax. 2014;69(5):409-14.
38. Petty TL. The history of COPD. International journal of chronic obstructive pulmonary disease. 2006;1(1):3-14.
39. Quaderi SA, Hurst JR. The unmet global burden of COPD. Global Health, Epidemiology and Genomics. 2018;3:e4.
40. Bourdin A, Burgel PR, Chanez P, Garcia G, Perez T, Roche N. Recent advances in COPD: pathophysiology, respiratory physiology and clinical aspects, including comorbidities. European respiratory review : an official journal of the European Respiratory Society. 2009;18(114):198-212.
41. Burrows B, Fletcher CM, Heard BE, Jones NL, Wootliff JS. The emphysematous and bronchial types of chronic airways obstruction. A clinicopathological study of patients in London and Chicago. Lancet (London, England). 1966;1(7442):830-5.

42. Castaldi PJ, Benet M, Petersen H, Rafaels N, Finigan J, Paoletti M, et al. Do COPD subtypes really exist? COPD heterogeneity and clustering in 10 independent cohorts. Thorax. 2017;72(11):998-1006.
43. Mannino DM, Gagnon RC, Petty TL, Lydick E. Obstructive lung disease and low lung function in adults in the United States: data from the National Health and Nutrition Examination Survey, 1988-1994. Archives of internal medicine. 2000;160(11):1683-9.
44. Petty TL. COPD in perspective. Chest. 2002;121(5 Suppl):116s-20s.
45. Viniol C, Vogelmeier CF. Exacerbations of COPD. European Respiratory Review. 2018;27(147).
46. Soler-Cataluña JJ, Martínez-García MÁ, Román Sánchez P, Salcedo E, Navarro M, Ochando R. Severe acute exacerbations and mortality in patients with chronic obstructive pulmonary disease. Thorax. 2005;60(11):925-31.
47. Sharma G, Goodwin J. Effect of aging on respiratory system physiology and immunology. Clinical interventions in aging. 2006;1(3):253-60.
48. Janssens JP, Pache JC, Nicod LP. Physiological changes in respiratory function associated with ageing. The European respiratory journal. 1999;13(1):197-205.
49. Vaz Fragoso CA, Gill TM. Respiratory Impairment and the Aging Lung: A Novel Paradigm for Assessing Pulmonary Function. The Journals of Gerontology: Series A. 2012;67A(3):264-75.
50. Lowery EM, Brubaker AL, Kuhlmann E, Kovacs EJ. The aging lung. Clinical interventions in aging. 2013;8:1489-96.
51. Verbanck S, Thompson BR, Schuermans D, Kalsi H, Biddiscombe M, Stuart-Andrews C, et al. Ventilation heterogeneity in the acinar and conductive zones of the normal ageing lung. Thorax. 2012;67(9):789-95.
52. Fletcher C, Peto R. The natural history of chronic airflow obstruction. British Medical Journal. 1977;1(6077):1645-8.
53. Kotz D, Wesseling G, Huibers MJ, van Schayck OC. Efficacy of confrontational counselling for smoking cessation in smokers with previously undiagnosed mild to moderate airflow limitation: study protocol of a randomized controlled trial. BMC public health. 2007;7(1):332.
54. Xu X, Dockery DW, Ware JH, Speizer FE, Ferris BG, Jr. Effects of cigarette smoking on rate of loss of pulmonary function in adults: a longitudinal assessment. The American review of respiratory disease. 1992;146(5 Pt 1):1345-8.
55. van Schayck CP, Kaper J. Smoking and COPD: will they ever vanish into smoke? Primary Care Respiratory Journal. 2006;15:81.
56. Peto R. The horse-racing effect. Lancet (London, England). 1981;2(8244):467-8.
57. Burrows B, Knudson RJ, Camilli AE, Lyle SK, Lebowitz MD. The "horse-racing effect" and predicting decline in forced expiratory volume in one second from screening spirometry. The American review of respiratory disease. 1987;135(4):788-93.

58. Berry CE, Drummond MB. The Horse-Racing Effect and Lung Function: Can We Slow the Fastest Horse? American journal of respiratory and critical care medicine. 2017;195(9):1134-5.
59. Lange P, Celli B, Agusti A, Boje Jensen G, Divo M, Faner R, et al. Lung-Function Trajectories Leading to Chronic Obstructive Pulmonary Disease. The New England journal of medicine. 2015;373(2):111-22.
60. Vestbo J, Lange P. Fletcher and Peto 40 Years On. A Tribute and Reflection. American journal of respiratory and critical care medicine. 2017;195(11):1420-2.
61. Burrows B, Knudson RJ, Lebowitz MD. The relationship of childhood respiratory illness to adult obstructive airway disease. The American review of respiratory disease. 1977;115(5):751-60.
62. Barker DJ, Godfrey KM, Fall C, Osmond C, Winter PD, Shaheen SO. Relation of birth weight and childhood respiratory infection to adult lung function and death from chronic obstructive airways disease. BMJ (Clinical research ed). 1991;303(6804):671-5.
63. Friedman GD, Klatsky AL, Siegelaub AB. Lung function and risk of myocardial infarction and sudden cardiac death. The New England journal of medicine. 1976;294(20):1071-5.
64. Schroeder EB, Welch VL, Couper D, Nieto FJ, Liao D, Rosamond WD, et al. Lung function and incident coronary heart disease: the Atherosclerosis Risk in Communities Study. American journal of epidemiology. 2003;158(12):1171-81.
65. Lange P, Nyboe J, Jensen G, Schnohr P, Appleyard M. Ventilatory function impairment and risk of cardiovascular death and of fatal or non-fatal myocardial infarction. The European respiratory journal. 1991;4(9):1080-7.
66. Engstrom G, Hedblad B, Janzon L. Reduced lung function predicts increased fatality in future cardiac events. A population-based study. Journal of internal medicine. 2006;260(6):560-7.
67. Sin DD, Wu L, Man SF. The relationship between reduced lung function and cardiovascular mortality: a population-based study and a systematic review of the literature. Chest. 2005;127(6):1952-9.
68. Mattila T, Vasankari T, Rissanen H, Knekt P, Puukka P, Heliovaara M. Airway obstruction and the risk of myocardial infarction and death from coronary heart disease: a national health examination survey with a 33-year follow-up period. European journal of epidemiology. 2018;33(1):89-98.
69. Lee HM, Liu MA, Barrett-Connor E, Wong ND. Association of lung function with coronary heart disease and cardiovascular disease outcomes in elderly: the Rancho Bernardo study. Respiratory medicine. 2014;108(12):1779-85.
70. Kannel WB, Hubert H, Lew EA. Vital capacity as a predictor of cardiovascular disease: the Framingham study. American heart journal. 1983;105(2):311-5.

71. Young RP, Hopkins R, Eaton TE. Forced expiratory volume in one second: not just a lung function test but a marker of premature death from all causes. The European respiratory journal. 2007;30(4):616-22.
72. Engstrom G, Hedblad B, Janzon L, Valind S. Respiratory decline in smokers and ex-smokers--an independent risk factor for cardiovascular disease and death. Journal of cardiovascular risk. 2000;7(4):267-72.
73. Hole DJ, Watt GC, Davey-Smith G, Hart CL, Gillis CR, Hawthorne VM. Impaired lung function and mortality risk in men and women: findings from the Renfrew and Paisley prospective population study. BMJ (Clinical research ed). 1996;313(7059):711-5; discussion 5-6.
74. Johnson LS, Juhlin T, Engstrom G, Nilsson PM. Reduced forced expiratory volume is associated with increased incidence of atrial fibrillation: the Malmo Preventive Project. Europace : European pacing, arrhythmias, and cardiac electrophysiology : journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology. 2014;16(2):182-8.
75. Silvestre OM, Nadruz W, Querejeta Roca G, Claggett B, Solomon SD, Mirabelli MC, et al. Declining Lung Function and Cardiovascular Risk: The ARIC Study. Journal of the American College of Cardiology. 2018;72(10):1109-22.
76. Engstrom G. The restrictive-obstructive continuum and the failing heart. Thorax. 2016;71(6):487-8.
77. Walter RE, Beiser A, Givelber RJ, O'Connor GT, Gottlieb DJ. Association between glycemic state and lung function: the Framingham Heart Study. American journal of respiratory and critical care medicine. 2003;167(6):911-6.
78. Yeh HC, Punjabi NM, Wang NY, Pankow JS, Duncan BB, Cox CE, et al. Cross-sectional and prospective study of lung function in adults with type 2 diabetes: the Atherosclerosis Risk in Communities (ARIC) study. Diabetes care. 2008;31(4):741-6.
79. Lange P, Groth S, Kastrup J, Mortensen J, Appleyard M, Nyboe J, et al. Diabetes mellitus, plasma glucose and lung function in a cross-sectional population study. The European respiratory journal. 1989;2(1):14-9.
80. Litonjua AA, Lazarus R, Sparrow D, Demolles D, Weiss ST. Lung function in type 2 diabetes: the Normative Aging Study. Respiratory medicine. 2005;99(12):1583-90.
81. Klein OL, Krishnan JA, Glick S, Smith LJ. Systematic review of the association between lung function and Type 2 diabetes mellitus. Diabetic medicine : a journal of the British Diabetic Association. 2010;27(9):977-87.
82. Davis TM, Knuiman M, Kendall P, Vu H, Davis WA. Reduced pulmonary function and its associations in type 2 diabetes: the Fremantle Diabetes Study. Diabetes research and clinical practice. 2000;50(2):153-9.
83. Eriksson KF, Lindgarde F. Poor physical fitness, and impaired early insulin response but late hyperinsulinaemia, as predictors of NIDDM in middle-aged Swedish men. Diabetologia. 1996;39(5):573-9.

84. Glaser S, Kruger S, Merkel M, Bramlage P, Herth FJ. Chronic obstructive pulmonary disease and diabetes mellitus: a systematic review of the literature. Respiration; international review of thoracic diseases. 2015;89(3):253-64.
85. Yeh F, Dixon AE, Marion S, Schaefer C, Zhang Y, Best LG, et al. Obesity in adults is associated with reduced lung function in metabolic syndrome and diabetes: the Strong Heart Study. Diabetes care. 2011;34(10):2306-13.
86. Polak M, Dorynska A, Szafraniec K, Pajak A. Cardiovascular risk assessment, cardiovascular disease risk factors, and lung function parameters. Kardiologia polska. 2018;76(7):1055-63.
87. Leone N, Courbon D, Thomas F, Bean K, Jego B, Leynaert B, et al. Lung function impairment and metabolic syndrome: the critical role of abdominal obesity. American journal of respiratory and critical care medicine. 2009;179(6):509-16.
88. Engstrom G, Hedblad B, Valind S, Janzon L. Increased incidence of myocardial infarction and stroke in hypertensive men with reduced lung function. Journal of hypertension. 2001;19(2):295-301.
89. Margretardottir OB, Thorleifsson SJ, Gudmundsson G, Olafsson I, Benediktsdottir B, Janson C, et al. Hypertension, Systemic Inflammation and Body Weight in Relation to Lung Function Impairment—An Epidemiological Study. Copd. 2009;6(4):250-5.
90. Schnabel E, Nowak D, Brasche S, Wichmann HE, Heinrich J. Association between lung function, hypertension and blood pressure medication. Respiratory medicine. 2011;105(5):727-33.
91. Cirillo DJ, Agrawal Y, Cassano PA. Lipids and pulmonary function in the Third National Health and Nutrition Examination Survey. American journal of epidemiology. 2002;155(9):842-8.
92. Isacsson SO. Venous occlusion plethysmography in 55-year old men. A population study in Malmo, Sweden. Acta medica Scandinavica Supplementum. 1972;537:1-62.
93. Hedblad B, Nilsson P, Janzon L, Berglund G. Relation between insulin resistance and carotid intima-media thickness and stenosis in non-diabetic subjects. Results from a cross-sectional study in Malmo, Sweden. Diabetic medicine : a journal of the British Diabetic Association. 2000;17(4):299-307.
94. 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. The European respiratory journal Supplement. 1993;16:5-40.
95. Arborelius M, Rosberg HE, Wiberg R. Multiple breath nitrogen dead space. Clinical physiology (Oxford, England). 1988;8(6):561-76.
96. Bouhuys A, Hagstam KE, Lundin G. Efficiency of pulmonary ventilation during rest and light exercise; a study of alveolar nitrogen wash-out curves in normal subjects. Acta physiologica Scandinavica. 1956;35(3-4):289-303.

97. Soderholm M, Zia E, Hedblad B, Engstrom G. Lung function as a risk factor for subarachnoid hemorrhage: a prospective cohort study. Stroke; a journal of cerebral circulation. 2012;43(10):2598-603.
98. Engstrom G, Melander O, Hedblad B. Population-based study of lung function and incidence of heart failure hospitalisations. Thorax. 2010;65(7):633-8.
99. Ekberg-Aronsson M, Pehrsson K, Nilsson JA, Nilsson PM, Lofdahl CG. Mortality in GOLD stages of COPD and its dependence on symptoms of chronic bronchitis. Respiratory research. 2005;6:98.
100. Engstrom G, Wollmer P, Valind S, Hedblad B, Janzon L. Blood pressure increase between 55 and 68 years of age is inversely related to lung function: longitudinal results from the cohort study 'Men born in 1914'. Journal of hypertension. 2001;19(7):1203-8.
101. Kristenson H, Trell E. Indicators of alcohol consumption: comparisons between a questionnaire (Mm-MAST), interviews and serum gamma-glutamyl transferase (GGT) in a health survey of middle-aged males. British journal of addiction. 1982;77(3):297-304.
102. Song JW, Chung KC. Observational Studies: Cohort and Case-Control Studies. Plastic and reconstructive surgery. 2010;126(6):2234-42.
103. Gordis L. Epidemiology. 4th edition ed: Saunders 2008.
104. Katz MH. Multivariable Analysis: A Practical Guide for Clinicians and PublicHealth Researchers2011.
105. Bewick V, Cheek L, Ball J. Statistics review 12: Survival analysis. Critical Care. 2004;8(5):389-94.
106. Kleinbaum DG, Klein M. Extension of the Cox Proportional Hazards Model for Time-Dependent Variables. In: Kleinbaum DG, Klein M, editors. Survival Analysis: A Self-Learning Text, Third Edition. New York, NY: Springer New York; 2012. p. 241-88.
107. Noordzij M, Leffondré K, van Stralen KJ, Zoccali C, Dekker FW, Jager KJ. When do we need competing risks methods for survival analysis in nephrology? Nephrology Dialysis Transplantation. 2013;28(11):2670-7.
108. Lau B, Cole SR, Gange SJ. Competing Risk Regression Models for Epidemiologic Data. American journal of epidemiology. 2009;170(2):244-56.
109. Logan BR, Zhang MJ, Klein JP. Regression models for hazard rates versus cumulative incidence probabilities in hematopoietic cell transplantation data. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2006;12(1 Suppl 1):107-12.
110. Austin PC, Lee DS, Fine JP. Introduction to the Analysis of Survival Data in the Presence of Competing Risks. Circulation. 2016;133(6):601-9.
111. Warnock DG. Competing risks: you only die once. Nephrology Dialysis Transplantation. 2016;31(7):1033-5.

112. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. Journal of the American Statistical Association. 1999;94(446):496-509.
113. Lunn M, McNeil D. Applying Cox regression to competing risks. Biometrics. 1995;51(2):524-32.
114. Uno H, Cai T, Pencina MJ, D'Agostino RB, Wei LJ. On the C-statistics for Evaluating Overall Adequacy of Risk Prediction Procedures with Censored Survival Data. Statistics in medicine. 2011;30(10):1105-17.
115. Austin PC, Steyerberg EW. Interpreting the concordance statistic of a logistic regression model: relation to the variance and odds ratio of a continuous explanatory variable. BMC Medical Research Methodology. 2012;12(1):82.
116. Rahman MS, Ambler G, Choodari-Oskooei B, Omar RZ. Review and evaluation of performance measures for survival prediction models in external validation settings. BMC Medical Research Methodology. 2017;17(1):60.
117. Schunemann HJ, Dorn J, Grant BJ, Winkelstein W, Jr., Trevisan M. Pulmonary function is a long-term predictor of mortality in the general population: 29-year follow-up of the Buffalo Health Study. Chest. 2000;118(3):656-64.
118. Beaty TH, Cohen BH, Newill CA, Menkes HA, Diamond EL, Chen CJ. Impaired pulmonary function as a risk factor for mortality. American journal of epidemiology. 1982;116(1):102-13.
119. Baughman P, Marott JL, Lange P, Martin CJ, Shankar A, Petsonk EL, et al. Combined effect of lung function level and decline increases morbidity and mortality risks. European journal of epidemiology. 2012;27(12):933-43.
120. Lange P, Nyboe J, Appleyard M, Jensen G, Schnohr P. Spirometric findings and mortality in never-smokers. Journal of Clinical Epidemiology. 1990;43(9):867-73.
121. Mannino DM, Buist AS, Petty TL, Enright PL, Redd SC. Lung function and mortality in the United States: data from the First National Health and Nutrition Examination Survey follow up study. Thorax. 2003;58(5):388-93.
122. Sorino C, Sherrill D, Guerra S, Enright P, Pedone C, Augugliaro G, et al. Prognostic value of FEV1/FEV6 in elderly people*. Clinical Physiology and Functional Imaging. 2011;31(2):101-7.
123. Hutchinson J. On the capacity of the lungs, and on the respiratory functions, with a view of establishing a precise and easy method of detecting disease by the spirometer. Medico-chirurgical transactions. 1846;29:137-252.
124. Tockman MS, Pearson JD, Fleg JL, Metter EJ, Kao SY, Rampal KG, et al. Rapid decline in FEV1. A new risk factor for coronary heart disease mortality. American journal of respiratory and critical care medicine. 1995;151(2 Pt 1):390-8.
125. Enright PL, Kronmal RA, Smith VE, Gardin JM, Schenker MB, Manolio TA. Reduced vital capacity in elderly persons with hypertension, coronary heart disease, or left ventricular hypertrophy. The Cardiovascular Health Study. Chest. 1995;107(1):28-35.

126. Johnston AK, Mannino DM, Hagan GW, Davis KJ, Kiri VA. Relationship between lung function impairment and incidence or recurrence of cardiovascular events in a middle-aged cohort. Thorax. 2008;63(7):599-605.
127. Bäck M. Atherosclerosis, COPD and chronic inflammation2008. 60-5 p.
128. Eickhoff P, Valipour A, Kiss D, Schreder M, Cekici L, Geyer K, et al. Determinants of systemic vascular function in patients with stable chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 2008;178(12):1211-8.
129. Chandra D, Gupta A, Strollo PJ, Jr., Fuhrman CR, Leader JK, Bon J, et al. Airflow Limitation and Endothelial Dysfunction. Unrelated and Independent Predictors of Atherosclerosis. American journal of respiratory and critical care medicine. 2016;194(1):38-47.
130. Zhong Y, Rosengren A, Fu M, Welin L, Welin C, Caidahl K, et al. Secular changes in cardiovascular risk factors in Swedish 50-year-old men over a 50-year period: The study of men born in 1913, 1923, 1933, 1943, 1953 and 1963. European journal of preventive cardiology. 2017;24(6):612-20.
131. Wilhelmsen L, Welin L, Svardsudd K, Wedel H, Eriksson H, Hansson PO, et al. Secular changes in cardiovascular risk factors and attack rate of myocardial infarction among men aged 50 in Gothenburg, Sweden. Accurate prediction using risk models. Journal of internal medicine. 2008;263(6):636-43.
132. Luoto JA, Elmstahl S, Wollmer P, Pihlsgard M. Incidence of airflow limitation in subjects 65-100 years of age. The European respiratory journal. 2016;47(2):461-72.
133. O'Byrne PM, Rennard S, Gerstein H, Radner F, Peterson S, Lindberg B, et al. Risk of new onset diabetes mellitus in patients with asthma or COPD taking inhaled corticosteroids. Respiratory medicine. 2012;106(11):1487-93.
134. Aiman U, Najmi A, Khan RA. Statin induced diabetes and its clinical implications. Journal of Pharmacology & Pharmacotherapeutics. 2014;5(3):181-5.
135. Flannelly KJ, Flannelly LT, Jankowski KRB. Threats to the Internal Validity of Experimental and Quasi-Experimental Research in Healthcare. Journal of health care chaplaincy. 2018;24(3):107-30.
136. Schlesselman J. Case-control Studies: Design, Conduct and Analysis.: Oxford University Press 1982.
137. Ronmark E, Lundqvist A, Lundback B, Nystrom L. Non-responders to a postal questionnaire on respiratory symptoms and diseases. European journal of epidemiology. 1999;15(3):293-9.
138. Janzon L, Hanson BS, Isacsson SO, Lindell SE, Steen B. Factors influencing participation in health surveys. Results from prospective population study 'Men born in 1914' in Malmo, Sweden. Journal of epidemiology and community health. 1986;40(2):174-7.

139. Berglund G, Nilsson P, Eriksson KF, Nilsson JA, Hedblad B, Kristenson H, et al. Long-term outcome of the Malmo preventive project: mortality and cardiovascular morbidity. Journal of internal medicine. 2000;247(1):19-29.

140. ATS statement--Snowbird workshop on standardization of spirometry. The American review of respiratory disease. 1979;119(5):831-8.

141. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. The European respiratory journal. 2005;26(2):319-38.

142. Engström G, Segelstorm N, Ekberg-Aronsson M, Nilsson PM, Lindgärde F, Löfdahl C-G. Plasma markers of inflammation and incidence of hospitalisations for COPD: results from a population-based cohort study. Thorax. 2009;64(3):211-5.

143. Engström G, Melander O, Hedblad B. Population-based study of lung function and incidence of heart failure hospitalisations. Thorax. 2010;65(7):633-8.

144. Mannino DM, Diaz-Guzman E, Buist S. Pre- and post-bronchodilator lung function as predictors of mortality in the Lung Health Study. Respiratory research. 2011;12:136.

145. Mannino DM, Doherty DE, Sonia Buist A. Global Initiative on Obstructive Lung Disease (GOLD) classification of lung disease and mortality: findings from the Atherosclerosis Risk in Communities (ARIC) study. Respiratory medicine. 2006;100(1):115-22.

146. Mannino DM, Buist AS, Petty TL, Enright PL, Redd SC. Lung function and mortality in the United States: data from the First National Health and Nutrition Examination Survey follow up study. Thorax. 2003;58(5):388-93.

147. Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015 [Available from: http://www.goldcopd.org/.

148. Clarke R, Shipley M, Lewington S, Youngman L, Collins R, Marmot M, et al. Underestimation of risk associations due to regression dilution in long-term follow-up of prospective studies. American journal of epidemiology. 1999;150(4):341-53.

149. Inghammar M, Engstrom G, Lofdahl CG, Egesten A. Validation of a COPD diagnosis from the Swedish Inpatient Registry. Scandinavian journal of public health. 2012;40(8):773-6.

150. Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim JL, Reuterwall C, et al. External review and validation of the Swedish national inpatient register. BMC public health. 2011;11:450.

151. Lykkegaard J, dePont Christensen R, Davidsen JR, Støvring H, Andersen M, Søndergaard J. Trends in the lifetime risk of COPD exacerbation requiring hospitalisation. European Respiratory Journal. 2013;42(4):964-71.

152. Engstrom G, Berglund G, Goransson M, Hansen O, Hedblad B, Merlo J, et al. Distribution and determinants of ischaemic heart disease in an urban population. A study from the myocardial infarction register in Malmo, Sweden. Journal of internal medicine. 2000;247(5):588-96.

153. Hansen O, Johansson BW, Gullberg B. Circadian distribution of onset of acute myocardial infarction in subgroups from analysis of 10,791 patients treated in a single center. The American Journal of Cardiology. 1992;69(12):1003-8.
154. Brooke HL, Talbäck M, Hörnblad J, Johansson LA, Ludvigsson JF, Druid H, et al. The Swedish cause of death register. European journal of epidemiology. 2017;32(9):765-73.
155. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabetic medicine : a journal of the British Diabetic Association. 1998;15(7):539-53.
156. Andersson T, Ahlbom A, Carlsson S. Diabetes Prevalence in Sweden at Present and Projections for Year 2050. PloS one. 2015;10(11):e0143084.
157. Last J. A dictionary of Epidemiology. 4th edition ed: Oxford University Press 2001.
158. BR Kirkwood JS. Essential Medical Statistics 2nd Edition ed: Blackwell 2003.
159. Concato J, Peduzzi P, Holford TR, Feinstein AR. Importance of events per independent variable in proportional hazards analysis. I. Background, goals, and general strategy. J Clin Epidemiol. 1995;48(12):1495-501.
160. Peduzzi P, Concato J, Feinstein AR, Holford TR. Importance of events per independent variable in proportional hazards regression analysis. II. Accuracy and precision of regression estimates. J Clin Epidemiol. 1995;48(12):1503-10.
161. Vittinghoff E, McCulloch CE. Relaxing the rule of ten events per variable in logistic and Cox regression. American journal of epidemiology. 2007;165(6):710-8.
162. Textor J, van der Zander B, Gilthorpe MS, Liśkiewicz M, Ellison GTH. Robust causal inference using directed acyclic graphs: the R package 'dagitty'. International Journal of Epidemiology. 2016;45(6):1887-94.
163. Marmot MG, Shipley MJ, Rose G. Inequalities in death--specific explanations of a general pattern? Lancet (London, England). 1984;1(8384):1003-6.
164. Hegewald MJ, Crapo RO. Socioeconomic status and lung function. Chest. 2007;132(5):1608-14.
165. Becklake MR. Occupational Exposures: Evidence for a Causal Association with Chronic Obstructive Pulmonary Disease. American Review of Respiratory Disease. 1989;140(3_pt_2):S85-S91.
166. Wang Y, Rimm EB, Stampfer MJ, Willett WC, Hu FB. Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. The American journal of clinical nutrition. 2005;81(3):555-63.
167. Borne Y, Nilsson PM, Melander O, Hedblad B, Engstrom G. Multiple anthropometric measures in relation to incidence of diabetes: a Swedish population-based cohort study. European journal of public health. 2015.

168. Kestenbaum B. Methods to Control for Confounding. Epidemiology and Biostatistics: An Introduction to Clinical Research. New York, NY: Springer New York; 2009. p. 101-11.
169. López-Campos JL, Tan W, Soriano JB. Global burden of COPD. Respirology (Carlton, Vic). 2016;21(1):14-23.

Printed by Media-Tryck, Lund 2018 NORDIC SWAN ECOLABEL 3041 0903

Lung function and the prediction of health outcomes in an urban population

Photo by:
Zeeshan Janjua

Suneela Zaigham is a medical graduate from the UK. She completed her medical degree at St George's, University of London (UK) and has completed 2 years of specialist training (ST) in Public Health in the UK. Suneela has a Master's degree in Public Health (MPhil Public Health) from the University of Cambridge (UK) and is also a Diplomate member of the Faculty of Public Health in the UK (DFPH).

The focus of this doctoral thesis was to investigate the role of lung function in the prediction of different health outcomes in the population of Malmö. This thesis shows that low levels of lung function in middle age can increase the risk of adverse health outcomes later in life. This knowledge can be useful to guide the identification of such high-risk individuals earlier on in life when the disease course and prognosis can potentially be altered.

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Clinical Sciences Malmö

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2019:01
ISBN 978-91-7619-718-9
ISSN 1652-8220