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Doctoral thesis

Sigrid Anna Aalberg Vikjord

Chronic obstructive pulmonary disease (COPD): Influence of comorbidities on health status, morbidity and mortality in a 25 years follow-up population-based study. The HUNT Study.

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Trykket av NTNU Grafisk senter

Hva påvirker utviklingen av kols?

Kronisk obstruktiv lungesykdom (kols) er en vanlig forekommende tilstand både i Norge og globalt. Her hjemme er det estimert at omkring 6% av individer over 40 år har kols, mens estimatene på verdensbasis er høyere; opp mot 13%. Kols er per 2020 den tredje ledende ikke-smittsomme dødsårsaken i verden, kun slått av iskemisk hjertesykdom og slag. Videre er kols en viktig årsak til sykdomsbyrde, tap av livskvalitet og økte helsekostnader for individ og samfunn. Komorbiditeter, forekomsten av ulike sykdommer eller tilstander hos samme person, opptrer hyppig ved kols, og kan øke sykdomsbyrden og dødeligheten ytterligere. Dette er til dels forårsaket av felles risikofaktorer som aldring, røyking, kosthold og fysisk inaktivitet, men kan også representere en felles, underliggende sykdomsmekanisme som foreløpig er ukjent. Vi har undersøkt hvordan enkelttilstander både sammen og hver for seg påvirker risikoen for alvorlige kols-forverringer og død.

Vi brukte data fra den andre (HUNT2) og tredje (HUNT3) undersøkelsen til Helseundersøkelsen i Trøndelag (HUNT), og koblet disse opp mot data fra lokale sykehus og Folkeregisteret. Individer som fikk påvist kols via en pusteprøve (spirometri), og som var mellom 40 og 85 år, ble inkludert i studiet. Vi gjorde også bentetthetsmålinger for å påvise benskjørhet, og undersøkte deltagerne for symptomer på angst og depresjon, høyt blodtrykk, kronisk nyresvikt, høye fettstoffer i blodet, diabetes, hjerteinfarkt og over-/undervekt.

Vi identifiserte 2076 deltagere med kols i våre data. Vi fant en positiv sammenheng mellom benskjørhet og dødelighet. Det samme ble funnet hos dem med symptomer på angst og depresjon. Vi identifiserte også fem ulike sykdomsklynger (sykdommer som opptrer hyppig sammen), og fant at to slike klynger; et psykologisk og et kakektisk (vevstap i ulike organer), var assosiert med økt dødelighet og økt risiko for sykehusinnleggelser på grunn av alvorlig kols-forverring. Resultatene viser at enkelttilstander kan fortelle oss noe om dødeligheten av kols, men at det er mer nyttig å se på alle sykdommer som opptrer samtidig hos individer med kols, og vurdere dem som en helhet. Ved å forebygge og behandle slike sykdommer, kan man bedre overlevelsen hos disse pasientene.

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for graden PhD (Doctor Philosophiae) i medisin.
Disputas finner sted ved HUNT forskningssenter
fredag 11. sept. 2020, kl. 12:15.

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List of Papers

The thesis includes three papers listed below, indicated by roman numerals.

Paper I: Vikjord SAA, Brumpton BM, Mai XM, Bhatta L, Vanfleteren L, Langhammer A. The Association of Bone Mineral Density with Mortality in a COPD Cohort. The HUNT Study, Norway. COPD. 2019:1-9.

Paper II: Vikjord SAA, Brumpton BM, Mai XM, Vanfleteren L, Langhammer A. The association of anxiety and depression with mortality in a COPD cohort. The HUNT Study, Norway. Respiratory Medicine. 2020.

Paper III: Vikjord SAA, Vanfleteren L, Brumpton BM, Mai XM, Langhammer A. The association of comorbidity clusters with long-term mortality and incidence of exacerbations in a COPD cohort. The HUNT Study, Norway.

Acknowledgements

This thesis was undertaken at HUNT Research Centre, Department of Public Health and Nursing, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, NTNU. The funding was provided by the National Association for Heart and Lung Diseases, LHL, through the DAM Foundation, and the Liaison Committee of the Central Norway Regional Health Authority (Helse Midt-Norge HF). The HUNT project is made possible by the effort of the population of Nord-Trøndelag, which has contributed to and supported the study for almost four decades.

This thesis would not have been possible without all the help from my wonderful team of supervisors, as well as invaluable colleagues, family and friends. The finishing stage of my thesis work was done amidst one of the largest public health challenges in modern history, with the COVID-19 epidemic locking down the entire world. Even though this introduced some extra hurdles in the finishing phase of the PhD, with home office and closed kindergartens, it has also emphasized the importance of epidemiological research as not merely an academic exercise, but one of real importance for people and society.

First, I want to thank my main supervisor, professor Arnulf Langhammer, for teaching me everything I know about COPD, epidemiology, spirometry, HUNT and the Lung Study, and a little bit about life as well. Thank you for letting me into the world of research, which has become such an important part of me. You have channeled all my frustrations, supported me through ups and downs, been supportive when it was needed and strict when it was necessary. We have shared many experiences over these three years, and I appreciate both our professional relationship and our friendship. I feel lucky to have had you as my supervisor, and I am looking forward to our future endeavors.

A warm thank you to my supervisor, professor Xiao-Mei Mai, for all your help, thoughtful comments, deep insight into epidemiology and statistics, and for cheering me on through this project. Your knowledge and advise have been invaluable and I am forever grateful for everything you have taught me. An equally warm thank you to my supervisor, associate professor Ben Brumpton, for all your help on statistics, for your input and comments on drafts and analysis plans (even with all my last-minute deadlines), and for your pleasant and approachable spirit. Your insights and knowledge will never cease to impress me. A huge thank you to my supervisor, researcher Lowie Vanfleteren, who I was so lucky to include in my supervisor team one year into my PhD. Your expertise on the field of pulmonology, new analysis methods and your never-ending source of new research ideas have been of great importance to me.

I deeply appreciate our friendship, and I am looking forward to all our future projects together.

I want to thank my PhD colleagues Kristin Hestmann Vinjerui, Sofie Lauvås Hjorthen, Anna Hjort-Hansen, Lars Saxhaug, Jens Horn, Theresa Valle Aguilar, Aivaras Cepelis and others for fruitful academic discussions, venting of joy and frustrations and a lot of fun. I am particularly grateful to my fellow PhD student, now postdoctoral fellow and researcher Laxmi Bhatta, for teaching me all things Stata, and always being willing to help me with a syntax problem (there have been many throughout the years…). Also, thank you for your pleasant company to meetings and conferences. I am looking forward to working with you on our upcoming projects. Also, a great thank you to postdoc and researcher Julie Horn, for all your input, encouragement, good conversations and collaborations so far.

A big thank you to all my colleagues at the HUNT Databank and rest of the HUNT Research Centre for all your help, support and friendly working environment during my PhD. I have felt included from the very first day, and that has meant a lot to me during these three years.

I also want to thank my colleagues at the pulmonary department at Levanger hospital for having patience with me during this project, and for making room for me in the “lung family”. I also want to thank the head of the medical department, Carl Platou, for your flexibility and accommodation.

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Last, but not least, I want to thank my great family and friends for all your support, time and patience during this time. Thank you, Alexander, my husband, partner in crime and best friend, for helping me cope, manage, and achieve something that resembles a work-life balance. Thanks to Abel, our amazing son, and your little brother or sister, for being so patient with your mom. Thank you to my amazing parents, who have always seen me for who I am, and not for my achievements. Thank you to my dearest siblings, Ellen and Vemund, and my dear Besse, parents- and brothers-in-law, for proof-reading, support, much-needed distraction, hugs, a lot of baby-sitting, and for reminding me that there are things that matters more than numbers, figures and the latest deadline.

Levanger, May 2020

Sigrid Anna Aalberg Vikjord

Acronyms and Abbreviations

ATS:	American Thoracic Society
BD:	Bronchodilator
BMD:	Bone mineral density
BMI:	Body mass index
BOLD:	Burden of Obstructive Lung Disease
DAG:	Directed acyclic graph
DXA:	Dual X-ray absorptiometry
COPD:	Chronic obstructive pulmonary disease
CI:	Confidence interval
ECG:	Electrocardiography
FEV_1:	Forced expiratory volume in first second
FVC:	Forced vital capacity
GLI:	Global Lung Initiative
GBD:	Global Burden of Diseases Study
GOLD:	Global Initiative for Chronic Obstructive Lung Disease
GWAS:	Genome-wide association studies
DALY:	Disability-adjusted life year
HADS:	Hospital Anxiety and Depression Scale
HR:	Hazard ratio
HUNT:	The Trøndelag Health Study (previously the Nord-Trøndelag Health Study)
HUNT1:	Nord-Trøndelag Health Study, survey 1 (1984-86)
HUNT2:	Nord-Trøndelag Health Study, survey 2 (1995-97)
HUNT3:	Nord-Trøndelag Health Study, survey 3 (2006-08)
HUNT4:	Nord-Trøndelag Health Study, survey 4 (2017-19)
NICE:	National Institute for Health and Care Excellence

ICD-9:	International Statistical Classification of Diseases and Related Health Problems, 9th rev.
ICD-10:	International Statistical Classification of Diseases and Related Health Problems, 10th rev.
ISCD:	International Society of Clinical Densitometry
LABA:	Long-acting β-agonist
LAMA:	Long-acting muscarinic antagonists
LLN:	Lower limit of normal
mMRC:	Modified Medical Research Council Dyspnea Scale
NHANES:	The National Health and Nutrition Examination Survey
NCD:	Non-communicable disease
NIPH:	Norwegian Institute of Public Health (Folkehelseinstituttet)
PA:	Posteroanterior
PH:	Proportional hazard
ROI:	Region of interest
RNS:	Reactive nitrogen species
ROS:	Reactive oxygen species
SABA:	Short-acting inhaled β2-agonists
WHO:	World Health Organization

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Summary

Background

COPD is a complex, heterogeneous disease characterized by airflow obstruction. It is a highly common disease worldwide, with a global prevalence of 13.1%. COPD is estimated to be the third leading cause of death by 2020 and is an important driver behind reduced quality of life, increased disease burden and health care costs. Comorbidities are prevalent in COPD and leads to increased morbidity and mortality.

Aims

The aim of this project was to investigate the association between osteoporosis and all-cause mortality in individuals with COPD (paper I), investigate the association between symptoms of anxiety and depression and all-cause mortality in individuals with COPD (paper II) and to investigate clusters of comorbidities in individuals with COPD, and their association with all-cause mortality and severe COPD exacerbations (paper III).

Methods

The data were collected from the second and third cycle of the HUNT Study (Helseundersøkelsen i Trøndelag), a population-based survey situated in the northern part of Trøndelag, Mid-Norway. Flow-volume spirometry was collected in the Lung Study of HUNT2 (1995-97) and HUNT3 (2006-08), and individuals were identified as having COPD upon having a FEV_1/FVC Z-score < –1.64 according to Global Lung Initiative (GLI) criteria, as well as having respiratory symptoms. In paper I, Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria were also used, defining COPD as a FEV_1/FVC <0.70. Individuals between 40-85 years were included. Bone mineral density (BMD) was measured using dual-energy X-ray absorptiometry (DXA) of distal forearm (HUNT2+3) or total hip (HUNT3), and osteoporosis was defined as BMD T-score <–2.5. Symptoms of anxiety and depression were assessed by the Hospital Anxiety and Depression Scale (HADS), and the cut-off for caseness of anxiety or depression was defined as ≥8. For the cluster analysis, we included seven additional conditions, including hypertension (systolic pressure >140 mm Hg or diastolic pressure >90 mm Hg); chronic renal failure (eGFR<60 ml/min/1.73 m^2 + presence of moderately increased albuminuria or eGFR<45 ml/min/1.73 m^2); hyperlipidemia

(triglyceride level >1.7 mmol/L or HDL <1.03 mmol/L [males] or <1.29 mmol/L [females]); obesity (body mass index [BMI] ≥30.0 kg/m^2); underweight (BMI <21.0 kg/m^2); any diabetes mellitus (based on glutamic acid decarboxylase antibodies (anti-GAD) and protein tyrosine phosphatase-like protein IA-2 (anti-IA-2) status, fasting C-peptide levels and self-reported insulin treatment) and myocardial infarction (ECG changes, dynamic changes in blood levels of cardiac enzymes and coronary symptoms). Outcome measures included all-cause mortality (collected from the Norwegian National Registry), and additionally for paper III hospital admissions due to COPD exacerbations (ICD-9- and -10 codes collected from two local and one regional hospital covering the area of former Nord-Trøndelag). We used multivariable Cox proportional hazards regression in all papers, obtaining hazard ratios with 95% confidence intervals. In paper II, we modeled the association of change in HADS score from HUNT2 to HUNT3 with all-cause mortality using joint models. In paper III, we also did cluster analysis using machine learning software (Viscovery SOMine) to identify comorbidity clusters. All models were adjusted for relevant confounders, identified using directed acyclic graphs.

Results

2076 individuals were identified as having COPD according to GLI criteria. For GOLD criteria, the corresponding number was 3239. We found that both osteoporosis and symptoms of anxiety and depression showed a weak positive association with all-cause mortality among the COPD patients. We found a positive association between low BMD T-score, osteoporosis and all-cause mortality. Further, a positive association between symptoms of anxiety and depression and all-cause mortality was seen. The mortality risk decreased if the symptoms diminished during the 11-year period between two study cycles. Using objectively identified comorbidities, we identified five distinct comorbidity clusters within our COPD cohort. Clusters dominated by symptoms of anxiety and depression (psychological cluster), as well as osteoporosis and underweight (cachectic cluster), were associated with increased all-cause mortality and higher risk of severe COPD exacerbations.

Conclusions

Both osteoporosis, symptoms of anxiety and depression were associated with all-cause mortality. Of five distinct comorbidity clusters identified in the HUNT COPD cohort, the cachectic and psychological clusters were associated with mortality and increased risk of exacerbations.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a complex, heterogeneous disease which is characterized by persistent airflow obstruction that is not fully reversible. Tobacco smoking is the main causative agent, but other environmental exposures as well as host factors also play a role in its development. COPD is highly prevalent, with an estimated worldwide prevalence of 13.1% in individuals above 40 years of age (1). Projections from the World Health Organization (WHO) show that COPD will be the third leading cause of death by 2030, although this estimate seems to be reached already this year (2). In Norway around 6-8% of the population above 40 years have COPD (3).

Individuals with COPD are at higher risk of developing coexisting diseases, which will negatively influence morbidity and mortality (4). Having concurrent COPD and other chronic diseases are found to negatively impact individual's health status, management of COPD and the development of COPD exacerbations, which is an important predictor of COPD-related death (5). In Norway COPD contribute to 0.7 % of the total health care expenditure (numbers from 2009) (6), and comorbidities was found to be the main predictor of excessive costs in COPD (7). The same trend has been found in international studies (8). The management of COPD is limited to symptomatic relief and prevention of exacerbations, as there is currently no curative treatment. This makes identification and management of comorbidities even more important, as failure to treat concurrent diseases may impair the treatment and outcomes of COPD (9).

There is an array of papers published on COPD and comorbidities, either as single disease associations or as co- and multimorbidity complexes. However, such studies are often performed on selected patient groups, not reflecting a COPD population that is true to life. The HUNT Lung Study has collected a vast amount of data on healthy and diseased individuals for almost 25 years, creating an ideal platform to study the impact of comorbidities on long-term COPD outcomes. Furthermore, a range of comorbidities have been objectively identified using diagnostic gold standards, improving the validity of the findings.

This thesis focuses on important and prevalent comorbidities in COPD and their association with all-cause mortality and severe COPD exacerbations, in a cohort with up to 25 years of follow-up time. Emerging statistical methods that were not widely available at the time when the protocol was written, have enabled us to expand the project to include comorbidity clusters, further exploring the complex associations of COPD and its comorbidities.

2. Background

2.1. Pathophysiology and pathology of COPD

COPD is characterized by irreversible airflow obstruction and abnormal inflammatory response in the lungs. This inflammatory response is often the result of long term exposure to noxious substances like cigarette smoking, and individuals with COPD have an enhanced immune response to such stimuli (10). Furthermore, oxidative stress and disruption in the balance between protease-antiprotease are involved. This response may lead to the typical pathological changes seen in COPD, including structural damage to the small airways leading to airway remodeling, destruction of the lung parenchyma (emphysema) and mucous hypersecretion. These changes lead to increased airflow resistance in the small airways (mainly small conducting airways <2 mm in diameter), increased lung compliance, air trapping and airflow obstruction. Micro-computed tomography (CT) studies have shown that the patency of terminal and transitional bronchioles is severely reduced in COPD, by as much as 40% in mild-to-moderate disease and 80% in severe disease (11). The pulmonary circulation is also affected in COPD, potentially leading to the development of pulmonary hypertension and cor pulmonale (12). Progressive airflow limitation and destruction of the capillary bed leads to ventilation/perfusion (V/Q) mismatch, the most important cause of hypoxemia in individuals with COPD (13). Increased ventilation of poorly perfused parts of the lungs of emphysematous individuals increases physiological dead space and thereby increase the V/Q ratio. In individuals with severe airway affection, a low V/Q ratio is more common, with perfusion of under-ventilated areas and physiological shunting as a result (13).

The loss of lung elastic recoil is due to the destruction of alveolar walls and alveolar attachments. Due to the airway obstruction, progressive air trapping results in hyperinflation. The main problem for individuals with COPD is the dynamic hyperinflation, i.e. the progressive air trapping due to a new breath being initiated before complete exhalation has occurred, leading to increase in end-expiratory lung volume and reduction of inspiratory capacity. This in turn increases the sensation of dyspnea and reduced exercise capacity. Static hyperinflation is less problematic and is caused by increased lung compliance as seen in emphysema (10).

A common feature in COPD is the COPD exacerbations, an acute worsening of respiratory symptoms requiring increase in treatment. Exacerbations are common in individuals with COPD, with an estimated incidence of 0.6–3.0 per year, depending on population and study type (14), and are associated with increased mortality, poorer quality of life and worsening of COPD (15, 16). Exacerbations are the greatest contributor to increased health care costs in COPD (15), due to more frequent visits to family

doctors, outpatient clinics and hospital admissions, as well as progression of the disease itself. Other comorbidities, like cardiovascular disease, may also progress after a COPD exacerbation (17, 18). Patients with severe and very severe disease have higher risk of exacerbations (15). Furthermore, the most important predictor of future exacerbations are prior exacerbations (19). COPD exacerbation may be caused by viral or bacterial respiratory infections, exposure to airway irritants or from unknown causes. The patient experiences increased dyspnea due to increased airway obstruction and increased mucus secretion leading to hyperinflation and air trapping. Further, increasing V/Q mismatch could lead to hypoxemia. Underlying infection contributes to increased purulence and volume of sputum and often increased cough. Exacerbations may span from mild worsenings that could be treated at home, to severe exacerbations requiring hospitalizations and the need for ventilatory support due to respiratory failure (16).

2.2. Definition of COPD

COPD is defined by airflow limitation that is not fully reversible, as measured by flow-volume spirometry. The diagnosis is defined by forced expiratory volume in one second divided by forced vital capacity (FEV_1/FVC) and the severity grade by FEV_1 percent predicted (ppFEV_1) or Z-scores. The cut-offs for determining airflow limitation have varied throughout the years, and an international consensus was not reached until the publication of the first GOLD report in 2001 (20). For a long time, COPD has been defined solely by the so-called fixed ratio criteria, defining COPD as post-bronchodilator FEV_1/FVC lower than 0.70 (15), which is still recommended by both GOLD and the National Institute for Health and Care Excellence (NICE). Previously, other cut-offs for the COPD diagnosis have been used, including the American Thoracic Society (ATS) definition of FEV_1/FVC <0.75 (21) and the British Thoracic Society (BTS) using FEV_1/FVC <0.70 in addition to FEV_1 % of predicted of <80 (22). These definitions have been surrounded by some controversy, as they do not consider physiological lung aging and consecutive fall in spirometric indices (23, 24). Furthermore, for ppFEV1 they do to fully take the variance of age and height into account (24). Due to this, the cut-off for COPD diagnosis has been suggested to be based on lower limit of normal (LLN) equal to the lower 5^{th} percentile from a population of healthy never-smokers; FEV_1/FVC less than LLN or below a z-score of −1.64. In 2012, the Global Lung Initiative (GLI) led by P. Quanjer developed reference values for all ages (3-95 years) and a number of ethnicities (25). Furthermore, severity of airway obstruction was now defined by Z-scores, using cut-offs of −2, < −2.5, < −3 and < −4 (26). Biological fluctuations in dynamic lung volumes could influence spirometry results

around the threshold between physiological and pathological lung function (27), and repeated measurements spaced in time are according to GOLD guidelines required to confirm the diagnosis (15).

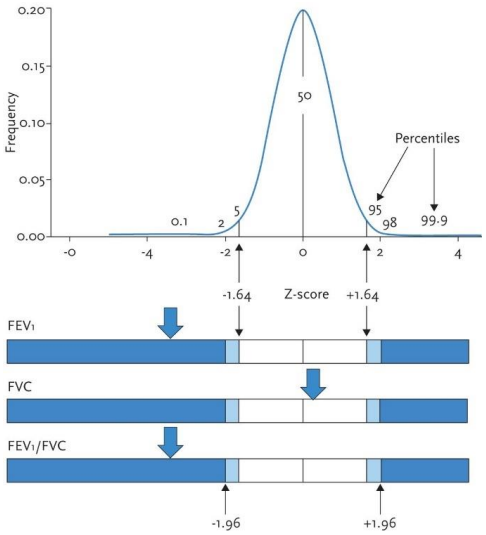

Figure 2.1 *The normal distribution with corresponding Z-scores and percentiles. The "Quanjer bars" below illustrate the distribution of Z-scores (white=normal range, light blue=1-5 percentile, blue=1 percentile). Arrows indicate where the measured observation lies and allows for easy visual interpretation of spirometry results. The 50th centile equals 0 Z-scores, or 100% of predicted (28).*

In the normal distribution of clinical measurements, 95% confidence interval (CI) is used to define the normal range, and the upper and lower 2.5-percentile defines pathological values (*Figure 2.1*). In lung function high levels are not considered pathological, and the lower limit is therefore defined as 5%. This corresponds to the lower limit 90% CI, with mean – 1.645 standard deviations (SD). A stricter criterium would be using the 95% CI, and the lower limit is then defined by the 1-percentile, with mean – 1.96 SD. There is an ongoing discussion on whether the 1- or 5-percentile should be used in population-based studies to reduce the proportion of false positives. In the HUNT Lung Study, the 5-percentile is used, since this is a stricter criterium than the fixed ratio cut-off (*Figure 2.2*) (A. Langhammer, personal communication).

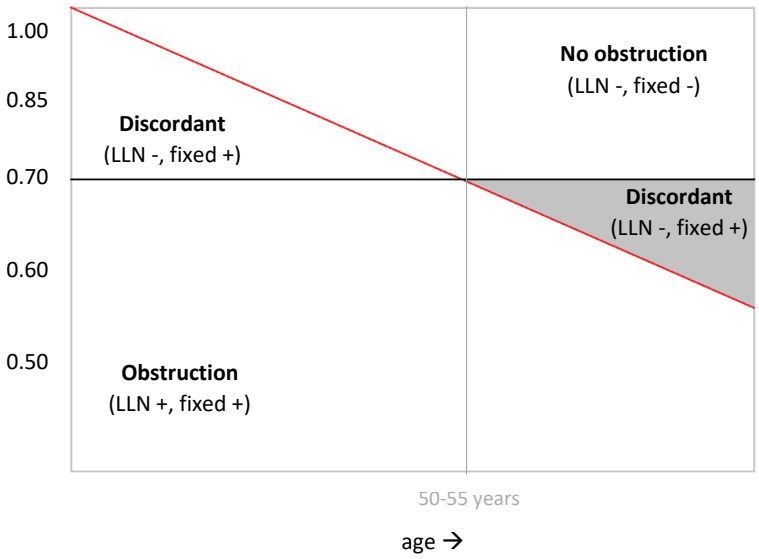

Figure 2.2 *Difference between using GOLD fixed ratio criteria (fixed) vs. LLN criteria (LLN). X-axis shows chronological age, and Y-axis shows the ratio of FEV_1/FVC. The red line shows the loss of lung function by age. The black line shows the fixed ratio cut-off at 0.70. The figure illustrates the problem of fixed ratio criteria: Upon increasing age, lung function will physiologically decline with age. Applying fixed ratio criteria leads to underdiagnosis in younger individuals, and overdiagnosis in older individuals (the discordant triangles). LLN criteria follow the age-related decline, reducing overdiagnosis among the elderly avoiding wrongful diagnosis. Figure adapted from Akkermans et al (29).*

Reference values

Reference values is of utmost importance upon the interpretation of spirometry results. Results from biological measurements cannot be interpreted in a vacuum, but relative to expected values in healthy, comparable individuals. Definition of "healthy" in the terms of lung function measurement have generally been limited to never-smoking individuals without lung- or heart diseases. Generally, such reference values are based on normal distribution of parameters in a representative population. Values outside the normal range, i.e. outside the central 95% of observations, are traditionally defined as pathological. Lung function measurements are complicated by significant biological variations by age, body height, sex and ethnicity (30), raising the need for parallel set of reference values based on these anthropometric and demographic characteristics.

A great number of reference values have been developed over time. The most common set of reference values for forced spirometry were derived from the European Community for Coal and Steel (ECCS) prediction equations (31). The reference population was aged 25 -70 years, and for age 20-25 patients were given the age 25. This oversimplifies the reality, including only the age span with linear relation between lung function parameters and age. Outside these, clinicians either had to shift to other age specific reference values or wrongly extrapolated predicted values beyond the reference groups' age and height. The ECCS-reference values have later been shown to underestimate predicted FEV_1 and FVC (32). Prediction equations are also sensitive to biological variability in the selected populations, measurement techniques and technician skills and cohort effects (33). Prediction equations for the Norwegian population was therefore developed from the HUNT2 Lung Study cohort (34), giving higher reference values compared to the ECCS. Currently, the GLI reference values are currently recommended and endorsed by most large respiratory bodies globally (25), as well in Norway by the Norwegian Lung Association, the Norwegian Society of Pediatricians and general practitioners (33). A validation study of the GLI 2012 reference equation has been done on an adult Norwegian population ≥20 years, and were shown to fit the data well (23).

Respiratory symptoms as diagnostic criteria

Respiratory symptoms including dyspnea, chronic cough and sputum production and recurrent lower respiratory tract infections have according to the latest GOLD guidelines been included as a criteria in addition to $FEV_1/FVC < 0,70$ in the definition of COPD (35). This probably will contribute to less overdiagnosis among elderly, healthy persons, and ensures that a diagnosis is given only if clinically relevant. Furthermore, a history of exposure to risk factors or a family history of COPD should be present (35).

Pre- and post-bronchodilator spirometry values

Another debated issue is the use of pre- vs. post-bronchodilator (pre-BD vs. post-BD) spirometry values for diagnosis. Post-BD spirometry is measured after administration of an inhaled bronchodilator (e.g., 400 µg salbutamol). GOLD guidelines recommend post-bronchodilator results (15). This is based on the hypothesis that some of the reversibility seen in individuals with COPD could be explained by variable smooth muscle tone (27). To eliminate this element of bronchial obstruction in pulmonary function testing, a smooth muscle relaxing inhalation is given prior to testing, avoiding such a lowered "ceiling" of lung function (20). However, post-BD testing in an epidemiological setting may be challenging and

resource-demanding, and many large population studies, including HUNT, have done post-BD measurements either in a smaller subsample, or not at all. A previous study on the Hordaland cohort found that the prevalence of COPD was 27% lower when using post-BD measurements compared to pre-BD (36). However, a more recent study found that pre- and post-BD predicted mortality with the same accuracy, and that post-BD measurements might not be needed in population studies predicting long-term outcomes (37). Except for the Hordaland study that also developed predicted values for post-BD spirometry, most studies have used prediction values based on pre-BD spirometry. From primary health care the need for post-BD spirometry is questioned; there are no other areas of medicine where diagnoses are determined by measures after inhalation or oral medication. As long as treatment is recommended only in patients experiencing symptoms, there should be minor over-treatment even if pre-BD spirometry was used for diagnosis (33).

2.3. Treatment of COPD

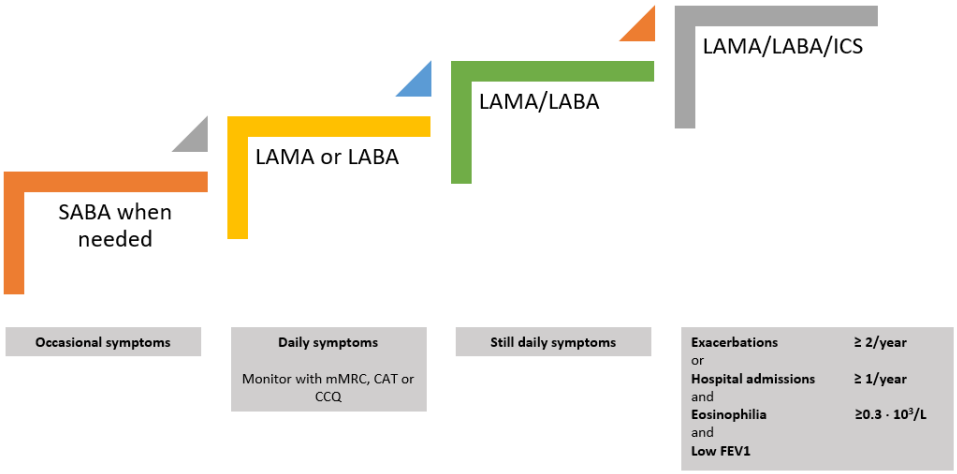

Figure 2.3 *Stairway approach to treatment of COPD. Note: COPD developed from an asthma should always be treated with ICS in combination with long-acting bronchodilators. Abbreviations: SABA, short-acting inhaled β2-agonists; LAMA, long-acting muscarinic antagonists; LABA; long-acting β2-agonist, ICS; inhaled corticosteroids, mMRC; modified Medical Research Council dyspnea scale, CAT; COPD Assessment Test, CCQ; Clinical COPD Questionnaire. Source: Langhammer et al, COPD guidelines from the Norwegian Institute of Public Health (NIPH).*

Figure 2.3 shows the stairway approach to COPD treatment according to Norwegian guidelines. Evidence has shown that long-acting muscarinic antagonists (LAMA) alone, or in combination with long-acting β_2-agonists (LABA), could reduce exacerbation rates (15, 38). For many years LABA-ICS fixed combinations have been used to decrease exacerbation frequency, but due to concerns of side effects, among these pneumonia, the role of ICS has been questioned (39). Currently, inhaled corticosteroids (ICS) is recommended as part of dual therapy ICS-LABA or triple therapy only in case of high symptom load (by mMRC), reduced health status (high COPD Assessment Test [CAT] score), recurrent exacerbations and increased blood eosinophils (15). Physical activity and pulmonary rehabilitation are recommended after acute exacerbations, despite moderate evidence (40). Other conditions, like worsening of chronic heart failure, thromboembolism and pneumonia may mimic COPD exacerbations (15), and should be ruled out. A growing field of current research work is the phenotyping of COPD exacerbations (41). Exacerbations are treated according to suspected etiology. Mild exacerbation could be treated by the individual at home by increasing bronchodilator intake, more specifically short-acting inhaled β_2-agonists (SABA) (38). More severe exacerbations could be treated ambulatory, with short courses (<5-7 days) of oral corticosteroids alone (42), or in combination with oral antibiotics when bacterial infection is suspected (43). Severe COPD exacerbations have high mortality (5) and could require hospitalization (44). At the hospitals, non-invasive ventilation should be given early in the course of acute respiratory failure (38), but invasive ventilation could be necessary .

2.4. COPD in a historical perspective

The clinical manifestations of obstructive lung disease have been known since the New Kingdom Period of Ancient Egypt and were described in one of the oldest medical papyri known. However, it was not before the mid 1960's that the word COPD was used to describe the clinical entity of symptoms. Before that, COPD often went under the flags of asthma, chronic bronchitis or emphysema. Not until 1997, when the Global Initiative for COPD was formed, did COPD get the attention it deserved. This is reflected in the PubMed/MEDLINE search history: As of March 17, 2020, there are 85.653 results on PubMed for the MeSH term "chronic obstructive pulmonary disease", whereas in 1945 the count was 11 (*Figure 2.4*).

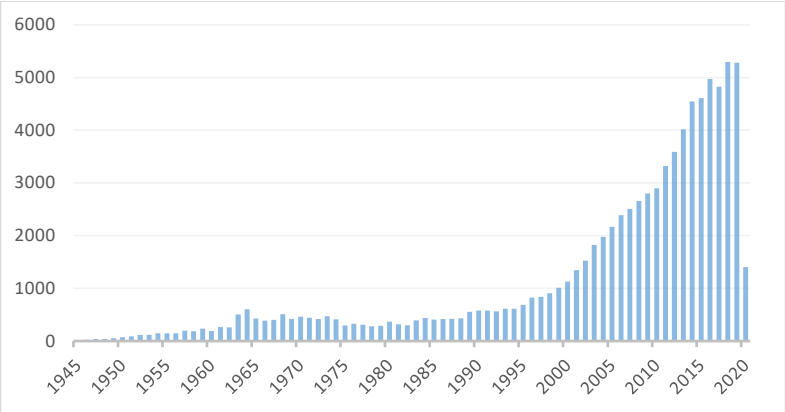

Figure 2.4 *Annual number of search results on COPD (MeSH) in PubMed from 1945-2020.*

In the table below some important milestones of obstructive lung disease are summarized (*Table 2.1*).

Table 2.1 *Important events in the history of obstructive lung disease.*

Year	**History of obstructive lung disease** (45, 46)
–1550	Asthma-like condition described in the Egyptian *Ebers* papyrus.
–1000	The oldest medical textbook in the world, the Chinese *Neu Ching*, describes asthma.
–480 - –370	*Hippocrates* teaches, according to humoral medicine beliefs, that asthma is caused by imbalance in the bodily fluids.
50-100	Greek physician *Pedanius Sioscorides* prescribed inhaled fumigation – the start of inhaled therapy.
1679	Swiss physician *Théophile Bonet* referred to “voluminous lungs”.
1691	Dutch anatomist *Frederik Ruysch* described a case of obstructed, hyperinflated lungs.
1769	Italian anatomist *Giovanni Morgagni* reported 19 cases of “turgid” (swollen and distended or congested) lungs.

1803	British physician *Matthew Baillie* describes and illustrates hyperinflated lungs in his book *The Morbid Anatomy of Some of the Most Important Parts of the Human Body*.
1814	British physician *Charles Badham* identified chronic bronchitis and used the term "catarrh" to describe excess mucus production in these individuals.
1821	*René Laënnec*, inventor of the stethoscope, recognized emphysema as part of COPD. He also identified environmental causes like air pollution as the main cause for the development of COPD (smoking was not common at that time). Furthermore, he divided catarrh into chronic and acute, the latter being synonymous to the common cold. He also described inflammation in COPD, as well as phenotypical characteristics of emphysematous patients.
1838	The first prevalence study of emphysema is conducted by French physician *Pierre Louis*, a pioneer of medical epidemiology, describing it as "one of the most frequent and remarkable affections to be found".
1846	*John Hutchinson* invented the spirometer, measured the vital capacity and developed the first reference values based on measurement of 1200 men. *Robert Tiffeneau* later developed this instrument to what it is today.
1868	Manchester's first Medical Officer of Health, *Dr. Leigh*, termed air pollution the main cause for chronic bronchitis and later emphysema.
1943	British physician *Ronald Christie* identified prolonged expiration as the most important physical sign of COPD.
1957	Australian physician *Bryan Gandevia* recommends the term FEV_1 to the British Thoracic Society.
1959	The *Ciba Guest Symposium* gathered and defined the criteria and definition of COPD.

1965	*Dr. William Briscoe* uses the term "COPD" for the first time, at the *9th Aspen Emphysema Conference*.
1976	*Dr. Charles Fletcher* linked smoking to COPD in his book *The Natural History of Chronic Bronchitis and Emphysema*. He found that smoking cessation helped slowing the progression of COPD, and vice versa.
1960s	First trial with oxygen therapy in COPD. Still, long-term oxygen therapy is the only intervention that is known to alter the course of COPD.
1997	United States (US) National Heart, Lung, and Blood Institute, US National Institutes of Health and World Health Organization forms the *Global Initiative for Chronic Obstructive Lung Disease (GOLD)* to increase awareness and policy making of the diagnosis and treatment of COPD.
2001	*First GOLD Report* was published, containing guidelines for diagnosis, classification and treatment of COPD. Spirometry alone was used to define COPD ("GOLD 1234").
2006	*Second GOLD Report* was published. GOLD grade 0 (symptoms without spirometric obstruction) was dropped based on data from the *Copenhagen City Heart Study*, as it did not predict the development to COPD. The COPD treatment ladder was introduced.
2011	A multidimensional approach to diagnosis and treatment, the GOLD ABCD classification, was released in the *third GOLD Report* of 2011 (published in 2013). This took respiratory symptoms and exacerbation history, as well as airflow limitation, into account.
2012	Multiethnic reference values were developed by the *Global Lung Initiative (GLI).*
2017	The *fourth GOLD Report* was published. Spirometric criteria were now removed from the ABCD classification, since a history of exacerbations were shown to predict future

	exacerbations better than the level of airflow obstruction.
2019	Modification of the use of ABCD, recommending the use to determine initial treatment only.

2.5. Epidemiology of COPD

2.5.1. Global prevalence and burden of disease

The prevalence of COPD has been increasing worldwide during the last decades. COPD causes premature death and is an important cause of disability-adjusted life years (DALYs) and health care expenditure globally (35). In 1990 it was the 6th leading cause of death from non-communicable disease (NCD) (47), as well as the 12th leading cause of DALYs worldwide (47), and in 2020 it is already the 3rd leading cause of death from NCDs, despite projections estimating this to not happen until 2030 (48). This is partly caused by the reduction in mortality from other NCDs, most importantly cardiovascular mortality. The global prevalence of COPD as of 2017 is estimated to be 13.1% (10.2–15.6%) (*Figure 2.5*) (1), varying across countries and groups of people within countries (35). However, a Burden of Obstructive Lung Disease (BOLD) study of 12 centers across the world showed strong heterogeneity in prevalence of COPD, as it ranged between 11.4% and 26.1% in individuals above 40 years (49). Generally, large differences in COPD prevalence may reflect the age range of included individuals, response rates, the prevalence of smoking and other risk factors, time periods and cohort effects. Prevalence of COPD also depends on diagnostic criteria (24), with the use of fixed ratio criteria overestimating airflow obstruction in the elderly (50), and underestimating it in young individuals (51). Pre- and post-BD measurements may also influence the prevalence, as described previously (36). Despite representing a substantial disease burden to the global society, COPD remains underdiagnosed and undertreated, making it a major global health threat (35).

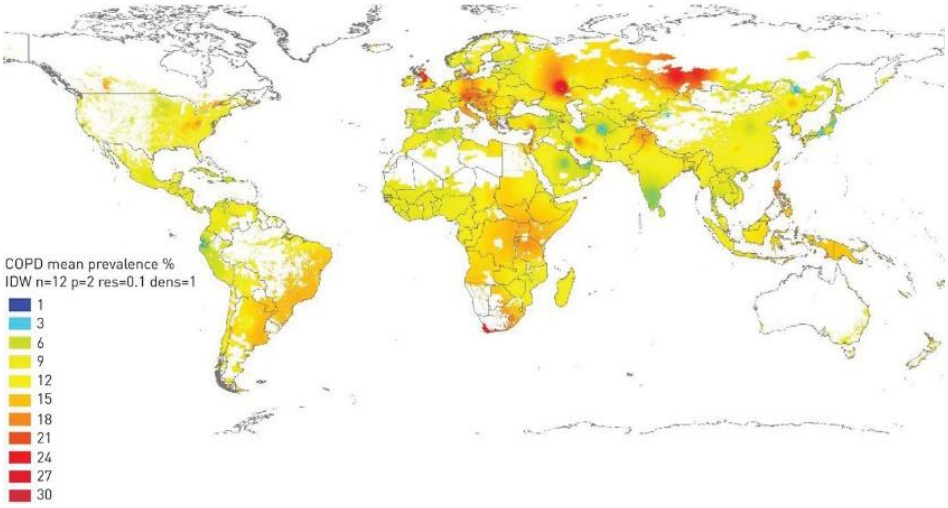

Figure 2.5 *Global COPD prevalence. Blanco et al, ERJ 2019. With permission.*

2.5.2. COPD prevalence in Norway

The COPD prevalence in Norway is similar to that of North America and other European countries (49, 52). Chronic diseases of the respiratory tract were the third leading cause of death in Norway in 2018, following malignant neoplasms and cardiovascular diseases (source: Norwegian Cause of Death Registry). From a previous study on the HUNT COPD cohort, the weighted prevalence of pre-bronchodilator COPD in adults ≥40 years using fixed-ratio criteria was 16.7% and 14.8% in HUNT2 and -3, respectively. The same percentages using LLN criteria were substantially lower; 10.4% and 7.3%. The prevalence was higher in men than in women regardless of criteria used. In the 11-year period between the two surveys the prevalence decreased in men but not in women (53). The smoking prevalence dropped in both sexes in the same time period (*Figure 2.6,2.7*), and on a national level the percentage of daily smokers decreased from 33% to 24% between 1995 and 2006 (53).

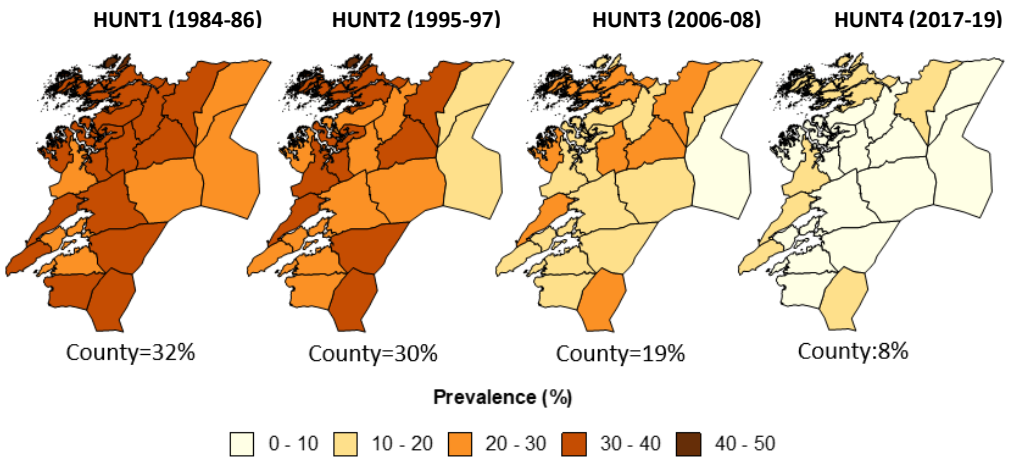

Figure 2.6 *Age-standardized smoking prevalence (%) in 23 municipalities for females, 1984-2019. Standard population: Norway, January 1, 2000. (Source: Erik R. Sund, HUNT Research Centre).*

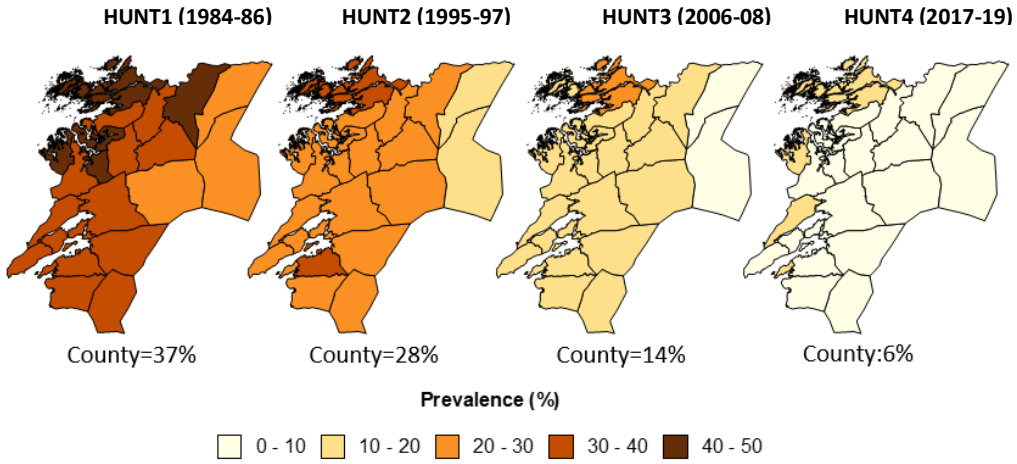

Figure 2.7 *Age-standardized smoking prevalence (%) in 23 municipalities for males, 1984-2019. Standard population: Norway, January 1, 2000. (Source: Erik R. Sund, HUNT Research Centre).*

In the Tromsø Study, a cohort study from the Northern part of Norway, a recent study showed that the age-standardized COPD prevalence using LLN criteria dropped from 7.6% to 5.6% in men and 7.3% to 5.6% in women between 2001 and 2017 (54). Furthermore, there were less individuals with moderate and severe COPD, and the rate of hospitalization due to exacerbations of COPD had decreased. In the same time period, the use of COPD maintenance treatment had increased. The study found a

corresponding substantial drop of daily smoking in the study period, and this might at least partly explain the results (54). An even larger reduction of age- and sex-adjusted prevalence of COPD defined by LLN was found in a Swedish study, with a drop from 8.1% to 3.1% between 1994 and 2009 (55). A corresponding change in risk factor pattern could partly explain these findings. However, in the Hordaland County Cohort Study located to the Western part of Norway, the prevalence of COPD In individuals aged 35-90 years increased between two surveys from 1985 and 2003-05, from 7% to 14% (56). These findings are surprising, even though the estimates are not directly comparable to the studies from HUNT, Tromsø and Sweden. The timing of the surveys was somewhat different, and COPD was defined by GOLD criteria in the Hordaland Study. Furthermore, post-BD measurements were used. The prevalence of pre-BD COPD was even higher; 21% (56). On the contrary, a Spanish study comparing individuals with COPD from two population-based studies in 1997 and 2007, found decreasing prevalence from 9.1% to 4.5% (57). The age range in the latter study was narrower; 40-69 years, and the definition of COPD deviated from the Hordaland study: post-BD $pFEV_1$ <88% in males and <89% in females (57), which could explain some of the differences. Furthermore, methodological differences and cohort effects could influence the estimates.

2.6. Risk factors for development and progression of COPD

The strongest risk factor for COPD is tobacco smoking. However, smoking cannot account for all cases of COPD alone (58). Other risk factors like aging, level of education, childhood respiratory infections, genetics, environmental and occupational exposure may influence its development.

2.6.1. Tobacco smoking

Smoking is the most important risk factor for COPD both in developed and developing countries, and as smoking prevalence is decreasing worldwide (59), it is expected that COPD incidence and prevalence will drop accordingly (60). A Global Burden of Disease Study from 2017 indeed found decreasing age-adjusted prevalence of COPD in 195 countries (2). However, 25% of those developing COPD throughout the lifetime have never smoked (61, 62), indicating that COPD is not a unifactorial disease, but rather the result of a complex interplay between environmental factors and host factors like genetic makeup (63), airway hyper-responsiveness (64) and suboptimal lung function development early in life (35, 65). In Norway, smoking has been shown to increase the risk of COPD more than four times (66). The last decades there have been no difference in smoking prevalence among men and women (*Figure 2.6, 2.7 and 2.8*). Smoking cessation slows the rate of decline of lung function (67, 68), making it the single most

important intervention in COPD prevention. This effect it seen in all COPD patients, regardless of severity (69).

The pathogenesis of tobacco-induced lung injury is thought to be mediated by reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated by oxidant-rich tobacco smoke and inflammatory cells. ROS and RNS induce inflammation in the lungs and airways through oxidative damage, resulting in chronic inflammatory changes in susceptible individuals. Structural remodeling following repeated injury leads to morphological changes in the pulmonary tissue (11, 70). Regrettably, studies have shown a persistent inflammation in the airways even one year after smoking cessation (59, 71), leading to speculation about an autoimmune component of COPD (11, 72, 73).

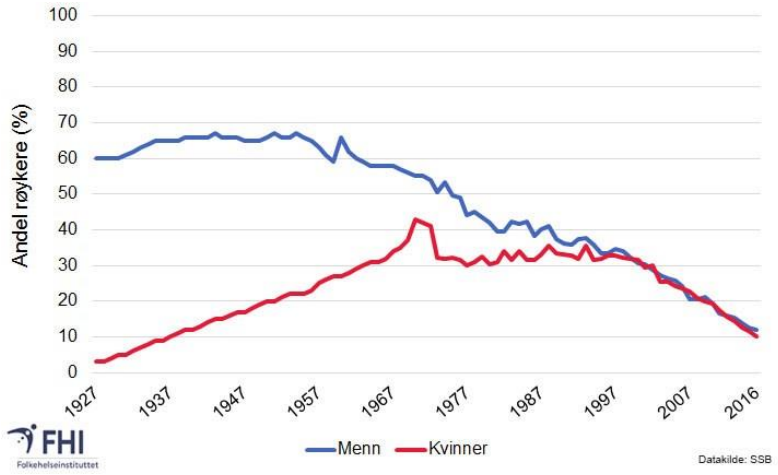

Figure 2.8 *Percentage of daily smokers among women and men aged 16-74 years in Norway in the period 1927-2016. Source: Statistics Norway / Norhealth. Reprinted with permission from NIPH.*

2.6.2. Occupational exposure and ambient pollution

Ambient pollution and occupational exposure to noxious gases, dusts and fumes remains important causative agents for COPD worldwide (52, 74, 75). The pathophysiological mechanisms behind occupational exposures are highly variable and depends of type of exposures. Inorganic or mineral dust exposure as seen in mining, construction and metallurgic industries are shown to increase the prevalence of COPD through long-standing exposure to fine particles (76). Organic or biological dust,

seen in farming, textile, wood and rubber industries also increase the risk of COPD (76). The same is found in occupational exposure to gases or fumes, commonly through oxidative damage (76).

Inhaled pollutants are known to affect lung function development (74, 77). The common sources are emissions caused by combustion from motor vehicles, road dust, atmospheric reactions, fuel burning and industry. The most common air pollutants in ambient air include particulate matter, ozone (O_3), nitrogen dioxide (NO_2), carbon monoxide (CO) and sulphur dioxide (SO_2). Together with NO_2, particulate matter with a diameter smaller than 2.5 µm ($PM_{2.5}$) is considered the most important pollutant, as the fine particles can penetrate deep into the lung. PM pollution may lead to exacerbations and increased mortality in COPD, but there is conflicting evidence as to whether inhaled pollutants lead to COPD (78). Recent papers from the Global Burden of Diseases (GBD) Study found that ambient air pollution was the second most important cause of morbidity and mortality in COPD in 2015 (79), and the leading cause in 2017 (80). However, these results cannot be extrapolated to say anything about the causative mechanisms behind COPD (81). Further, no significant associations have been found between exposure to smoke from biomass and development of airflow obstruction (81), despite wide research effort.

2.6.3. BMI and body composition.

BMI, both low and high, is an important predictor of outcomes in COPD. According to a study conducted by the Canadian National Health Survey, the prevalence of obesity was higher in individuals with COPD compared to controls: 24.6% and 17.1%, respectively (82, 83). Several other studies report the same findings (82). Obesity contribute to other chronic respiratory diseases like sleep apnea and asthma (84), and the association between obesity and loss of lung function has been extensively studied (82). Furthermore, the role of circulating adipokines, including leptin and adiponectin, and their influence on lung function development has gained increasing interest (85). In the general population there is a dose-response relationship between BMI and mortality (86), and a large proportion of the global burden of disease is attributable to overweight and obesity (86). However, in several meta-analyses overweight and obesity were associated with lower risk of all-cause mortality in COPD, the so-called “obesity paradox” (87, 88), which is also seen in other chronic diseases like diabetes mellitus type II and chronic kidney disease (88).

Low BMI (<21.0) is a known predictor of mortality in COPD, independent of disease severity (89, 90). However, a population-based study has found that only severe COPD is associated with being underweight (91). The reason is probably multifactorial, with appetite loss due to reduced physical

activity, dyspnea interfering with eating, enhanced energy expenditure due to increased work of breathing, and the effect of humoral factors like inflammatory cytokines, adipokines and different hormones (92).

Cachexia denotes a loss of muscle mass, with or without fat loss, in individuals with chronic disease (93). Calculation of BMI is insufficient to diagnose cachexia, as it might occur regardless of body weight. Low fat-free mass has been found to be an independent predictor of mortality in COPD (94, 95). Furthermore, it deteriorates quality of life and exercise capacity in these individuals, and increase the risk of acute exacerbations (92). The cause of cachexia in COPD patients is uncertain, but might be due to impaired protein synthesis and impaired exercise tolerance of skeletal muscle (96-98).

Composite measures as the BODE (BMI, airflow Obstruction, Dyspnea, Exercise capacity) index utilize the relationship between BMI and mortality to generate prediction models for mortality. This index includes FEV_1% of predicted, 6-minute walking distance (6MWD) and the modified Medical Research Council (mMRC) dyspnea scale in addition to BMI, to predict all-cause mortality in individuals with COPD (90). These are widely used in research but have uncertain value in clinical practice.

2.6.4. Physical activity

Few studies have examined the effect of physical activity on lung function development. In a prospective cohort study, no association was found between levels of physical activity in childhood and asthma development later in life (99). It has been suggested that physical activity could have a beneficial effect on lung function development due to its anti-inflammatory effects (100). However, a recent review on physically modifiable risk factors for asthma in children found highly inconsistent evidence for the association between physical activity and long-term lung function outcomes (101). On the contrary, in a study of individuals aged 28-80 years, high levels of physical activity were found to prevent age-related decline in FEV_1 (102).

Inactivity is common in individuals with COPD (103). Physical inactivity has been found to precede the development of advanced COPD, indicating that it is not merely a feature of deterioration due to severe disease (103). It may even precede the onset of respiratory symptoms (103). Decline in levels of physical activity as the disease progresses is found to increase the mortality in individuals with COPD (104), and exercise capacity is an important prognostic predictor of mortality in COPD (90). Individuals with COPD show an accelerated loss of aerobic capacity (V_{O2} max), impairing the physical functioning. Further,

physical inactivity has been found to be more strongly associated with the presence of comorbidities in COPD than airflow obstruction (105).

2.6.5. Socioeconomic status

In epidemiology, socioeconomic status (SES) is usually measured as level of education, occupation or income (106). Adults with low SES have higher chances of having lower lung function and accelerated lung function decline (107). This could be explained by higher prevalence of health behaviors like smoking and low physical activity. However, these differences may begin in early phases of life (108), and factors influencing lung function development in early years, including prematurity, low birth weight, poor nutrition and a higher prevalence of childhood infections, is associated with lower socioeconomic status (107).

2.6.6. Gender

COPD was traditionally viewed as a disease affecting mainly men. However, this is not the case. Increasing COPD also in women is partly explained by women adopting men's smoking behavior. The increasing morbidity and mortality of COPD is also partly driven by female COPD patients (109), and studies have shown that women have more severe COPD (110), with higher risk of hospitalization and respiratory-related death (111). Women are found to be more sensitive to the development of COPD upon exposure to cigarette smoking (110), and the reason have been theorized to be caused by genetic and hormonal differences, morphological differences in the airways and difference in smoking behavior (109). There has also been a substantial gender bias in diagnosis of COPD in women (112), even though women are found to report more respiratory symptoms at the same level of lung function as men (113). A more recent study found gender difference in reported respiratory symptoms, with women reporting more dyspnea and cough, whereas men reported more phlegm and sputum production (114).

2.6.7. Age

COPD is a disease of aging. Aging is a physiological process with a progressive decline in the body's ability to restore homeostasis, due to deterioration of the function of cells, tissue and organ. This loss of homeostasis reduces the ability to adapt to stress responses, which in turn increases the vulnerability to disease and the risk of death (115, 116). The process of aging is highly complex, and not yet completely understood, although hallmarks like genomic instability, telomeric attrition, epigenetic drift and cellular senescence are thoroughly studied components in this process (116). COPD has been termed a disease of accelerated lung aging (117). It is mainly a slowly progressive disease, and the prevalence is highly age

dependent (118). One of the theories behind aging points to an accumulating build-up of DNA damage throughout the life span. Inflammatory reactions are thought to exacerbate such damage via the generation of ROS and increased oxidative stress, as well as faulty DNA repair and telomere shortening (replicative senescence), all known to take part in the aging process (115). Mice with homozygous lack of klotho, a gene known for its antiaging coding properties, are shown to have more pulmonary emphysema, other age-related changes and shorter lifespan (119). Other genetic markers of senescence have also been found to cause age-related pulmonary changes when studies together with cigarette smoke exposure, indicating that genetic variants could protect or increase vulnerability to smoking (117). Structural changes due to age, like loss of recoil and dilatation of airways, may be due to the loss of elastin, a well-known feature of age. Loss of muscular tissue results in decreased strength of respiratory musculature, and decreased sensitivity of the respiratory centers in the central nervous system may impair ventilatory response (117). Inflammation seems to be a key player in the difference between physiological and accelerated lung aging. The accumulation of ROS, either by senescence or through environmental exposures like cigarette smoking, induces mitogen activated protein kinase (MAPK) and phospho-inositide 3-kinase (PI3K)/AKT pathways, which in turn activate NF-κB, induce oxidative stress, inhibit SIRT1 which downregulates the anti-senescence protein Sirtuin, and other molecular and genetic processes (117). This cascade of activation and inactivation of important signaling pathways induces apoptosis, increases inflammatory responses and the risk of cancer.

2.6.8. Poor lung function development and childhood respiratory infections

Intrauterine growth restriction (IUGR), preterm birth, bronchopulmonary dysplasia (BPD) and the need for assisted ventilation are risk factors for altered lung growth in utero and after birth (75). Further, a study found low birth weight and preterm birth to be risk factors for development of COPD later in life (120), but this association was only found in women.

Several longitudinal studies have shown that children who have lower respiratory tract diseases in early periods of life are at increased risk for later chronic respiratory symptoms and low FEV_1, often persisting into adult life (121). Pneumonia before 3 years of age is a strong predictor for later lung function deficits (122), as well as bronchiolitis caused by respiratory syncytial virus (RSV) (123, 124). One study have suggested a causal relationship between pneumonia and bronchiolitis in early childhood and COPD in late adult life (mean age 70 years) (125, 126). Another study found that adult smokers with a history of childhood pneumonia had increased risk of developing COPD, and showed phenotypical traits like more

exacerbations, chronic bronchitis, cardiovascular diseases, increased dyspnea and worse disease-related quality of life. Concomitant asthma strengthened this association (127).

2.6.9. Asthma

Childhood asthma is an established risk factor for chronic airflow obstruction in adulthood (124). In a study on children with mild-to-moderate childhood asthma, impairment of lung function in childhood and male sex were the most significant predictors of abnormal trajectories of lung-function growth and decline (128). Several studies have shown that children with persistent asthma are at increased risk for fixed airflow obstruction and resulting COPD in later in life (128-130). Furthermore, parental asthma is a risk factor for COPD in adulthood (65), and the impact of childhood disadvantage (parental and own asthma, maternal smoking and respiratory infections in childhood) was found to be as impactful as smoking on the future risk of COPD (65).

2.6.10. Genetic factors

Around 25% of COPD cases occur in never-smokers (131), and the heterogeneity in COPD phenotypes at least partly indicates a genetic or epigenetic background. The most well-known genetic risk factor is alpha1-antitrypsin deficiency (15). However, a recent genome-wide association study (GWAS) on the UK Biobank material identified 97 independent genetic associations with any lung function parameter, 43 of them novel. Signals showed enrichment in genes for development, elastic fibers and epigenetic regulation pathways (132). Importantly, most of the identified single-nucleotide polymorphisms (SNPs) seemed to influence lung function in both children and adults. Based on these findings, a polygenic risk score was developed, which showed a 3.7-fold higher risk for COPD for the highest compared to the lowest percentiles (132). However, in cross-sectional lung function studies, associated genes show little effect on longitudinal lung function decline (133), and few studies have investigated the genetic variants associated with lung function and lung function decline. This is an interesting field for future studies (11). Epigenetic changes may also influence the susceptibility to COPD (134). Studies on DNA methylation in lung tissue have shown differences in susceptibility to smoking exposure (11, 135-137). Histone deacetylase was found to be reduced proportionally to airflow limitation severity (138), possibly leading to increased inflammatory response through lack of cytokine downregulation (11), and changes in micro-RNA has also been seen (139). Network medicine, connecting levels of genetic markers with biological and clinical phenotypes as well as environmental factors, could provide a way forward in the investigation of the genetic risk of COPD (140, 141).

3. COPD as a systemic disease: part of a multimorbidity complex?

For many years, the idea was that there was a "spill-over" of inflammatory mediators generated by the effect of cigarette smoking on the lungs. This was thought to cause systemic inflammation, responsible for systemic effects like loss of fat-free mass, skeletal muscle dysfunction, weight loss, osteoporosis and depression (142, 143). Later, this concept was changed to view tobacco smoke exposure as a direct cause of systemic inflammation, not only through the lungs (144). Later studies have shown that the concept of systemic inflammation in COPD is more complex. In the Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE) cohort, systemic inflammation was only found in 16% of individuals with COPD (145), and another study found no association between pulmonary and systemic inflammation (146). Several studies have not found an association between inflammatory markers and comorbidities in COPD (147). However, comorbidities are highly prevalent in individuals with COPD. The presence of such comorbidities impact quality of life, health care burden, morbidity and mortality (4, 8, 15, 148). Furthermore, comorbidities are severely underdiagnosed in COPD (149).

Some diseases are more prevalent in COPD than others. This may be due to aging, shared risk factors and disease compounding, or it may have an underlying common causative mechanism. The complex network made up of risk factors and premorbid conditions like obesity, dyslipidemia, atherosclerosis and arterial stiffness, hypertension and prediabetic states makes it difficult to investigate the causative mechanisms behind COPD and comorbidities (105). However, it is clear that COPD often occurs as part of a multimorbidity complex , but it is unclear whether it predisposes to the development of other diseases as an independent risk factor (105). The most frequently occurring comorbidities in COPD are summarized in Figure 3.1 (9).

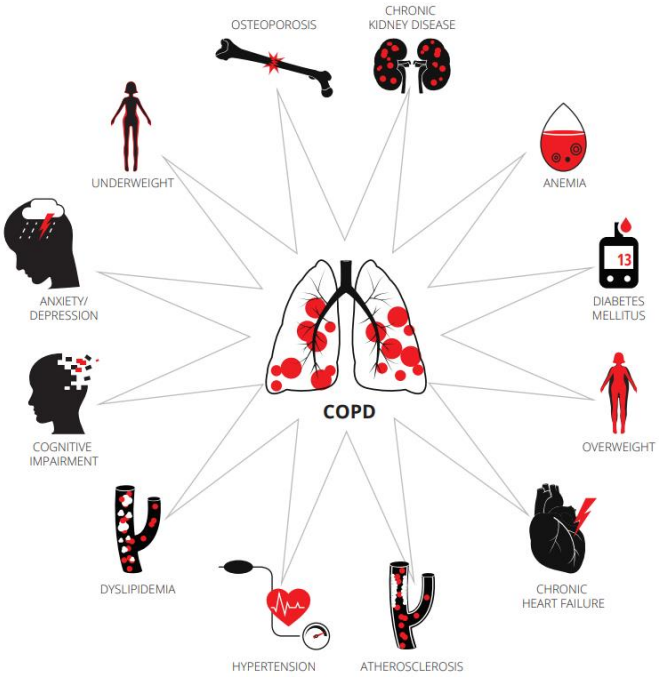

Figure 3.1 *Chronic obstructive pulmonary disease (COPD) as a component of the multimorbidity spectrum. Credit: NTNU Graphic Center.*

The concept of comorbidity can be defined as an illness co-existing with a disease of interest. Multimorbidity on the other hand, can be defined as multiple co-existing conditions in an individual (150). In a study done on almost 48.000 individuals from HUNT3, multimorbidity was found to be prevalent across genders and age-groups, with an overall age-standardized prevalence of 42% (151). Recent hypotheses are moving in the direction of viewing COPD as the pulmonary part of a multimorbidity complex (152). The concept of multimorbidity has, with an aging population with increasingly complex disease profiles, gained more interest during the last decade. This prompted the first guideline for multimorbidity, by the United Kingdom National Institute for Health and Care Excellence (NICE) in 2016 (153). This guideline highlights the need for coordinated care of the multimorbid patient. This could be extended to the care of the multimorbid COPD patient (154). The symptoms associated with COPD could be caused by coexisting diseases like heart failure (155), depression (156) and sleep disorders (157), and failure to treat these could mean failure of treating the patient.

3.1. COPD and osteoporosis

One of the most prevalent comorbidities in COPD is osteoporosis (158, 159), which is characterized by reduced bone strength and increased fracture risk. Due to methodological differences, the worldwide prevalence of osteoporosis is difficult to determine, but the overall prevalence in the United States among men and women 50 years and older was 10.3% in 2010 (160). Bone mass reaches the peak at an age between 25-30 years (161). After a plateau phase, physiological bone loss occurs by a rate of 0.5-1% per year, dependent on sex, age and skeletal site. For men the rate of bone loss is constant between 25-39 years, with an increase in loss at the hips ≥65 years (162), and loss of cortical bone from 75 years of age (163). In women the bone loss occurs later, between 40-44 years. However, a rapid loss of bone in the hip occurs earlier, between 50-54 years, and further acceleration after the age of 70 (162-164).

Osteoporosis is an important risk factor for fractures, with a 50% risk of life-time fractures in women >50 years, and 20% risk in men >50 years (164). Risk factors for osteoporosis include aging and female sex, with postmenopausal decrease in female sex hormones as the single most important risk factor. Further, the prevalence of osteoporotic fractures is highest among those with Northern European and Scandinavian descent. A family history of osteoporotic fractures, as well as previous fractures, are also important predictors of future fracture risk (164). Individuals with COPD have a 1.5–2-fold increased risk of osteoporosis (165), and a recent meta-analysis found a pooled global prevalence of osteoporosis in COPD to be 38% (166). Shared risk factors and an interplay between compounding elements of the two diseases may partly explain the high prevalence (*Figure 3.2*) (167).

Oral corticosteroid (OCS) is commonly used as treatment of COPD exacerbations and is also a risk factor for osteoporosis upon high cumulative doses. A definite cut-off of OCS and risk of osteoporosis has not been determined (164). However, sporadic use is associated with less adverse effects than continuous use (164). On the other hand, frequent high-dose courses are worse than low (2.5-5 mg) continuous doses (164). Use of OCS affects mainly the collagen structure and bone microarchitecture, and to a lesser degree mineralization of bone, and fracture rates are therefore higher on higher levels of BMD (164). Corticosteroid (CS) treatment affects trabecular bone, which is metabolically active, and the cortical rim of vertebral bodies more severely than the cortical part of long bones (168-170). Due to this, CS-related fractures are more common in areas with high proportion of trabecular bone, like the spine (171, 172). The fracture risk upon CS treatment doubles in hip and distal radius, and quadruples in the vertebrae (173). There is conflicting evidence regarding the association between inhaled corticosteroids

(ICS) and BMD. However, in a HUNT study ICS use was associated with lower BMD, but no dose-response relationship was found between ICS dose and BMD (174).

BMD is commonly used as a surrogate measure of bone quality, and osteoporosis is defined by this. Osteoporosis is an important risk factor for fractures, but other factors reflecting bone metabolism and bone quality are also important (165). Vertebral fractures are common but underdiagnosed to a large extent. These might add a restrictive component to COPD (175), further worsening the respiratory capacity. Hip fractures increase the risk for subsequent fractures and mortality (165). In women, but not in men, low BMD was found to account for a large proportion of post fracture mortality risk (176). Few studies have addressed potential associations between BMD and all-cause mortality in COPD, but an association between BMD and COPD mortality has been reported (177, 178).

Figure 3.2 *Aggravating and protective factors of the development of osteoporosis in COPD. Everyone is born with a genetic potential for peak bone mass. However, several life-style factors could influence this development. Among protective factors, physical activity, sun exposure and a diet rich in vitamin D and calcium and balanced nutrients are important. Among aggravating factors are tobacco smoking, excessive alcohol consumption, medicaments affecting bone metabolism and increasing age, as well as factors giving secondary amenorrhea in female adolescents (like excessive physical training and anorexia nervosa) preventing optimal peak bone mass development. Protective factors are often low in COPD, as reduced physical activity, less sun exposure and a diet lacking in calcium and other important nutrients. Furthermore, the link between smoking and COPD, the frequent use of prednisolone in the treatment, as well as the advancing age of individuals with COPD, contribute to a downward spiral of disease progression of both COPD and osteoporosis.*

3.2. COPD and symptoms of anxiety and depression

Anxiety and depression are highly prevalent in the general population. The global prevalence of depression was estimated to be 4.4% in 2015, and it is more common in females than in males, 5.1 vs. 3.6% (179). Prevalence varies among regions and age groups and are highest in the age group 55-74 years. The global prevalence of anxiety was 3.6% in 2015 and was also more prevalent in women; 4.6 vs. 2.6% (179). Contrary to depression, the prevalence rate does not vary between age groups, however, the prevalence is slightly lower in the older age groups. Depressive disorders are the largest cause of non-fatal health loss (years lived with disability [YLD]), with 7.5% of all YLD. Anxiety disorders are the 6th leading cause of YLD globally (179). A study done on the general HUNT population found that depression was a risk factor for mortality, comparable in strength to smoking (180). Having comorbid anxiety reduced mortality. Furthermore, the relationship between symptoms of anxiety and mortality was found to have a U-shape, with highest mortality among those with lowest symptom load (180).

Anxiety and depression often co-exist and are two of the most common comorbidities in COPD, with an estimated prevalence of up to 40% and 25%, respectively, in a clinical population (181). Despite the high prevalence in COPD, these conditions are under-recognized and undertreated (182, 183). Symptoms of anxiety and depression impact on quality of life, coping strategies and adherence to treatment of both COPD and comorbid diseases (181). Changes in breathing patterns, hyperventilation and dynamic hyperinflation associated with anxiety also contribute to a downward spiral of disease progression (*Figure 3.3*) (184). Both anxiety and depression have been identified as major determinants of dyspnea scores (185, 186). The relationship between anxiety and dyspnea is unclear, and although some studies suggest an element of bidirectionality in this association (185), several studies have suggested that anxiety in itself cause dyspnea (187). In a study done on the same cohort as the present study, it was reported that anxiety symptoms were strongly associated with reporting dyspnea (185). Further, biological pathways including hyperactivity of the hypothalamic-pituitary-adrenal axis and chronic activation of the somatic nervous system is suggested to increase the vulnerability for lower respiratory tract infections (156), thereby increasing the risk for infectious exacerbations.

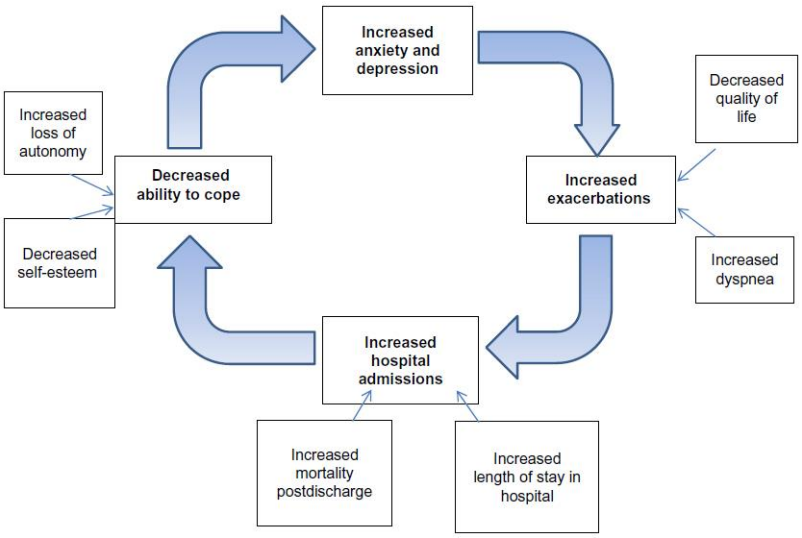

Figure 3.3 *The vicious cycle of anxiety and depression in COPD (188).*

3.3. COPD and myocardial infarction

Individuals with COPD are at a higher risk of developing a range of cardiovascular diseases, including heart failure, ischemic heart disease (IHD), stroke and myocardial infarction (MI) (155). IHD, a spectrum of heart disease generalized by atherosclerosis of coronary arteries causing myocardial ischemia, is shown to be more prevalent in individuals with COPD than in matched controls (155). An MI is one of the acute presentations of IHD, with sudden obstruction of one or more coronary arteries leading to ischemia and eventually necrosis of the cardiac muscle (189). The mechanism behind such obstruction could be rupture of atherosclerotic plaque (type I MI) or imbalance of supply and demand of blood to the cardiac muscle (type II MI), resulting from severe acute illness. A diagnosis of acute MI is made based upon international consensus criteria (189): clinical presentation of acute myocardial ischemia with the detection of rise and/or fall of cardiac troponin at least > 99th percentile of the upper reference limit, and at least one of the following: cardiac symptoms, new ischemic electrocardiographic (ECG) changes or development of pathological Q waves, imaging finding of regional dyskinesia or evidence of a coronary thrombus upon angiography.

MI is shown to increase morbidity and mortality in COPD compared to individuals without COPD (190). Death by cardiac causes is estimated to constitute 20-30% of all-cause mortality in COPD (191-193). Individuals with COPD are shown to receive suboptimal pharmacological treatment in the form

of secondary prevention after an MI (194), which could explain some of the increased mortality compared to non-COPD individuals (195). Furthermore, they have worse outcomes after percutaneous coronary intervention (PCI) and coronary artery bypass graft (CABG) (194, 196, 197), higher in-hospital mortality and rehospitalization rates (155). Delayed diagnosis of MI due to overlapping symptoms with COPD may also be a factor (155, 198), even though COPD is not associated with increased risk of atypical presentation of MI (199). The risk of an MI have been found to increase following an COPD exacerbation (200), possibly due to processes in the immune system including macrophage uptake of low-density lipoprotein (LDL) and increased leucocyte adhesion in the endothelium of the arteries (200). The increased risk of MI in COPD could be explained by shared risk factors like aging, tobacco smoking and unhealthy diet. However, a common mechanistic background, like generalized inflammation, has also been investigated, but no conclusions have been made (155).

3.4. COPD and diabetes

Diabetes is a metabolic disorder affecting around 422 million individuals worldwide, and the global prevalence in adults >18 years was estimated to be 8.5% in 2014 (201). Hyperglycemia and diabetes are important causes of morbidity and mortality, partly due to direct clinical consequences, but also due to secondary complications including cardiovascular and kidney disease (202). Diabetes is characterized by hyperglycemia due to defects in insulin secretion or -function. This hyperglycemia leads to a chronic hyperglycosylated state that could lead to secondary damage, including retinopathy, peripheral and autonomic neuropathy, and increased risk of peripheral arterial, cardio- and cerebrovascular disease. There are several subtypes of diabetes mellitus, the most common being type I, where autoimmune destruction of the pancreatic β-cells leads to deficient insulin secretion, and type II, where peripheral insulin resistance is the underlying cause (203).

Diabetes has been found to be more prevalent in individuals with COPD compared to controls, and was associated with increased mortality and incidence of cardiovascular diseases and stroke (204, 205). In a database study, both pre-existing and incident diabetes mellitus were associated with an increased risk of all-cause mortality in COPD patients (206).

3.5. COPD and lipid abnormalities

The key players of dyslipidemia are increased plasma triglycerides (TG), low levels of high-density lipoprotein (HDL), and an excess of low-density lipoprotein (LDL). Dysregulation of cholesterol and

lipoprotein metabolism is involved in the multifactorial development of atherosclerosis (207), a risk factor for development of coronary and peripheral artery disease, stroke and kidney disease.

Dyslipidemia and hyperlipidemia are related terms, sometimes used interchangeably. However, they represent different nuances of a similar condition: the levels of lipids and lipoproteins in the blood. Dyslipidemia is used to describe the presence of abnormal amounts of lipids in the blood, either in high or low amounts. HDL, the "good" cholesterol, may be low, and in this case, we term the condition dyslipidemia. Hyperlipidemia simply states that blood lipid levels are increased.

Dys- and hyperlipidemia are common in COPD, and a major risk factor for cardiovascular disease (208). However, the presence of these conditions in COPD has not uniformly been found to be negative. In a multicenter study, hyperlipidemia was associated with less hyperinflation and airway obstruction in individuals with COPD (209). Interestingly, individuals with COPD and hyperlipidemia have been found to have decreased incidence of pneumonia and reduced mortality (4, 210). Furthermore, a retrospective database study found that the incidence of cardiovascular disease was not increased in individuals with COPD and hyperlipidemia (210). It is not clear whether this is mediated by better nutrition and higher BMI. However, it represents an interesting field for future studies.

Metabolic syndrome is not a disease, but a set of risk factors for disease, and is defined as having three or more of the following traits: waist circumference ≥89 cm for women or ≥102 cm for men; increased triglyceride levels (1.7 mmol/L); reduced HDL cholesterol (<1.04 mmol/L in men <1.3 mmol/L in women); hypertension (≥130/85 mm Hg and; elevated fasting blood sugar (≥5.6 mmol/L) (211). The association between COPD and metabolic syndrome has been investigated in several cross-sectional and longitudinal studies (212). Metabolic syndrome has also been identified as an independent risk factor for adverse effects on the lung, including increase of respiratory symptoms and worsening of lung function (213). It is unsure how much each of the components of the metabolic syndrome contribute to these effects. However, of the individual components of the metabolic syndrome, abdominal obesity shows the strongest association to lung function decline (214). A role of adipokines, leptin and insulin-like growth factor have been suggested to take part in these mechanisms, however, studies have shown conflicting results, and more research is needed to establish the causal mechanisms (213).

3.6. COPD, chronic kidney disease and microalbuminuria

Chronic kidney disease (CKD) is defined as abnormalities in kidney function or structure lasting for more than three months (215). According to KDIGO (Kidney Disease: Improving Global Outcomes) guidelines, the criteria for CKD are 1) One or more markers of kidney damage (e.g. albuminuria, urine sediment or structural abnormalities) and 2) GFR <60 ml/min/1.73 m^2 (216). Worldwide prevalence of CKD is estimated to be between 10-16% (216), and a recent systematic review and meta-analysis found a mean global prevalence of 13.4% (217). A systematic review found that CKD was prevalent in COPD with an odds ratio of 2.20 (218). Another study reported the prevalence of overt CKD to be 20.8%, and concealed CKD 22.2% in individuals with COPD. This was twice as high as compared controls (219). The mortality of CKD has increased the last decades, from being ranked the 27th leading cause of death in 1990 to the 13th in 2013 (220). CKD as defined by GFR and microalbuminuria has been found to be an independent predictor of mortality in the general population (221).

Several studies have identified COPD as an independent predictor of reduced renal function (219, 222, 223). Furthermore, the severity of emphysema is found to be associated with reduced glomerular filtration rate (GFR) (224). Poor kidney function has been shown to coexist with osteoporosis, underweight and loss of free fat mass in cluster analysis of patients with COPD (159). A recent study found concurrent lung and kidney damage in smokers with COPD, with systemic damage to the endothelium as probable cause (200). This association was also found in mice exposed to cigarette smoke (225).

Moderately increased albuminuria (previously microalbuminuria, MA) reflects excretion of albumin in urine and is a predictor for cardiovascular morbidity and mortality in individuals with or without diabetes mellitus. Endothelial dysfunction leading to increased permeability of the vascular wall permits leakage of albumin through the vessel wall. MA is a potent marker for generalized damage and systemic inflammation in the vascular system (226). A previous study on the HUNT COPD cohort found that MA was positively associated with mortality in a 12-year follow-up study (226).

4. Aims

4.1. Main objective

The aim of this project was to examine the association of prevalent comorbidities in COPD with severe COPD exacerbations and all-cause mortality in the HUNT COPD cohort.

4.2. Specific aims

The specific aims of the papers constituting the thesis was as follows:

Paper I: To examine the association of bone mineral density (BMD) and osteoporosis with all-cause mortality in individuals with COPD recruited in a population-based study.

Paper II: To examine the association of symptoms of anxiety and depression with all-cause mortality in individuals with COPD recruited in a population-based study. Further, we aimed to assess the impact of longitudinal change in HADS score on mortality.

Paper III: To examine how clusters of comorbidities are associated with risk of severe exacerbations and mortality in COPD, in individuals recruited from a general population.

5. Material and methods

5.1. The Trøndelag Health Study (HUNT)

The Trøndelag Health Study (previously the Nord-Trøndelag Health Study) (HUNT) is a comprehensive population-based study that have taken place in four study surveys: HUNT1 (1984-86); HUNT2 (1995-97); HUNT3 (2006-08) and HUNT4 (2017-19). The HUNT Study invited all inhabitants above 19 years of age living in the former Nord-Trøndelag county (from January 1. 2019 fused with Sør-Trøndelag County to form Trøndelag county), geographically situated to Mid-Norway (*Figure 5.1*).

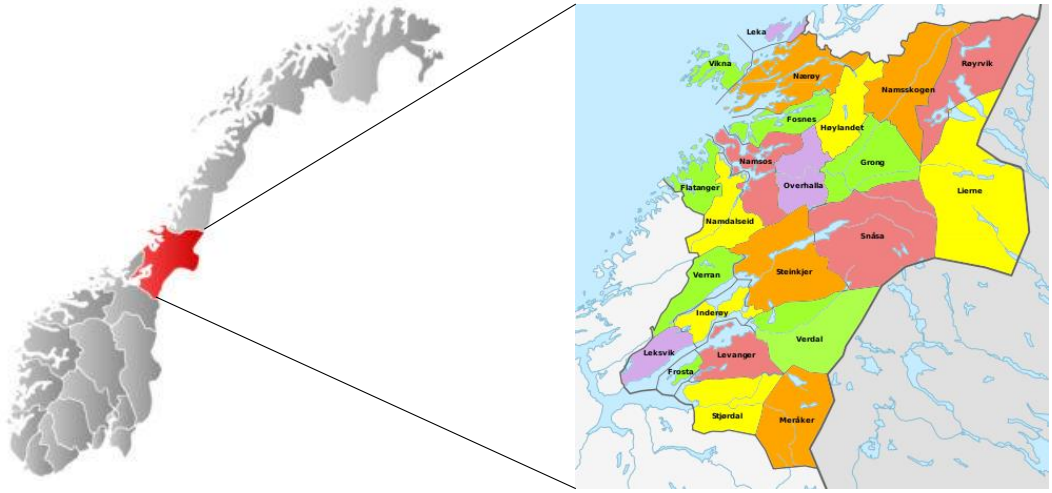

Figure 5.1 *Map over former Nord-Trøndelag (left) with its 23 municipalities (right). Source: Wikipedia.*

The population is stable with little migration in and out of the country. The population of Nord-Trøndelag is demographically representative of the Norwegian population concerning mortality and health status, and is considered representative of Norway as a whole, expect for lack of large cities, low degree of pollution and lower prevalence of current smokers. Furthermore, there is a lower level of education due to high proportion working in agriculture and fishing. Income level has also been lower, however, great increase in coastal municipalities due to fish farming have been seen the last years (34, 227). The HUNT Study had overall high participation rates: 89% in HUNT1, 69% in HUNT2 and 54% in HUNT3-4. The highest participation rates were among the middle aged and elderly (aged 50–79), and lower in the younger and oldest age groups.

HUNT has collected data from questionnaires, interviews, objective measurements and biological samples including blood, urine, saliva and feces. DNA has been extracted from participants in HUNT2-4,

and GWAS analyses have been conducted in 70.000 participants so far. Based on the experience from HUNT2 and HUNT3, we expect that complete genotyping will be available for around 75% of those who participate in HUNT4, yielding genotype data for around 88.000 individuals.

5.1.1. The HUNT Lung Study

The HUNT Lung study was a sub-study taking part in HUNT2, -3 and -4. Participants were recruited to the HUNT2 Lung Study if they gave affirmative answer to questions on ever having had asthma, used asthma or COPD medication or had attacks of wheezing or breathlessness during the last 12 months before participation. In the Lung Study of HUNT 3 and 4, questions on ever COPD, emphysema and chronic bronchitis also were included for selection. Furthermore, random samples of all participants were invited: 5% in HUNT2 and 10% in HUNT3-4. In our study we included individuals participating in the HUNT Lung Study of HUNT2 and -3.

5.1.1.1. HUNT2 Lung Study

The HUNT2 Lung Study took part during HUNT2 (1995-97).

5.1.1.1.1. Methods

In HUNT 2 the county consisted of 24 municipalities. Spirometry was performed at the HUNT screening stations in the five larger municipalities. Participants with indications of bronchial obstruction (FEV_1/FVC < 0.75) were invited to phase II with pre- and postbronchodilator spirometry. In the 19 smaller municipalities, spirometry was not performed at the screening station due to logistic limitations. Those being selected were invited to pre- and postbronchodilator spirometry after 6-8 weeks (study parts NT2Lu2M1 and -M2). For these municipalities, the prebronchodilator results of spirometry should be used as baseline results.

5.1.1.1.2. Known challenges and quality assurance

In a few peripheral municipalities a higher proportion of spirometry maneuvers did not reach adequate quality. The main problem was too short expiration time, giving too low FVC. A thorough quality assurance was performed after completion of data collection. Furthermore, measurements were compared to measurements performed at the local hospital (n=160) and general practice (n=300) and measurements performed in phase 2-4 of the Lung Study. For about 5 % of individuals the spirometry measurements were deleted due to unacceptable quality.

Lack of optimal FVC would underestimate COPD (defined by FEV_1/FVC). In the HUNT databank COPD has been defined as FEV1/FVC < 0.7 for both pre- and postbronchodilator spirometry, the latter according to GOLD recommendations (15). These variables have been compared to the diagnosis of all patients having been examined (n=1560) due to obstructive lung disease at the only lung department in the county. There was a high level of agreement of diagnosis between HUNT and the hospital registry. However, in case of forced expiratory time less than 6 seconds in HUNT2 and disagreement of diagnosis, the diagnosis has been changed in accordance with the hospital diagnosis.

Due to the study design with different phases, around 20% of participants were lost between phase 1 and 2 in both large and small municipalities. This implies that there was a loss of 1/5 of participants for spirometry measurements in the latter, which might induce selection bias, since individuals with respiratory symptoms could be more eager to participate. In large municipalities only post-BD measurements would be lost.

5.1.1.2. HUNT3 Lung Study

The HUNT3 Lung Study took part during HUNT3 (2006-08).

5.1.1.2.1. Selection criteria and method

Participants from the random and symptom sample of HUNT2 and HUNT3, as well as all HUNT3 participants previously having participated in the Young-HUNT Lung Study of HUNT2 (228), were invited. The latter were invited as everyone in Young-HUNT1, -2 and -3 had performed spirometry.

5.1.1.2.2. Known challenges and quality assurance

Absence due to disease among the staff, long duration of the total program at the field stations and logistical challenges led to loss of 30-40% of individuals selected for the Lung Study. This non-response was considered to be at random and should not induce selection bias.

Curves that not fulfilled the ATS criteria included in the software were deleted at the screening station. After the data collection all spirometry curves was manually checked and was scored for FEV1, FVC, peak expiratory flow (PEF) and forced vital capacity at inhalation (FVCIN). Outliers was checked. Errors in predicted values stemming from wrong age or height were recalculated.

5.1.2. The HUNT Osteoporosis Study

In HUNT2 bone densitometry was performed by the Osteoporosis Study (n=7000) and the Lung Study (n=11.000). In HUNT3 and -4 all bone densitometries were organized and performed by the Lung Study. To avoid confusion, all bone densitometries in HUNT4 are part of the Osteoporosis Study and all spirometries are part of the Lung Study, both with the same principal investigator.

In HUNT2 dual-energy X-ray absorptiometry was performed at the non-dominant forearm by three DTX100 machines. In HUNT3 the non-dominant forearm was measured by two DTX100 and one DTX200, and in addition hip and lumbar spines were measured by Lunar Prodigy in the five larger municipalities (see 5.2. *Instruments and variables*).

5.1.2.1. Selection criteria

In HUNT2 the Lung Study invited all participants from the symptom- and random samples also having been invited to spirometry. The Osteoporosis Study invited 50% of women in the age groups 32-41 years and 51-60 years, as well as 100% of women 65-85 years having participated in HUNT1.

In HUNT3, all individuals having participated in bone densitometry in HUNT2 were invited, as well as new participants in the Lung Study. Around 70% responded.

As part of HUNT4, individuals having participated in HUNT2 or -3 living in the five larger municipalities have been invited to bone densitometry of hip and lumbar spines. These measurements are currently ongoing.

5.1.2.2. Known challenges

In the five larger municipalities during HUNT2, the measurement was performed the same day as the screening, while in the 19 smaller municipalities, the measurements were performed 6-8 weeks after the screening due to logistical challenges. Due to this, the participation rate was lower in smaller municipalities, and could induce selection bias. However, this was mainly young people from the random sample, and should not affect the current study.

In HUNT3 only two of the three DTX100 having been used in HUNT2 worked adequately, and the upgraded version, DTX200, was bought. The two DTX100 were used in the smaller municipalities and the DTX200 in the larger municipalities in addition to Lunar Prodigy measuring hips and spines. The Lunar Prodigy equipment was moved between the five larger municipalities

5.2. Instruments and variables

For an overview of all instrument variables, refer to Appendix 5.

5.2.1. Questionnaires

Everyone received an invitation by mail with information about the HUNT Study, consent forms and a printed baseline questionnaire (Q1). Q1 contained questions on quality of life, own and family diseases, health-related behaviors and use of health care services. Upon attendance at the field stations, participants answered an interview regarding work, and for females; reproductive factors, and were delivered a sex and age stratified questionnaire (Q2) which contained a common part for everyone as well as age and sex specific questions. Selected groups, either through reporting of diseases or symptoms or randomly selected, were given additional questionnaires (Q3) specific for different sub-studies. In HUNT2, the Lung Study included Q3 Lung, which contained questions on airway symptoms and allergies in addition to an interview with additional questions on airway symptoms, diagnoses and use of medication. In HUNT3 all questions in the Lung Study were included in an interview (see Appendix 3-4).

5.2.2. Instruments

5.2.2.1. Spirometry

5.2.2.1.1. Equipment

In HUNT2 flow-volume spirometry was performed using three pneumotachographs (MasterScope Spirometer, version 4.15, Erich Jaeger GmbH, Wuerzburg, Germany). Calibration procedures, including ambient conditions (surrounding temperature, air humidity and barometric pressure), volume calibration using a 3,0 L syringe (Series 5550, Hans Rudolph Inc., Shawnee, KS) and biological calibration by the technician was performed twice a day. In HUNT3 similar spirometers were used (version 5.1).

5.2.2.1.2. Acceptability and reproducibility criteria

American Thoracic Society (ATS) 1994 criteria was used in HUNT2 (229), and ATS 2005 criteria was used in HUNT3 (230).

<u>Acceptability:</u>

1. Satisfactory start of test (maximal effort curve and back-extrapolated volume less than 5% of FVC/0.150 L).

2. Forced expiratory time of at least 6 seconds.
3. End of test criteria (end of plateau achieved, or volume change less than 40 ml the last 2 seconds of expiration).

Reproducibility:

1. No more than 150 mL difference between highest and lowest FEV_1.
2. No more than 150 mL difference between highest and lowest FVC.
3. At least three acceptable curves.

This was the gold standard, but in quality check of spirometry curves we also accepted curves with criteria D or better according to suggestions of John Hankinson (*Figure 5.2*).

Grade	Number of Measurements	Repeatability: Age >6 yr
A	⩾3 acceptable	Within 0.150 L
B	2 acceptable	Within 0.150 L
C	⩾2 acceptable	Within 0.200 L
D	⩾2 acceptable	Within 0.250 L
E	⩾2 acceptable	>0.250 L
	OR 1 acceptable	N/A
U	0 acceptable AND ⩾1 usable	N/A
F	0 acceptable and 0 usable	N/A

Figure 5.2 *Grading system for FEV_1 and FVC (231). The repeatability grade is determined for the set of prebronchodilator maneuvers and the set of post-bronchodilator maneuvers separately. The repeatability criteria are applied to the differences between the two largest FVC values and the two largest FEV_1 values. Grade U indicates that only usable but not acceptable measurements were obtained. Although some maneuvers may be acceptable or usable at grading levels lower than A, the overriding goal of the operator must be to always achieve the best possible testing quality for each patient.*

Studies have shown that about 1.6% of patients have problems achieving the quality and repeatability criteria. It is important to take into account that patients with lung disease have greater challenge with this and that curves, at least in the clinic, should be considered even if ideal criteria are not achieved (232).

5.2.2.2. Bone densitometry

Forearm bone densitometry was performed in both HUNT2 and HUNT3, and bone densitometry of hip and lumbar spine was performed in HUNT3.

5.2.2.2.1. Forearm bone densitometry

Forearm bone densitometry was performed using three single x-ray absorptiometry (SXA) Osteometer DTX-100 (Osteometer MediTech, Inc., Hawthorne, California). The X-ray source current was 29 KeV. To reduce effect of leisure physical activity and manual work, non-dominant forearm was measured, unless in case of previous fractures, when the dominant arm was measured (2.5% of measured). In individuals reporting fractures of both arms (0.1%), the non-dominant forearm was measured. Due to a single x-ray source the measured limb had to be submerged in a water-filled container. This makes it possible to separate two components, water/soft tissue and bone.

For all analyses, the measured region of interest (ROI) was defined as the distal site. With the starting point where the distance between radius and ulna was 8 mm, distal site was defined as the 24 mm proximal to the starting point, and the ultradistal part as the area between the 8 mm site and tangent of the end plate of radius (*Figure 5.3*). The 8 mm point identifies a transition zone between two different segments of the radius, with distal site consisting of mainly cortical and ultradistal of mainly trabecular bone. Due to problems with the automatic computer identification of the endplate in individuals with high or low BMD, all measurements were recalculated after manual reposition of ROI. Calibration of the equipment was performed daily before the first measurement. Standardized phantoms were used. Additionally, a cross calibration study was performed including the three apparatuses used in HUNT and the two similar used in the Tromsø Study. Correction factors for each equipment was estimated and results corrected according to this, and the mean bone mineral content (BMC) was stable in all three densitometers (174).

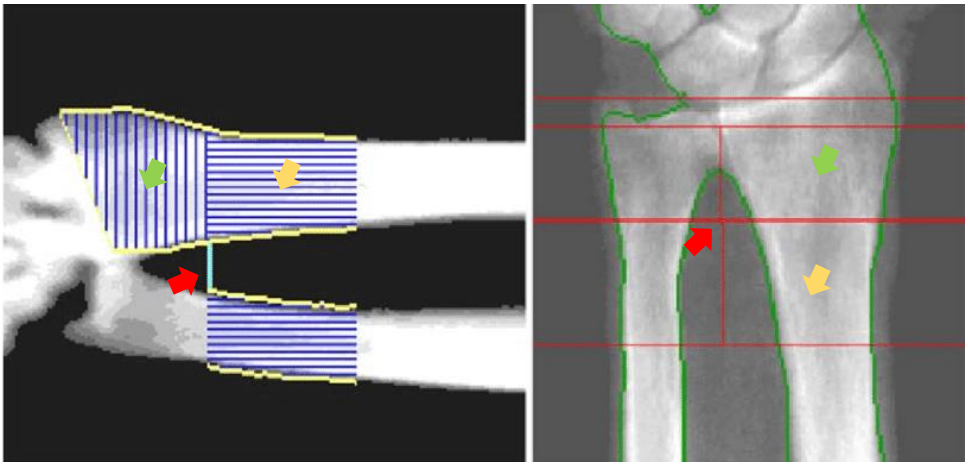

Figure 5.3 *Distal part of ulna (yellow arrow) and ultradistal part (green arrow). The red arrow marks the 8 mm. starting point.*

In HUNT3 an updated version of DTX100, DTX200 was also used. DTX200 estimated the bone mineral content (BMC, grams) and bone mineral density (BMD, grams/cm^2) in the distal section of the forearm (radius and ulna). The current of the dual X-ray source was 55 kV, and the beam was filtered with tin (Sn), yielding two effective energy levels for separation of bone and soft tissue (fat, muscle). Due to two energy levels it was not necessary to fill the container with water as it was for DTX100. Quality control had to be performed every day before the first patient scan.

5.2.2.2.2. Bone densitometry of hip and lumbar spine (central DXA)

Central DXA was performed using Lunar Prodigy Advance (GE Healthcare, Little Chalfont, UK) with dual X-ray absorptiometry. The Lunar Prodigy measured bone mineral content (BMC, grams) and calculated bone mineral density (BMD, grams/cm^2) in anteroposterior (AP) lumbar spine, hip, femoral neck, proximal femur and distal forearm, as well as total body composition. The instrument utilized a narrow-angle fan beam. The X-ray tube gave a constant peak X-ray energy of 76 kV and a current of 3 mA. The beam was filtered by a rare-earth samarium K-edge filter system producing energies at 38 and 70 kV, and detected by a cadmium zink-telluride (CZT) detector (233, 234). Quality control had to be performed every day before the first patient scan. Calibration was done with a European Spine Phantom (ESP, QRM GmbH, Germany). It consists of three geometrically defined semi-anthropomorphic vertebrae (L1-L3)

with calcium hydroxyapatite densities of 50, 100 and 200 mg/cm^3 for spongious bone and 400 and 800 mg/cm^3 for cortical bone, embedded in water-equivalent resin-based plastic.

In central DXA the total hip and lumbar spine regions of interest (ROI) were measured. The total hip ROI was defined as the entire region of bone covered by femoral neck, trochanter and intertrochanter bone areas (235). Lumbar columna ROI included lumbar vertebrae 1-4 (L1-L4), and the mean of these measurements were calculated. In case of > 1 SD to the neighboring vertebra, the vertebra in question was removed from the calculation.

5.2.2.2.3. DXA reference population

The reference population used in both HUNT2 and -3 was taken from the US National Health and Nutrition Examination Survey (NHANES). For both male and female participants, female reference values were used according to ISCD guidelines for adults (236). For paper I and III we calculated T-scores using a healthy Caucasian female reference population aged 20-39 years from the reference population in HUNT as recommended by ISCD (236). We excluded individuals with self-reported osteoporosis, arthritis, hip or wrist fractures, hyper- or hypothyroidism and use of corticosteroids.

5.2.2.2.4. Diagnostic criteria for osteoporosis

The WHO international reference standard for osteoporosis diagnosis is a T-score of -2.5 or less at total hip. Femoral neck and posteroanterior (PA) spine may also be used (237). This definition was used in both paper I and III. In lack of total hip T-score, lumbar T-score was used, and in lack of both, distal forearm T-score was used. No correction factor between these scores was developed. DXA at the total hip is the best predictor of hip fractures, but spine and hip BMD has similar prediction value for spine fractures. Forearm BMD predicts general fracture risk as well as other ROIs, but each ROI predicts fracture at the given site best (164). However, age related calcification of spine and aorta may introduce artefacts, giving artificially high values for BMD after 60-65 years. The total hip ROI is less affected by degenerative changes. Due to these factors, total hip measurements are used as the standard for diagnosis (164).

BMD is widely used in clinical practice to predict fracture risk. However, a majority of fragility fractures occurs in women who does not fulfill the osteoporosis criteria (238). The success of prediction of fractures increases if the temporal change between two measurements are used (162). However, in practice researchers and clinicians use only one measurement to estimate fracture risk.

5.2.2.3. Hospital Anxiety and Depression Scale (HADS)

The Hospital Anxiety and Depression Scale (HADS) is a validated (239, 240), two-factor scale with two 7-item subscales (see Appendix 1) (241). Developed as a self-assessment questionnaire by psychiatrists Zigmond and Snaith in 1983, it was constructed to assess possible and probable cases of anxiety and depression in non-psychiatric hospital outpatients, excluding physical symptoms of anxiety and depression, like headaches, insomnia and fatigue, from its structure (241). HADS is one of the most frequently used instruments to assess symptoms of anxiety and depression worldwide and is widely used within the field of epidemiology. Seven items relate to symptoms of anxiety (HADS-A), and seven to symptoms of depression (HADS-D). Each item was scaled by 0-3, resulting in a total score between 0–21. The clinical cut-off of either of the two scales was defined ≥8 for caseness of both anxiety and depression (180, 240), which has been shown to have the most optimal sensitivity and specificity, both of around 0.80 (240).

The instrument was part of the baseline assessment of all participants in HUNT2-4. In HUNT2 it was part of Q1, but in HUNT3 it was placed in Q2 (Appendix 1.1. and 1.2). Due to this, there is a loss of 15.3% in HUNT3 made up of individuals not returning Q2 in the prepaid envelope. Due to space limitations in Q1 of HUNT2, one item of HADS was dropped: "I feel tense or 'wound up'"/ "Jeg føler meg nervøs og urolig". This was replaced by a similar question from the Cohort of Norway (CONOR) Mental Health Index (MHI): "During the last 2 weeks I have felt nervous or restless". The time dimension of the latter item is not included in HADS. However, in a study comparing CONOR MHI and HADS, a high correlation with HADS of $r > 0.7$ was found (0,8 corrected for attenuation) using Pearson correlation (242). The small difference in wording of one item of the HADS-A subscore should not influence estimates. HUNTs version of HADS was not the officially validated version in Norway at the time. However, since HUNTs version is the most studied, the copyright owner has accepted this as a validated Norwegian translation.

5.2.2.4. Modified Medical Research Council (mMRC) dyspnea scale

The mMRC is a symptom questionnaire with five statements on various physical activities graded 0-4 (Appendix 2). It measures perceived respiratory disability and is used to grade the effect of dyspnea on daily activities. It is validated for assessment of the degree of breathlessness (dyspnea) (243). The mMRC is a modification of the original MRC dyspnea scale (last revised in 1986) (244).

Internationally the most commonly used modified British Medical Research Council Dyspnea Scale (mMRC) includes five items with increasing dyspnea as a scale where the participant tick off the alternative that best describes her/his dyspnea. This version was used in HUNT4 and has also been used in Tromsø7. In HUNT2 and HUNT3 all items were included but asked as five separate questions to be answered “No” or “Yes”. This was done in order to increase compatibility with the Hordaland Study, another Norwegian population-based study. Based on these answers an mMRC was constructed choosing the affirmative answer to the highest dyspnea level of the five questions as level of the scale. Inconsistencies in reported level of symptoms were also cross-checked with other questions on limitations of respiratory disease/symptoms in the interview.

5.2.2.5. Blood pressure measurements

In the five larger municipalities Dinamap Critikon apparatus was used (model 845XT in HUNT2 and model 8100 in HUNT3). In the 19 smaller municipalities the Dinamap XL model 9301 was used (Johnson & Johnson Medical Inc.). Blood pressure was measured in a sitting position according to standardized methods. Three consecutive automatic oscillometric measurements were recorded at 1-min intervals and the mean of the second and the third readings were calculated. Blood pressure was registered to the nearest 2 mmHg. Cuff size was adjusted after measuring the arm circumference. The measurements were started after the participant had been seated for two minutes with the cuff on the arm, and the arm resting on a table. Blood pressures measured with the Dinamap device are slightly lower than those measured with a sphygmomanometer, especially for diastolic blood pressure (245).

5.2.2.6. Moderately increased albuminuria (microalbuminuria)

In HUNT2, all participants who answered "yes" to at least one of the questions: "Do you have diabetes?” or “Do you use antihypertensive medication now?” were invited to the microalbuminuria (MA) screening. In addition, a 5% randomly selected sample of the total population was included. Of these, around 33% had also participated in the HUNT2 Lung Study (226). In HUNT3, the selection criteria included all who participated at the MA screening in HUNT2, as well as participants who answered "yes" to the question: "Do you have diabetes?” and a 10% random sample of all participants (identical with the 10% random sample of the HUNT3 Lung Study). When attending the field station, each participant received a unit with three plastic containers for three first morning urine samples, three transport tubes and one pre-stamped envelope for return by mail to HUNT biobank. They were informed by a trained nurse about the MA study and a written instruction was included in the unit, containing how to collect

urine, information about the MA screening and a questionnaire asking about urine tract infection in the previous week, persistent hematuria in the previous year and menstruation at time of collection. No reminders were given.

In the period of January-February 2008 there were errors in measured urine albumin and albumin/creatine ratio due to the use of inappropriate calibration kits at the laboratory at Levanger Hospital. Outliers identified in this period have been labeled "1" in the variable *UrAlbCreaDev* and was not included in analyses. The analysis instrument used in HUNT2 was Hitachi 911 Autoanalyzer (Hitachi, Mito, Japan) and in HUNT3 Architect ci8200 (Abbott Diagnostic, Longford, Ireland). Urine analyses were done by Levanger Hospital, Levanger, Norway.

MA was previously defined as a urine albumin creatinine ratio (ACR) ≥2.5 mg/mmol. However, this criterium is now abandoned, and the degree of albuminuria is now defined as low-graded albuminuria (formerly called normoalbuminuria) (ACR < 30), including normal to mildly increased albuminuria (ACR < 3) and moderately increased albuminuria (formerly called MA) (3-30), or severely increased albuminuria (formerly called macroalbuminuria) (ACR > 30) (216). For simplicity, we have used the abbreviation MA to denote moderately increased albuminuria, since most of the referenced studies on the topic have used this term.

5.2.2.7. Blood samples

Non-fasting blood samples were drawn from all participants at the baseline examinations in HUNT2 and HUNT3. Triglyceride and high-density lipoprotein (HDL) levels was measured by applying an enzymatic coulometric method with the instrument Hitachi 911 Autoanalyzer (Hitachi, Mito, Japan). Glucose was analyzed by the same instrument, using an enzymatic hexokinase method. Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease Study (MDRD) equation (SI Units) on corrected serum creatinine, adjusting by x 0,742 in women (246), and the Chronic Kidney Disease Epidemiology collaboration (CKD-Epi) equation in individuals >70 years of age or if eGFR was >60 mL/min/1.73 m^2 (247).

Anti-GAD/anti-IA-2 status and C-peptide levels were analyzed in HUNT2 and HUNT3 as part of the HUNT Diabetes Study. Participants at baseline with Finnish Diabetes Risk Score (FINDRISC) score >14 points (i.e. 33% risk for development of diabetes during the next 10 years) (248) and participants with known diabetes (answered yes to the question in baseline Q1) were selected. Analyses were performed on a Microbeta Counter (PerkinElmer, Massachusetts, USA) by the Hormone Laboratory, Department of

Medical Biochemistry, Oslo University Hospital, Oslo, Norway. A classification variable based on these measurements, called *DiaDiagTyp*, was developed for both HUNT2 and HUNT3, classifying subtypes of diabetes including type I and type II, latent autoimmune diabetes of adults (LADA), maturity-onset diabetes of the young (MODY) and unclassifiable. For Paper III these were dichotomized to diabetes yes/no.

5.2.3. Other study variables

5.2.3.1. Anthropometric measurements

All measurements were performed by trained health personnel upon attendance on the field stations. Standardized scales and metric tape for circumferences were used. Standing height and weight was measured with light clothes without shoes and given in whole centimeters (cm) in HUNT2 and cm with one decimal place in HUNT3, and kilograms rounded to the nearest half kg. Outliers were checked, and thorough quality assurance was performed based on measurements from HUNT1 and HUNT3, as well as from the measurement done at the HUNT Lung Study (n=11.000). BMI was calculated using the formula weight/height^2, and classified according to WHO cut-offs (86): <18.5 (underweight), 18.5–24.9 (normal range), 25.0–29.9 (overweight) and ≥30.0 (obese) in paper I-II. Underweight was defined as BMI <21.0 in paper III, since this cut-off is associated with mortality in COPD (87).

5.2.3.2. Exposure variables

For paper I, BMD T-score was categorized into normal (>–1.0), osteopenia (–1.0 to –2.5) and osteoporosis (≤–2.5) according to WHO criteria (237). To avoid loss of information due to categorization, T-score was also used as a continuous variable. Restricted cubic spline terms were also inserted into the models.

For paper II, HADS subscores (A and D) was dichotomized with a cut-off of 8, with score 0-7 indicating non-case, and score 8-21 indicating caseness of either anxiety or depression. HADS subscores were also used as continuous variables, and spline terms were also used in these models.

For paper III, clusters of comorbidities were generated. The input included data on nine objectively measured comorbidities using established cut-offs (see Appendix of paper III): hypertension (systolic pressure >140 mm Hg or diastolic pressure >90 mm Hg); chronic renal failure (eGFR <45 ml/min/1.73 m^2 or eGFR 45-60 ml/min/1.73 m^2 + presence of albuminuria); hyperlipidemia (triglyceride level >1.7 mmol/L or HDL <1.03 mmol/L [males] or <1.29 mmol/L [females]); obesity (body mass index [BMI] ≥30.0 kg/m^2); underweight (BMI <21.0 kg/m^2); osteoporosis (T-score ≤–2.5 at total hip); anxiety (HADS-A ≥8);

depression (HADS-D ≥8), diabetes mellitus (determined using anti-GAD/anti-IA-2 status, fasting C-peptide levels and self-reported insulin treatment) and myocardial infarction (ECG changes, dynamic changes in blood levels of cardiac enzymes and coronary symptoms). Data on myocardial infarction were collected from hospital records. Microalbuminuria was defined as a urine albumin creatinine ratio (ACR) 3.0-30.0 mg/mmol in at least two out of three urine samples, collected as morning urine at three consecutive days (249).

5.2.3.3. Outcome variables

5.2.3.3.1. Registry status and all-cause mortality

Data on all-cause mortality was provided by the Norwegian National Registry. These data are collected quarterly by the HUNT Databank and rasterized to the 1. and 15. day of each month, meaning that those dying between the 1. and the 15. will have date of death at the 15., and those dying between 15. and 1. will have date of death at the 1. in every month. The National Registry also provide registry status, including date of emigration or still alive and living in Norway. During the time of data collection in HUNT4, the data from the National Registry was updated every month and from March 2019 quarterly, meaning that for this project the follow-up ended at February 1. 2019 for paper I-II, and April 1. 2020 for paper III.

5.2.3.3.2. Severe exacerbations

Data on COPD exacerbations were retrieved from the North-Trøndelag Health Trust (HNT) and St. Olav's Hospital patient data. We asked for admissions to the department of internal medicine at the hospitals of Trondheim, Levanger and Namsos to obtain the ICD codes listed below. These three hospitals cover the entire catchment area of the HUNT population. The data were coded by physicians and quality controlled by audit of the patient journals done by trained ICD coding secretaries after discharge.

- ICD-10 (January 1, 1999-current): A presence of codes J40-J47 combined with any of the other codes will indicate admission for a COPD exacerbation, as will J44 alone:
    - J40-J47. Chronic diseases of lower airways AND
    - J09-J18. Influenzae and pneumoniae AND/OR
    - J20, J22. Acute infection in lower airways AND/OR
    - J44. Exacerbation of chronic obstructive lung disease

- ICD-9 (HUNT2-December 31, 1998): A presence of codes 490-493 or 496 and 466 was the coding practice for COPD exacerbations (Thor Naustdal, personal communication).
    - 460-466. Acute respiratory infections.
    - 481-488. Pneumonia and influenzae.
    - 490-496. COPD and allied conditions.

5.2.3.4. Covariates

Covariates were identifies using directed acyclic graphs (DAGs) to evaluate possible confounding (250). Sex, age at participation, COPD severity and level of education were common confounders used in Paper I-II. In addition, in Paper I tobacco smoking, alcohol consumption, BMI, level of physical activity, functional limitations and comorbidities at baseline were included as confounders. Of these, only functional limitation was included in Paper II. In Paper III, only sex and age were considered being confounders. Data on these covariates were collected at baseline, either by questionnaires or by clinical measurements. All variables on comorbidities were dichotomized to yes/no and included as separate variables in the final models.

Severity of COPD by GLI criteria was graded using ppFEV1 calculated by the GLI-2012 software, using cut-off values by z-scores at <−2 (mild), < −2.5 (moderate) , < −3 (severe) and < −4 (very severe) (26).

Severity of COPD by GOLD criteria was graded using; ppFEV1≥80 (GOLD1/mild), 80>ppFEV1≥50 (GOLD2/moderate), 50> ppFEV1 ≥30 (GOLD3/severe) or 30> ppFEV1 (GOLD4/very severe) (251).

Level of education was used as a proxy measure for socioeconomic status and was categorized into low (≤9 years), medium (10–12 years) and high (≥ 13 years) based on the question: "What is your highest level of education?" from HUNT2. Level of education in HUNT3 was assessed using the occupational classification in HUNT according to the Erikson Goldthorpe Portocarero (EGP) social class scheme, based on the question "What kind of paid work do you do?" (252).

BMI was calculated in kg/m^2 and categorized according to the WHO classification: <18.5 (underweight), 18.5–24.9 (normal range), 25.0–29.9 (overweight) and ≥30.0 (obese) (86).

Tobacco smoking was categorized as never-, former- or current smoker. The latter two categories were subdivided into <10, 10-20 and >20 smoking pack years.

Alcohol consumption was categorized by weekly consumption: low (<14 unit alcohol [U.A.] for men and <10 U.A. for women), intermediate (14–21 for men and 10–14 U.A. for women) and high intake (>21

alcohol units (U.A.) for men and >14 for women). One U.A. was defined as 13 grams of alcohol. The definition of a standard drink varies widely between countries, ranging from 8-20 grams (253). Norway has not developed a standard drink definition (253).

Physical activity was categorized according to hours of self-reported physical activity per week: inactive (≤1 hour light physical activity and no hard activity), low (>1 hour light and <1 hour hard), medium (1-2 hour of hard regardless of light activity) and high (≥3 hour of hard regardless of light activity).

Self-reported cardiovascular disorders included previous myocardial infarction, angina pectoris and ischemic-/ hemorrhagic brain infarction. We identified participants with hypertension as those answering yes to prior or current use of antihypertensive medication or having hypertension (defined as ≥140 and/or ≥90) upon blood pressure measurements at baseline.

Chronic musculoskeletal diseases included rheumatoid arthritis, ankylosing spondylitis, spondyloarthritis, osteoarthritis and "other long-term skeletal or muscular diseases".

Chronic diseases affecting ADL was assessed by the following question from Q1 in HUNT2-3: "Do you suffer from any long-term illness or injury of a physical or psychological nature that impairs your functioning in your everyday life?". This variable was included to assess the impact of unmeasured comorbidities that were not included separately in the model.

5.3. Ethical considerations

All participants in HUNT have given signed consent to data collection, storage in HUNT data bank and linkage to a wide specter of national and local registers. The Data Inspectorate has given concession for storage of HUNT data in the HUNT databank, and the Regional Ethics Committees have approved data collection and are asked for approval of all studies including the data. From 2018, HUNT is covered by *Regulations on population-based health surveys* (https://lovdata.no/dokument/SF/forskrift/2018-04-27-645). After this data collections do not need concession by the data inspectorate, but the owner is responsible for adherence to regulations. The Regional Committee for Medical and Health Research Ethics (REC) Central Norway have approved the current study with the project no. 2014/1114-1. Updated REC approvals were collected before the linkage to HNT hospital data, and after changes were done to the protocol because of the altered analysis plan of paper III.

The people in previous Nord-Trøndelag (now northern part of Trøndelag) are continuously informed of the research activities at HUNT by local newspapers and information at the HUNT website (ntnu.no/hunt).

5.4. Statistics

Observational studies, often with large samples, produce several statistical challenges. Missing data is a major concern and need to be handled correctly. Confounders must be identified, correctly classified and adjusted for. When investigating survival, problems like loss to follow-up, censoring and time-varying covariates need to be addressed. Specific methods need to be applied in case of analysis of repeated data. Furthermore, to utilize the richness and complexity in the HUNT data, novel techniques like machine learning and clustering are exciting tools to discover new patterns and correlations.

5.4.1. The Cox proportional hazards model

The Cox proportional- hazards model is a semi-parametric regression model that investigates the association between survival time and one or more predictor variables. The dependent variable is the time-to-event (e.g. survival time). Using a Cox model, we can simultaneously assess the impact of several covariates on an outcome, e.g. death. The resulting hazard is a function of these covariates. The Cox model is flexible and allows both continuous and categorical variables. It is a semiparametric method because the baseline hazard function (h0) does not have to be specified.

It can be expressed on the following form:

$h(t) = h_0(t)\, e^{\beta' xi}$

where,

- t is the survival time.
- h(t) is the hazard function determined by a set of covariates (x_1, x_2,...,x_i).
- the coefficients (b_1, b_2..., b_i) measure the impact (the effect size) of covariates.
- h(0) is the baseline hazard. It corresponds to the value of the hazard if all the x_i are equal to zero ($e^0 = 1$). The t reminds us that the hazard may vary over time.
- The quantities $e^{\beta' xi}$ are called hazard ratios. A value of $b_i > 0$, or equivalently a hazard ratio >1, indicates that as the value of the i^{th} covariate increase, the event hazard increases, and the length of survival decreases.

One of the main advantages with the Cox model compared to other survival methods, like log-rank, Mantel-Haenzel and life-tables, is that Cox models take censoring into account. Censoring occurs when individuals are still alive at the end of follow-up, before the event of interest could occur.

The main assumption of the Cox proportional hazards (PH) model is that the hazard ratio remains constant with time. This implies that the hazard curves for the compared groups of observations should be proportional. Using age at the time axis, this assumption takes in to account that there is no effect modification by age (254).

There are several ways to test the PH assumption, including graphical methods and statistical tests (255). Plots of scaled Schoenfeld residuals, complimentary log-log plots and Kaplan-Meier curves are the most frequently used graphical methods. The Schoenfeld residuals are independent of time and are used to assess the relationship between the residuals and time, and non-significant results support PH. Statistical tests include goodness-of-fit tests. The PH assumption can further be relaxed by fitting interactions with time. This is done fitting two level models where between subject variation (level 2) and within subject variation (level 1) are incorporated.

Further assumptions of the Cox model include linear covariate relationships. This assumption can be tested by plotting the Martingale residuals against continuous covariates to assess the functional form of the covariate in order to detect nonlinearity. Non-linearity is not an issue with categorical values.

5.4.2.Joint models

Joint models represent a flexible parametric method to model longitudinal and survival data within the same model (256). This is an ideal method for modelling the impact of repeated measurements on survival, and was used in paper II, where we wanted to assess the impact of change in HADS scores from HUNT2 to HUNT3 with mortality. The model could be viewed as two component models: one longitudinal part with subject-level covariates measured at several time points, utilizing a linear mixed effects model (Stata -mixed- command), and one survival part, where a parametric survival model (Stata -streg- command) is applied. These components share one common parameter, which is dependence through shared random effects (256). Restricted cubic splines are used to model the log cumulative hazard function, allowing a highly flexible assessment in case of complex hazard functions. This was achieved through the community-contributed Stata suite -stjm- (256). We used a Weibull survival submodel, and further adjusted using time-depending covariates (measured at both HUNT2 and

HUNT3), comparing individuals with change in HADS score during the 11-year period compared to those who had no change, and assessed the impact on all-cause mortality.

5.4.3.Poisson regression

Poisson regression is used for count variables and was used to model exacerbations as an outcome in Paper III. It is distinguished from multiple regression in that the dependent variable is an observed count that follows Poisson distribution. Ordinary least squares (OLS) regression works best on real numbers and using such techniques would potentially be problematic with count variables. Furthermore, the highly skewed distribution of the number of exacerbations excluded OLS regression in its regular form. Finally, the rate of exacerbations could be expected to be changing over time, creating even more problems for linear regression models. Poisson regression would overcome these obstacles. The goal of a Poisson regression model is to find the values of the regression coefficients β which makes the vector of the observed outcome most likely. This is obtained through maximum likelihood estimation (MLE). Goodness-of-fit tests could estimate how well the obtained model works (257).

5.4.4.Directed acyclic graphs

Control of confounding requires careful a priori assessment of potential confounding factors in a model. Directed acyclic graphs (DAGs) can be helpful in order to assess the model build (258). A DAG is a systematic way of showing assumptions about relationships between variables. The term *directed* indicates that the arrowheads in the DAG point in one direction; they cannot be bidirectional or without direction. The term *acyclic* indicates that there are no feedback loops in the system; a variable cannot point back to itself. The DAG diagram (*Figure 5.4*) shows the assumed causal effect of the exposure on the outcome, as well as the effect of a confounding factor on both the exposure and the outcome. The concept of confounders will be discussed in more detail under the *Discussion* section.

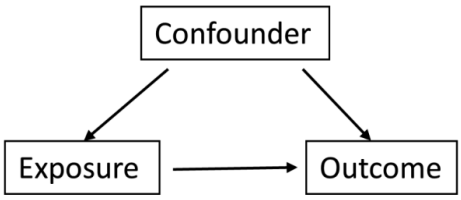

Figure 5.4 *Simple DAG showing the relationship between an exposure, and outcome and a confounder. The confounder is related to both the exposure and the outcome but is not an effect of the exposure.*

5.4.5.Cluster analysis using self-organizing maps

Cluster analysis for paper III was performed using Viscovery SOMine by Viscovery Software GmbH (www.viscovery.net; Vienna, Austria). Self-organizing maps (SOMs), also called Kohonen maps, were used to create an ordered representation of the comorbidity data. The SOM method, a form of an unsupervised neural network, is based on non-parametric regression techniques. It produces a two-dimensional representation of an input vector, called a map, by competitive learning in order to achieve dimensionality reduction of complex input data.

Machine learning is a branch of artificial intelligence, where machines are trained to learn from data and identify patterns. The types of learning schedules are subdivided into supervised and unsupervised learning, and the learning process may be deep or shallow learning. Supervised learning happens when the outcome is known; e.g. recognition of handwriting, objects etc. Within the medical field, supervised learning is the method behind automated ECG interpretation and radiological software able to separate benign and malignant nodules (259).

Clustering is a form of unsupervised learning. In unsupervised learning, the computer tries to identify patterns or groupings within the data. As a contrast to supervised learning, there are no outcomes to predict. This is useful when trying to make sense of heterogeneous diseases like COPD – complex data can be fed into a machine learning software, and hidden patterns or clusters can be detected. This could aid identification of distinct phenotypes as well as novel therapeutic targets. Clustering could be used on all types of data, including genomics and clinical data (260).

5.4.5.1. The SOM process

Self-organizing maps are a type of unsupervised learning, using so-called nodes, or neurons (processing units) on an adjustable map; termed a self-organizing map. In this lattice of neurons, a neighborhood concept is utilized to develop the map during a process of training. The goal of this training process is to find weight values so that neighboring units have similar values, i.e. to adapt the lattice as good as possible to the underlying data. The neurons are assigned weights, and each input vector is examined for similarity to the weight of the node. The winning node is commonly known as the Best Matching Unit (BMU). Initially, each node is treated as an individual cluster.

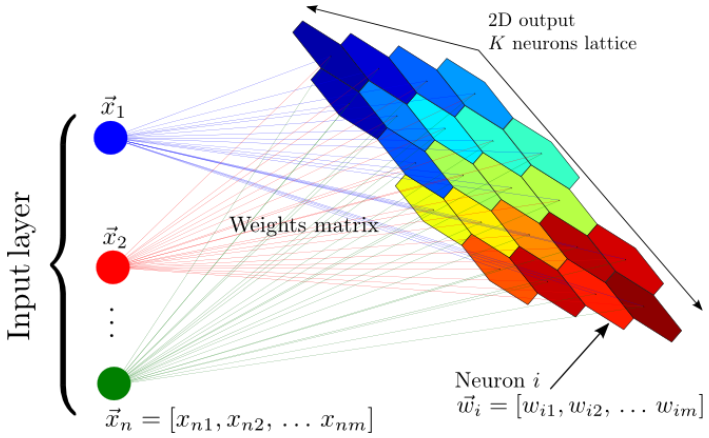

Figure 5.5 *An overview of a SOM. The input layer to the left contains the input data x. Weights are given to the input vectors, resulting in a lattice of neurons presenting as a two-dimensional map (261).*

In other terms, given input x, the computer finds the i^{th} unit with the closest weight vector by competition. The neurons are fixed, and the data are moving to adapt. For each unit *j* in the neighborhood N(i) of the winning neuron *i*, the weights of *j* (Wj) are updated. This is the process of training, learning to adjust the weights. Weights outside of N(i) are not updated (*Figure 5.5*).

The training process of a SOM has three stages. The first stage is competition, where all neurons compete. In the start of the training process, the SOM does not know the structure of the data. Therefore, random placement of the nodes based on the input data is performed. Euclidian distance is used to determine the best-fitting node. When the SOM has started to make some sense of the data, a second phase of collaboration is initiated. Here the concept of neighborhood comes into play. Gaussian functions are used to create clusters, creating a smooth transition from perfect weight, to less perfect weights, to weights outside of the neighborhood. At the start of the training process, the nodes share more weights with their neighbors (weight closer to 0 than 1) but get more competitive towards the end of the training, when the clusters are getting more defined. The vectors are hence a function of time. During the third phase, adjustment of the weights takes place. The training process stops when there is no noticeable change in weights (262).

Based on the resulting SOM model, clusters are generated using the SOM-Ward Cluster algorithm of Viscovery, a hybrid algorithm that applies the agglomerative hierarchical Ward's method (with a goal of least increase in total variance) on top of the SOM topology (*Figure 5.6*).

Summary variables on comorbidities and clinical characteristics for the study sample and for each cluster are presented as mean ± standard deviation for quantitative variables, and percentage for discrete variables. Variables with highly skewed distribution were sigmoid transformed to reduce the impact of outliers on the training process.

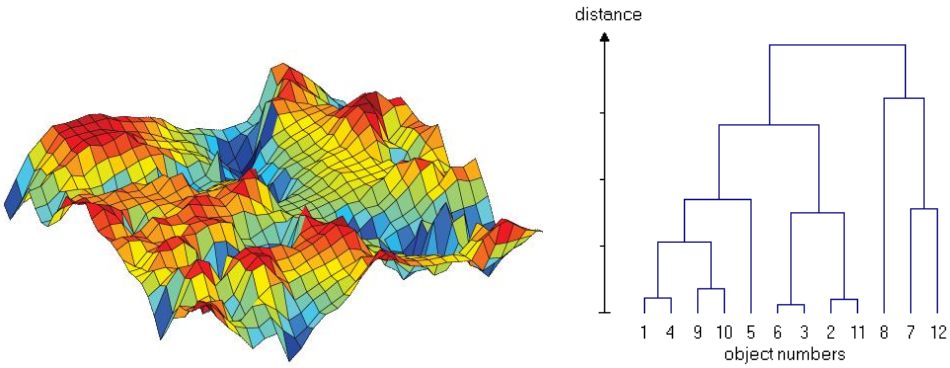

Figure 5.6 *Left: The topology of a SOM. Source: viscovery.net. Right: Hierarchical method of Ward.*

Clustering analysis using SOM technology allows intuitive graphical interpretation of complex data. However, the generated clusters are sensitive to the data input. Categorized data does not perform well with the SOM model. Due to this, we also generated continuous variables for all categorical input variables. Doing this, the SOM will integrate these two approaches. The map created by continuous input are harder to interpret, and thus the dichotomized attributes are often presented in papers. However, both maps will aid in interpretation of the clusters.

5.4.6.Restricted cubic spline regression

When the association between dependent and independent variables is non-linear, restricted cubic splines can be used to both test the non-linearity, and to summarize non-linear associations. Furthermore, it can be used to avoid categorization of data (e.g. by age groups, cut-off points for diagnosis). Categorization forces the relationship with the outcome in each category to be flat, resulting in a step function at the end of each category resulting in loss of information. Using splines this can be avoided, given that the categorical variable could be made continuous.

Restricted cubic splines are transformations of an independent variable, and can be used in any regression model, but requires a continuous independent variable. The range of values of the

independent variable is split up into piecewise polynomials, with knots defining the end of each polynomial. Separate regression curves are then fitted between the knots, and the splines works as statistical smoothers to ensure that the regression curve is continuous. To apply restricted cubic splines in a regression model, number and positions of knots as well as the degree of the polynomial must be decided. Five knots have been shown to be sufficient to uncover any likely pattern (263). However, if the sample size is small, three knots may be enough (264). If too many knots are fitted, there might not be enough observations to fit the polynomials. In our paper we used cubic splines (a polynomial of degree 3), requiring $k + 3$ coefficients. Since cubic splines tend to perform poorly at the tails, restricted cubic splines are used. These are constrained to be linear at the two tails, providing a better fit to the data, and reduces the degrees of freedom. Polynomials could be an alternative to achieve modeling of non-linear associations but are not flexible at the ends of each curve (264).

5.4.7.Missing data and multiple imputation

Missing data is an important issue in population-based studies. The risk of bias depends on the reason of missingness of the data. There are three main mechanisms causing missing data: missing completely at random (MCAR); missing at random (MAR); and missing not at random (MNAR) (*Figure 5.7*):

- MCAR: The mechanism causing missing data depends neither on observed nor missing data, and hence does not cause systematic error.
- MAR: The mechanism depends on the observed data. There is a systematic relationship between the missingness and the observed data, but not the missing data. This allows prediction of the missing values based on participants with complete data.
- MNAR: There is a relationship between the missing value and its reason for being missing.

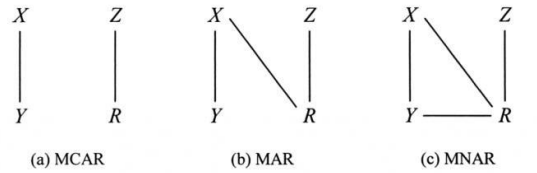

Figure 5.7 *Graphical representation of univariate missingness patterns: a) missing completely at random (MCAR), b) missing at random (MAR), and c) missing not at random (MNAR). X: variables that are completely observed. Y: variables that are partly missing. Z: the component of the causes of missingness unrelated to X and Y, R: missingness. Adapted from Schafer and Graham 2002 (265).*

If the mechanism of missingness is MCAR, no imputation is needed, as complete case analysis will be unbiased. If MAR, then multiple imputation or full information direct maximum likelihood may lead to unbiased results. The MAR assumption could be hard to assess, and sensitivity analyses would be needed to assess the impact as if the data were MNAR. If MNAR, multiple imputation would in itself be introducing bias, and should not be used. There are no definitive formal tests for missingness, but missingness pattern analysis using auxiliary variables could help in distinguishing MAR and MNAR. However, the most important thing is to use a priori knowledge about the data, the sample and the data collection process to uncover the mechanism of the missing data. After HUNT3, an extensive non-responder study was done (266), which could also tell us something about the missingness. Furthermore, each field of study may have well-known sources of missingness, e.g. reporting of cigarette smoking and alcohol consumption, respiratory symptoms in women (114) and depressive symptoms in men (267).

Multiple imputation (MI) is a statistical approach to account for the uncertainty generated by missing data. It can be viewed as an iterative form of stochastic imputation, where a residual term is drawn from a normal distribution with a mean of zero and variance equal to the residual variance of the regression model in question (268). But instead of filling in single missing values, the imputation procedure creates several (denoted *M*) different imputed datasets, runs the statistical procedure of interest on each dataset, and then pools the results for inference. The imputed values are sampled from their predictive distribution based on the observed, complete data, using a Bayesian approach (269). The missing data do not represent the true missing values, as the goal of the imputation procedure is to reflect the underlying variance/covariance matrix of the observed data. MI is considered necessary if some or all of the variables have >5% missing (270). However, the mechanism of missingness is important to consider

first, as discussed above, to avoid introducing bias due to the imputation procedure itself (269). In cases with missingness <5% for each variable, or a clear MCAR pattern, complete case (CC) analysis could be justified. In CC analysis, individuals with missing data on one or more variables are removed by pairwise deletion, leaving only those with full information on all variables in the analyses. Since the missingness is non-informative, CC analysis will not induce bias to the estimates.

5.5. Study design and sample

All three papers used the same study sample. Participants were recruited from their first participation in either HUNT2 or HUNT3 (*Figure 5.8*). For those participating in both surveys, HUNT2 was used as baseline. All participants aged 40-85 years with acceptable pre-bronchodilator spirometry were recruited to the study cohort. This age range was used because a previous study on the same cohort found increased prevalence of COPD in individuals <40 years (n=451), possibly due to skewed recruitment or few participants in this age group (53). Individuals >85 years were generally excluded from the spirometry measurements of the HUNT Lung Study, however, a few individuals above this age have participated (n=21). These were healthier than their peers, and to avoid survivorship bias they were excluded from the sample. Participants were identified as having COPD if they had 1) a z-score of $FEV_1/FVC < -1.64$ according to Global Lung Initiative (GLI-2012) (25); and 2) respiratory symptoms (chronic cough, dyspnea, wheezing) (35). In addition, GOLD criteria ($FEV_1/FVC < 0.70$) were used in paper I (15).

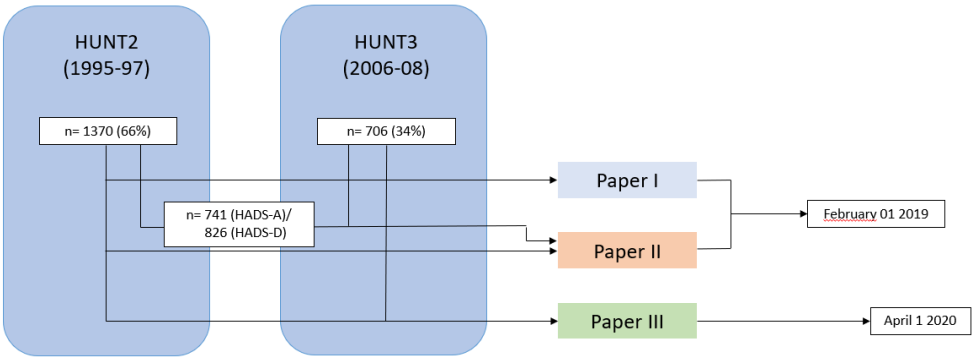

Figure 5.8 *Analysis cohort of papers I-III. In all papers the cohort was the same: individuals with COPD according to GLI between 40-85 years (n=2076). For paper II, those with repeated measurements in both HUNT2 and -3 were included in joint analyses. The follow-up time for paper III was 14 months longer than in the remaining papers.*

5.6. Statistical methods used in individual studies

5.6.1. Paper I

For the first paper we included participants identified as having COPD by both GOLD and GLI criteria in HUNT2 or -3 and did separate analyses with these criteria. The slightly larger sample achieved using GOLD criteria is caused by GOLD criteria identifying more individuals with COPD in the older age groups. BMD T-score was included in analyses as a continuous variable but was also categorized according to the WHO criteria into T-score > –1.0 (normal), –1.0 to –2.5 (osteopenia) and ≤ –2.5 (osteoporosis). We used multivariable Cox proportional hazards regression models to examine the association between T-score and all-cause mortality and adjusted for relevant confounders. In addition, we performed non-parametric regression using restricted cubic splines to assess the functional form of the age and sex adjusted relationship between BMD T-score and all-cause mortality. Missing values were completed using multiple imputation with chained models.

5.6.2. Paper II

For the second paper we included participants identified as having COPD by GLI criteria only. HADS subscores were analyzed as continuous scores, as well as dichotomized by a cut-off of 8. We chose to use a cut-off of 8, which has been shown to have good discriminatory effect between caseness and non-case (209), and also analyzed the HADS subscales on a continuous scale as well as with the use of restricted cubic splines to avoid loss of information. We used multivariable Cox proportional hazards regression models to examine the association between HADS score, both as separate scores and combinations of HADS subscores, and all-cause mortality. Models were adjusted for relevant confounders. Furthermore, we analyzed the impact of change in HADS score between HUNT2 and HUNT3 with mortality using joint models. The functional form between HADS subscores and all-cause mortality was visualized using restricted cubic spline regression.

5.6.3. Paper III

For the third paper we included participants identified as having COPD by GLI criteria only. We included data on ten objectively identified comorbidities and identified comorbidity clusters in the cohort using unsupervised cluster analysis. The association between these clusters and all-cause mortality was assessed using multivariable Cox regression, adjusted for relevant confounders. The association between clusters and the cumulative number of severe COPD exacerbations requiring hospitalization was assessed using multivariable Poisson regression models.

6. Results – review of papers I-III

6.1. Descriptive statistics

In GLI-COPD 1370 (66%) of the participants were recruited from HUNT2, and 706 (34%) from HUNT3. The corresponding numbers for GOLD-COPD were 54% and 46%, respectively. No cohort effect was found when adjusting for survey recruitment. GLI LLN criteria identified 2076 participants with COPD with a mean age of 62.1 years, contributing to 26 370 person-years of follow-up (median 12.7, maximum 23.4 years) (Paper I-II) and 27 443 person-years of follow-up (median 15.7, maximum 24.6 years) (Paper III). The severity distribution was as follows: 40.4% had mild, 34.8% moderate, 19.8% severe and 5.1% very severe airway obstruction. There were 44.8% current smokers in the cohort, and the mean cumulative tobacco exposure was 24.7 pack-years. 47.2% had low education (≤ 9 years). 943 individuals (45.4%) were women.

6.2. Results from paper I

We found a prevalence of osteoporosis of 15.7% in individuals with GLI-defined COPD. A higher proportion of females had osteopenia or osteoporosis compared to males. Participants with osteoporosis had lower BMI, were less physically active and had shorter education compared to individuals with COPD and normal BMD. There were more never-smokers among those with osteoporosis, but a larger proportion of participants with osteoporosis had severe or very severe airway obstruction.

Analyses using BMD T-score as a continuous exposure showed 5% increased mortality per one unit decrease in BMD T-score (HR 1.05, 95% CI 1.01-1.09) in GLI-COPD. Sensitivity analyses using HUNT2 post-BD spirometry values showed similar results as the main analysis. Using WHO criteria for osteoporosis (BMD T-score ≤–2.5), a sex- and age-adjusted model gave a HR of 1.23 (95% CI 1.01-1.50) for osteoporosis compared to normal T-score. Further adjustment for severity of airflow obstruction, BMI, smoking status and cumulative smoking exposure, self-reported PA, level of education and alcohol consumption, resulted in a statistical non-significant HR of 1.13 (95% CI 0.91-1.41) for osteoporosis, probably due to power issues. Smoking, COPD severity and age seemed to be the strongest confounders. Sex-stratified analyses showed the same direction and magnitude as the non-stratified models, and no interaction between sex and T-score was found upon a priori testing of the models.

6.3. Results from paper II

The prevalence of caseness of anxiety (HADS-A ≥8) was 16.2%, and caseness of depression (HADS-D ≥8) was 15.9%, hence present in around 1/6 subjects. Thirty percent of participants had either caseness of

anxiety or depression, and 10% had both caseness of anxiety and depression. More women had caseness of anxiety, and more men had caseness of depression. Compared to caseness of depression, those with caseness of anxiety were more physically active, had higher education, lower alcohol intake and higher mean ppFEV$_1$. More individuals with caseness of depression had cardiovascular diseases and musculoskeletal disorders.

We observed a positive association between HADS scores as a continuous measure and all-cause mortality. The age and sex adjusted risk of death increased by 3% (95% CI 1.01-1.05) and 4% (95% CI 1.02-1.06) per one unit increase in HADS-A and D, respectively. When adjusting for severity of airflow limitation, levels of education, PA and BMI, the association between caseness of anxiety and all-cause mortality disappeared (HR 1.01, 95% CI 0.98-1.03), while the association with caseness of depression was slightly attenuated (HR 1.03 95% CI 1.01-1.11). For HADS-A and -D dichotomized by ≥8, the corresponding results were 1.30 (95% CI 1.09-1.55) and 1.28 (95% CI 1.10-1.50) in baseline models, and 1.25 (95% CI 1.05-1.50), and 1.22 (95% CI 1.04-1.44) in fully adjusted models.

Having caseness of depression alone increased mortality with 31% (4-68%), and both caseness of anxiety and depression gave a 37% (7-75%) increase. Only caseness of anxiety was not significantly associated with mortality (HR 1.14 [95% CI 0.88-1.47]).

For participants with a repeated measures of HADS-A (n=741) and HADS-D (n=826), we found a reduced risk of mortality in those who went from HADS-A ≥8 in HUNT2 to <8 in HUNT3 (HR 0.97 [95% CI 0.94-1.00]), and the same reduction was found using HADS-D, although the estimate was less precise (HR 0.97 [95% CI 0.93-1.18]). A HADS score change from <8 to ≥8 was associated with an increase in mortality for HADS-A (HR 1.03 [95% CI 1.01-1.05]). A similar association was observed for HADS-D, but the estimate was less precise (HR 1.03 [95% CI 0.99-1.18]).

6.4. Results from paper III

In a cohort of individuals with mild to very severe COPD recruited from a general population, we identified five distinct clusters of objectively identified comorbidities: a less comorbidity cluster, a metabolic cluster, a cardiorenal cluster, a psychological and a cachectic cluster. The degree of airflow limitation was similar among the clusters, however, we found notable differences in long-term survival and the risk of severe COPD exacerbations, with the psychological and cachectic clusters significantly associated with poor outcomes. The less comorbidity cluster was used as reference. In adjusted analyses, only the psychological and cachectic clusters were significantly associated with mortality, with

a HR of 1.23 (95% CI 1.04-1.45) and HR 1.83 (95% CI 1.52-2.20), respectively. Adjusting for age and sex, the associations were attenuated, giving a HR 1.19 (95% CI 1.00-1.40) for the psychological cluster, and HR 1.42 (95% CI 1.17-1.71) for the cachectic cluster.

There was a significant variability in the cumulative number of COPD exacerbations between the clusters. The lowest mean frequency of severe exacerbations was found in the metabolic cluster (4.3±7.3), whereas the cachectic cluster had the highest mean (8.0±9.6). The pattern was the same as with all-cause mortality: the psychological and cachectic clusters had the highest risk of hospital admission for COPD exacerbation, with an unadjusted IRR of 1.24 (95% CI 1.04-1.48) and 1.50 (95% CI 1.23-1.83), correspondingly. The association between severe COPD exacerbations and the metabolic and cardiorenal clusters were non-significant. The associations remained unchanged when adjusting for sex and age.

7. Discussion

7.1. Summary of main findings

All three papers included in this thesis investigated the association between COPD and comorbidities with all-cause mortality. COPD exacerbations requiring hospitalization was included as an additional endpoint in paper III. We studied osteoporosis and its impact on long-term mortality (Paper I). Furthermore, we studied the association of symptoms of anxiety and depression, and longitudinal change in such symptoms, on long-term mortality using HADS subscores alone or combined with each other (Paper II). Finally, we investigated the impact of comorbidity clusters in COPD with long-term mortality and severe COPD exacerbations in paper III. Our main findings are briefly summarized as follows:

- We found a positive association between low BMD T-score, osteoporosis and all-cause mortality.
- A positive association between symptoms of anxiety and depression and all-cause mortality was seen. Furthermore, the mortality risk decreased if the symptoms diminished during the 11-year period between two study cycles.
- Using objectively identified comorbidities, we identified five distinct comorbidity clusters within our COPD cohort. Clusters dominated by symptoms of anxiety and depression (psychological cluster), as well as osteoporosis and underweight (cachectic cluster), were associated with increased all-cause mortality and higher risk of severe COPD exacerbations.

7.2. Methodological considerations

A cohort study, one of three main types of observational studies next to case-control and cross-sectional studies, is a longitudinal study design following a defined group of individuals for a given time. More specifically, it follows two groups, exposed and not exposed, and compare the incidence of a given outcome of interest after a certain time. Advantages with cohort studies include the possibility to examine multiple outcomes for an exposure, as well as calculating rates like incidence and relative risk. However, this type of study design is expensive, may require long follow-up (years to decades), and is susceptible to loss of follow-up and withdrawals (271).

The main goal of a longitudinal study design is to study temporal effects of certain exposure variables on a given outcome. Due to the potential biases introduced by this study design, it is important to ensure that effect estimates are valid and precise, with high accuracy. To reach this goal, attention needs to be given to the sources of two types of uncertainty: random and systematic error. *Systematic error* are errors threatening the validity of the study, whereas *random errors* are caused by statistical fluctuations affecting the degree of precision. Random errors will be any direction, whereas systematic error is consistently to one direction.

Validity and reliability are often illustrated using target models (*Figure 7.1*). In general, a method that is neither reliable nor valid will not measure what is intends to measure, and the spread of the results is wide. A method with validity but no reliability will measure what it intends to measure, but the results are still scattered. A method that is reliable but not valid will get the same measurements each time, but all of them are wrong. And finally, a good method with high reliability and validity will measure what it intends to measure each time.

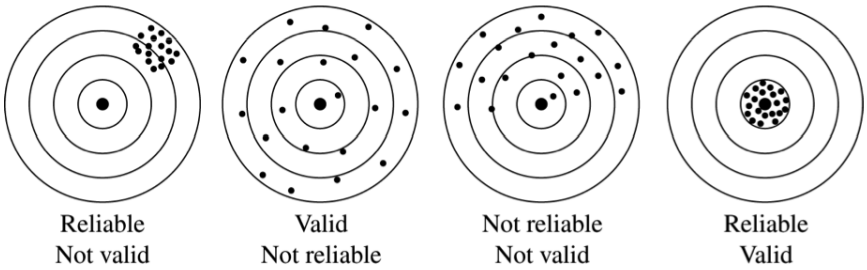

Figure 7.1 *Target models describing the difference between validity and reliability. Source: Encyclopedia of Occupational Health and Safety, 4th Ed.*

7.2.1. Precision/reliability (=lack of random error)

A measure is said to have a high reliability if it produces similar results under similar conditions, i.e. that it has good reproducibility or repeatability. The lack of reliability will increase the amount of random error. Random errors are often caused by precision limitation in instruments used to collect study data, and their impact could be reduced by increasing the sample size. Random errors are the uncertainty of the estimates remaining after elimination of systematic errors (272). The precision of a parameter is reflected in confidence intervals (CIs), and a 95% CI should be interpreted as, if a trial was repeated an infinite number of times, 95% of the results would fall within the limits of this range. The CI indicates how precise the obtain mean is as an estimate of the population it is meant to measure. A precise estimate has a small random error and narrow CI, reflecting small variance. A wide CI can be caused by a small sample, or large variance within the sample.

The potential sources of random error in the current study could arise from a variety of measurement methods used, most prominently the spirometry measurements. To decrease the risk of random error, strict quality criteria were applied to ensure the precision of the measurements. In spirometry the precision was determined by the magnitude of within-person variation of lung function. At least three similar measurements with a difference of no more than 150 mL between the best and worst FVC and FEV_1 must be performed in order to deem the measurement adequate (230). However, other factors like the quality of the curve must be evaluated before rejecting the test on basis of the repeatability criteria (230). A manual quality control of all spirometry curves was done in HUNT2 and -3, and some individuals were accepted on these terms, despite having only two acceptable trials. The precision of the measurements will also depend on the instruction and skills of the technician and the cooperation of the

study subject. Due to the extent of data collection of the HUNT Lung Studies, several technicians were involved in the measurements. This may also have introduced random errors. However, an inter- and intra-observer agreement test was performed in the Tromsø Study (which methods are comparable to the HUNT Study), showing excellent results (273).

The precision of DXA measurements is largely dependent of the technician and depends on the placement of the study subject on the DXA machine, as well as the placement of the measurement grid. When done correctly, precision of DXA measurements is high. Automated grid placement software was used in both HUNT2 and -3, and manual alteration and recalculation have been done, decreasing the risk of random error (274).

Test-retest reliability, or repeatability, is an important issue upon the use of instruments like HADS. This is a measure of the consistency of the instrument over time; in other words, if individuals score the same score when answering the test again, given that the circumstances or the condition have not changed. Correlation tests are used to assess the difference between the repeated tests scores, where a coefficient of stability indicates how reliable the test is. A coefficient of 1 indicates perfect reliability and a coefficient of 0 indicates no reliability. The internal consistency of the instrument, i.e. the correlation between items of a test as measured by Cronbach's alpha (α), is also used to measure reliability. In a Norwegian review of 19 studies including HUNT, Cronbach's α was found to be consistently high of ≥0.70 (275). In a separate study of 92.100 individuals in HUNT2, α was found to be 0.80 (good) for HADS-A and 0.76 for HADS-D (acceptable) (276).

7.2.2. Validity (=lack of systematic error)

A method or technique has high validity if it measures what it is thought to measure. Validity could be divided into *internal validity*: whether the results of a study is representative to the group of people being studied, and *external validity:* whether the results of a study is generalizable to other groups of people.

7.2.2.1. Internal validity

Systematic errors, or bias, happens when there is a systematic difference between what the study intends to measure, and what it measures. There are three main types of bias: selection bias, information bias and confounding, all of which are discussed below.

7.2.2.1.1. Selection bias

Selection bias are systematic errors that can stem from two sources: either the procedure used to select subjects, or from factors influencing study participation. It arises when the association between exposure and outcome differs between responders and non-responders.

In the HUNT Study, all inhabitants in the county of Nord-Trøndelag were invited. Thus, the selection method does not induce bias. However, non-participation is an issue also in large population-based studies. A non-responder study including questionnaires answered by nearly 7.000 individuals and extracted data for 27.000 persons from general practice records after HUNT3 found that nonparticipants had lower socioeconomic status, higher mortality and higher prevalence of several chronic diseases including COPD, compared to those who attended (266). This could influence the prevalence of COPD and other conditions found in our study, as the most severely ill individuals did not attend. For the biological associations with the outcomes, however, attendance should not introduce large bias (272).

To a certain degree, self-selection bias is an issue in the HUNT Lung Study. Some individuals with severe COPD, being selected in the symptom sample, chose not to perform spirometry on the field station due to dyspnea or other symptoms, or the perception of spirometry as an exhaustive maneuver (A. Langhammer, personal communication). However, the proportion of COPD severity grades resembled other large population-based studies (53), either indicating that self-selection would not induce large bias, or that the same bias is found in other population-based studies.

7.2.2.1.2. Information bias

Information bias are systematic errors that occur when the collected information about the subjects are wrong, often referred to as misclassified, when measured on a categorical scale. Such misclassification can be *differential* (related to the value of other study variables) and *non-differential* (not related to the value of other study variables). Both exposure and outcome variables can be misclassified.

7.2.2.1.2.1. Misclassification of exposure

According to Rothman, non-differential misclassification of the exposure occurs when the misclassification is unrelated to the occurrence or presence of the outcome (272). If it is related to the outcome, it is differential (277). Hence, non-differential misclassification occurs when all subjects have the same probability of being misclassified. Correspondingly, differential misclassification occurs when

subjects do not have the same probability of being misclassified. Misclassification can both over- and underestimate the real effect (regression away from/ towards the mean).

To avoid misclassification of COPD, strict criteria for diagnosis were used. Furthermore, the use of appropriate reference values is important to avoid diagnostic misclassification. In our study, GLI-2012 reference equations are used, which are found to fit the Norwegian population well (23).

The use of adequate reference values is equally important in DXA measurements. However, the use of a female reference population in men might underestimate the prevalence of osteopenia and osteoporosis in men. Although the International Society for Clinical Densitometry (ISCD) recommends use of the distal forearm measurement site for diagnosis if hip and PA spine BMD is unavailable (236), *r* values between 0.5 and 0.6 between central and distal measurement sites indicate that BMD at one site cannot be used to predict BMD at another site. This is due to site differences regarding accuracy errors, variance in the population and algorithms for edge detections (164). Generally, forearm BMD measurements are less predictive than spine BMD measurements for spine fracture risk, however this tendency is reduced with age (164). Some studies show that the use of forearm densitometry tends to overestimate the prevalence of osteoporosis compared to central densitometry measurements (278). This is reflected in the crude prevalence rate of osteoporosis in HUNT3, comparing forearm and total hip densitometry measurements in the same individuals. In 7951 individuals where both central and distal DXA were performed at the same occasion, 290 (3.7%) were identified as having osteoporosis using T-score derived from total hip, compared to 575 (7.2%) using forearm T-score (own analyses, unpublished). Other studies suggest that this is caused by the smaller scatter of forearm BMD compared to spine and hip making the forearm densitometry a more precise measurement site for diagnosis (279).

Forearm BMD have been shown to be as good as lumbar spine BMD to predict hip fracture, however inferior to total hip (164). BMD at any site is strongly related to fracture risk, and BMD of femur, spine and forearm have similar value in predicting non-spine fractures (164). In general, BMD measurements can predict fracture risk, but cannot identify individuals who will have a fracture (164). The predictive ability of BMD for fracture risk is a good as blood pressure for stroke and serum cholesterol for cardiovascular disease (164). An increase in fracture risk of 1.5 for each 1 SD reduction in BMD will give an individual with a bone densitometry of - 3 SD below average for that given age a 1.5^3 increased risk compared to individuals with normal BMD.

Osteoporosis cannot be diagnosed in men under age 50 based on BMD alone, but the above diagnostic criteria may be applied to women in the menopausal transition. For younger individuals the use of Z-score <–2 is recommended for making the diagnosis (236). The number of men <50 years with osteoporosis in our sample was negligible; n=2 (0.5%). The mean age of female participants was 62.7 years (± 9.8 SD), and the mean age of menopause in the same sample was 47.4 years (±5.8 SD). Based on this we viewed T-scores as being enough for a valid diagnosis of osteoporosis. Since we do not take vertebral fractures into account, using spinal DXA measurements only may misclassify elderly individuals as not having osteoporosis (280), underestimating the prevalence and attenuating the association with mortality.

Studies comparing HADS to structured diagnostic interviews for anxiety and depression disorders have shown good validity and case-finding abilities (240). Using a cut-off score ≥8, the risk of misclassification should be low (240).

Furthermore, the use of questionnaires to collect data could induce errors. In both HUNT2 and -3, the question: "Have you had, or do you have any of the following diseases: Chronic bronchitis, emphysema or COPD?" was included. Around 700 of individuals answering yes to having had chronic bronchitis confused this diagnosis with only having had recurrent lower respiratory tract infections as a child. This question had low validity, resulting in a rather high number of persons not fulfilling COPD criteria, even though they were invited to the symptom sample. Due to this, the question in HUNT4 was changed to "Have you, or have you had, COPD or emphysema?". However, the use of LLN criteria as well as reported respiratory symptoms for the diagnosis of COPD would minimize this misclassification error. *Recall bias*, the problem of recalling information, is a common problem in studies based on self-report, especially when there is a long time period between the event of interest and the study. Age, socioeconomic status and perceived importance of the information could also influence recall (281). Data on smoking or alcohol use are vulnerable to recall bias and tend to be underreported, but also previous diseases, medication and respiratory symptoms, among others, could be influenced by such recall problems.

Socioeconomic status (SES) is also prone to non-differential misclassification. Based on the available data, we used self-reported level of education from HUNT2, and self-reported occupation from HUNT3. To get a complete picture of an individual's SES, both level of education, occupation and income for the individual and the household should be taken into consideration (106). Generally, women, especially elderly individuals participating in HUNT2, may have been misclassified as having lower SES. This is

partly due to the concept of “borrowed” status, where the spousal income could result in high household SES despite the wife’s low education. Furthermore, more potentially high-income jobs prevalent in Nord-Trøndelag, including farming and fishing, requires low education. Thus, individuals from HUNT2 could be misclassified due to the use of educational level as a basis for SES. Originally, we planned to link our data file to Statistics Norway data on the abovementioned variables, but due to a cumbersome, lengthy and costly application process, this had to be dropped. SES was only used as a covariate in extended models, and the size of the potential bias would be small. If we assume that low SES will increase mortality in our sample, the bias would overestimate the association between exposure and outcome, making it a more serious threat to our results. However, in extended analyses where SES was included, the estimates were significantly reduced compared to baseline models, upon which we concluded that this misclassification would have minor effects on our estimates.

Finally, self-reported physical activity, smoking and alcohol use are frequently over- and underreported, increasing the risk of differential misclassification. The risk of misclassification of smoking could be especially high in COPD cohorts, since underreporting of smoking is found to be more common in individuals who are advised to quit due to disease, in studies of serum cotinine levels (a metabolite of nicotine) (282). There is a risk of differential misclassification of smoking, since individuals with severe COPD and a high cumulative tobacco exposure could be more inclined to report lower levels of smoking. However, in general when conducting prospective cohort studies, the misclassification tends to be non-differential (i.e. less severe), since the outcome of interest has not yet occurred.

7.2.2.1.2.2. Misclassification of outcome

In our study the main outcome is all-cause mortality. All-cause mortality is a robust endpoint, and due to the Norwegian system of personal identification numbers for all citizens, all deaths occurring nationwide are registered. The risk of misclassification is small upon the use of this endpoint. A few participants (n=5) were registered as emigrated, but these were correctly handled in the Cox regression models and should not induce misclassification bias.

Another endpoint used in Paper III was hospital admissions due to COPD exacerbations. Here, the risk of misclassification is higher. This endpoint depends on the registered ICD codes in the hospital system where they were admitted. COPD exacerbations may be underdiagnosed in individuals with atypical symptoms, undiagnosed COPD or concurrent heart disease. Heart failure and COPD exacerbations have overlapping symptoms and are often misdiagnosed (283, 284). Differential misclassification of this

outcome may occur if the presence of e.g. concurrent chronic heart disease results in a missing or non-valid diagnosis of COPD exacerbation.

7.2.2.1.3. Confounding

A confounder is a variable that is related both to the exposure and the outcome, but not an effect of the exposure (i.e. it is not an intermediate step in the causal pathway). To adjust for confounding, statistical methods including stratification, adjustment in regression models or propensity scores could be used. Stratification does not allow for direct comparison of estimates from different models due to different reference groups, whereas adjustment in regression models does.

Confounding by indication happens when the exposure is linked to or is a risk factor for the outcome of interest (285). Confounding by severity is by some considered a subgroup of confounding by indication, where not only the disease, but also its severity, is a confounder (286). E.g.: the use of hormonal replacement therapy was in HUNT2 associated with increased risk of osteoporosis; as people with osteoporosis at that time were given this as treatment. To avoid residual confounding adjustment should be performed both for the disease itself and its severity, as this may influence the associations. In COPD, the mortality of mild and severe disease differs substantially (251). If adjustment for disease severity is not done, the associations with e.g. mortality may have been overestimated.

Residual confounding is the remaining bias that is left after controlling for confounding in the analyses. Three main causes of residual confounding can be discerned: 1) Confounders were not considered, or not adjusted for due to lack of data (unmeasured confounders); 2) classification of variables were too coarse (e.g. age groups instead of continuous age); 3) Measurement error of explanatory variables in the model. In our study, all the suggested mechanisms could be present. As an example, the correlation between self-reported and objectively measured physical activity (PA), an important confounder of the association between osteoporosis and risk of death, was found to be poor in some studies (287). One study comparing self-reported and objectively measured sitting time found that daily sitting time was underestimated with as much as 2.4 hours (288). Other studies found a good agreement between self-report and objectively measured PA (289). As objectively measured PA was not available in our cohort, some residual confounding could be present here. Furthermore, to avoid loss of information by classification, we did spline analysis of exposure variables in both paper I and II and included covariates as continuous variables if it could be meaningfully interpreted. However, the need to reduce residual confounding must be balanced with the risk of overfitting the models, thereby diluting the real effects.

Model misspecification covers a range of modeling errors, and include measurement errors, unnecessary categorization of continuous variables and failure to include important covariates in the model (290). This could cause biased estimates in any direction, depending on the nature of the misspecification.

7.2.2.2. External validity/generalizability

The county of northern Trøndelag (Nord-Trøndelag until 01.01.2019) is considered representative of Norway, expect for the lack of large cities, low levels of ambient pollution, lower levels of education and income, and lower prevalence of active smokers (227). These factors could influence the prevalence of both COPD and respiratory symptoms. However, the external validity of the associations between exposure and outcome should be unaffected. Further, 97% of the residents of former Nord-Trøndelag are of Caucasian origin (34). The results from paper I-III are consistent with findings in previous studies and are viewed to have international generalizability to people of the same ethnicity and culture.

7.2.3. Missing data

The main consequences of missing data may be reduced precision and power problems as well as residual confounding, giving wider confidence intervals, and potential introduction of bias in the obtained estimates (291). However, the amount of bias is dependent on the mechanism of missingness.

In paper I, the proportion having missing data for BMD measurements were relatively high, with 8.6% missing in the GLI cohort and 16.6% in the GOLD cohort. The mechanism of missingness was thought to be partly MCAR due to malfunctioning of DTX-100 during HUNT3, and logistic challenges including long waiting lines at the field stations during HUNT2 and -3. On the other hand, missing data on smoking and alcohol consumption could have MNAR patterns: less willingness to answer such questions due to overconsumption, or non-drinkers regarding these questions as irrelevant. However, a missingness pattern analysis showed that a possible MNAR pattern, e.g. missing data on all lifestyle factors, made up less than 1% of the total data. We therefore decided to use multiple imputation to complete the missing values. Imputation was done with five iterations. The imputation model including all potential confounders as well as the outcome variable. No auxiliary variables were included. Perfect predictions were avoided using an augmented-regression approach (292).

In paper II, the amount of missing data for HADS-A and HADS-D were 24.1 and 14.6%, respectively. The high amount of missing data was partly caused by participants not answering any of the HADS items or answered only some of the items (subscale dependent missingness (293)) Furthermore, HADS was a part

of Q1 in HUNT2 but Q2 in HUNT3, causing more missing in the latter survey. It is recommended to complete the missing items using multiple imputation (294), or to replace the missing items with the mean of the answered items in the subscale if at least 50% has been answered (293). However, the mechanism of missingness of the HADS variable is unclear. Individuals with psychiatric symptoms could be more inclined to underreport their symptoms, leaving the HADS questionnaire blank. On the other hand, individuals with no psychiatric symptoms could deem these items irrelevant to them and leave them out. In our study, we found that participants with missing data on HADS were older, had lower levels of education, less cumulative smoking exposure but more airflow obstruction compared to those with non-missing HADS. Furthermore, non-responders of HADS had slightly higher mortality compared to responders, even when adjusting for age. This was in line with findings of the nonparticipation study after HUNT3. Even though a diagnosis of anxiety or depression given by a general practitioner is not directly comparable with the HADS score, non-responders in HUNT3 had higher disease burden and mortality compared to the background population. Furthermore, depression, to a higher degree than anxiety, was found to be a restricting factor for participation (266). Due to these factors, we could not exclude the potential for MNAR, and hence complete case analysis was performed. This would probably induce bias, underestimating the relationship between HADS score and mortality due to leaving out the more severely ill participants.

For paper III, 1373 (66%) had missing data on at least one comorbidity. In cluster analysis with SOMine, missing data are handled using information from complete cases to inform the learning process. Due to this, missing data do not induce bias like it would do with other types of methods like regression models. However, this requires missingness to be non-informative (not MNAR). Assessing the missingness pattern of the comorbidities using auxiliary variables including age, sex, self-reported health, smoking exposure and socioeconomic status, we deemed the missingness pattern to be at least MAR.

7.3. Other strengths and limitations of our study

Our study had a large sample with long follow-up time. It represents a stable, homogenous population, and individuals with COPD are recruited from a general population. The HUNT study has relatively high participation rates, and the rate of participation was highest for the age group included in our cohort. Furthermore, objectively measured comorbidities using gold standards or validated instruments were used for all analyses.

Post-bronchodilator spirometry was only measured in participants from large municipalities in HUNT2. Several covariates were included in our analyses, and to obtain a sufficient sample size, pre-bronchodilator spirometry was used. Theoretically, this could overestimate the prevalence of COPD in our sample. However, results from sensitivity analyses using only post-BD measurements did not show large deviation from pre-BD results.

We found diverging results using HADS subscores alone or in combination. This discrepancy was probably caused by the difference in reference groups in the analyses, and illustrated the symptom overlap of anxiety and depression, as well as the high correlation between the HADS-A and -D subscores. The bifactorial structure of HADS measures a general factor of negative affect common for both anxiety and depression, as well as two orthogonal group factors accounting for specific anxiety and depression variance (295). Item response theory (IRT) analysis have shown problems with the item structure, with anxiety items loading on depression factors, and depression factors loading on the general distress factor, suggesting that HADS fail to discriminate between anxiety and depression (296). A tripartite model dividing the symptoms of anxiety and depression into three groups (negative affectivity, anhedonic depression and physiological hyperarousal) has been suggested (297, 298), and more recently a unidimensional structure has been proposed (296). More recent systematic reviews suggest that HADS is a better measure of general psychological or emotional distress (239, 295). However, since most of the alternative models are generated using confirmatory factor analysis, the proposed structures were not generalizable to our cohort (299). Although the factor structure of HADS is debated, its validity and reliability in the general population and COPD samples are shown to be satisfactory (239, 240). Snaith et al suggested three categories for HADS scoring (241), but various cut-off levels have been reported from different study populations.

The term anxiety disorder is an umbrella term that includes both generalized anxiety disorder (GAD) and panic disorder (PD). The items of HADS-A cover symptoms of both disorders. Patients with PD have higher levels of negative cognitions than those not experiencing panic attacks (300), and a previous study have shown that COPD disease severity is not associated with levels of anxiety, indicating that anxiety found in COPD patients rather reflects negative cognitive pathways (300). To disentangle these effects, analyses assessing the autonomic anxiety construct of HADS-A (e.g. items: 'I get a sort of frightened feeling as if something awful is about to happen'; 'I get a sort of frightened feeling like 'butterflies' in the stomach'; and 'I get sudden feelings of panic') separately should be performed, using factor or item response theory analysis, but this was beyond the scope of our study.

8. Conclusions and implication for future research

The focus of this thesis has been the impact of comorbidities, both separate and combined, on important longitudinal outcomes in COPD. Our overall finding is that the presence of comorbidities increases mortality in COPD. We found minor increased mortality by osteoporosis and anxiety/depression in COPD patients. If true, this is encouraging for this group of patients, but does not rule out larger effect on disease burden and health status.

8.1. Conclusions of paper I-III

Individuals with COPD are at increased risk of osteoporosis due to shared risk factors and disease compounding. We found a small positive association between decreasing BMD T-score and mortality in COPD defined by both GLI and GOLD criteria. However, osteoporosis as defined as T-score ≤–2.5 was not associated with mortality in fully adjusted models, probably due to power problems. The reason behind this mortality increase was not explored in this study but may be caused by negative mechanical effects on the lungs due to vertebral compression fractures, increased mortality after surgery for osteoporotic fractures, associated general tissue loss and low BMI, or other unexplored factors.

Comorbid symptoms of anxiety or depression were associated with a minor increase in mortality per unit increase in HADS-A and -D. Similarly, caseness of anxiety as well as caseness of depression based on validated and accepted thresholds showed significantly increased mortality. When comparing combinations of HADS subscores, the risk of mortality was more pronounced for depression. Further, there appeared to be an association of change in HADS-A or –D scores between the two surveys with mortality. No causative conclusions can be made based on our study, but lower treatment adherence, less self-care or dysregulation of biological stress pathways have been pointed out as possible reasons for the increased mortality in individuals with symptoms of general distress.

Five distinct comorbidity clusters could be identified in our cohort: less comorbidity, metabolic, cardiorenal, psychological and cachectic clusters. The two latter were associated with increased mortality and risk of severe COPD exacerbations. Furthermore, the participant's characteristics like symptom burden, exposure profile and airflow obstruction varied significantly between clusters.

8.2. General conclusions

COPD is a complex, heterogenous and multi-component condition. Comorbidities, here defined as concurrent diseases coexisting with COPD, seem to increase the risk of death and hospitalizations, both separately and in combination. However, the effects of single comorbidities on mortality, including osteoporosis and symptoms of anxiety and depression, were small. This is probably because the effects of comorbidities work in concert, with a summarizing effect of more comorbidities. Hence, the focus should be shifted towards the concept of multimorbidity, i.e. two or more coexisting diseases, in the management of COPD and its comorbidities. We saw a high prevalence of concurrent diseases in our cohort, with as much as 49% being defined as multimorbid when assessing only ten coexisting conditions. The development of comorbidity in COPD is a complex interplay between genetic and epigenetic effects, aging, shared risk factors, and probably also unidentified pathomechanisms that needs to be further explored. Our findings underline the importance of addressing comorbid conditions in COPD, as it may have substantial implications on outcomes of this patient group.

8.3. Future perspectives

The concept of multimorbidity has gained increasing attention the last year. Is seems evident that individuals not only follow a trajectory of lung disease, but also a trajectory of multimorbidity. To be able to achieve early detection of individuals with potential poor outcomes in COPD, it would be of interest to investigate such trajectories in the HUNT cohort. This cohort gives a unique opportunity to explore the effects of genetics, epigenetics, exposures and pheno- and endotypes on such trajectories. Extensive opportunities for linkage to external registries further expands the possibilities. Furthermore, it would be of interest to explore the different comorbidity clusters. What is the role of BMI, body composition, fat distribution and circulating adipokines in the development of COPD? Why does high BMI seem to be protective against mortality (as in the metabolic cluster), while low BMI and tissue loss increase the risk (as in the cachectic cluster)? Patient-related outcomes like quality of life, symptom burden and use of health care services would also be interesting endpoints to explore in relation to the comorbidity clusters.

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Papers I-III

Paper I

Paper I

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The Association of Bone Mineral Density with Mortality in a COPD Cohort. The HUNT Study, Norway

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ABSTRACT

In individuals with chronic obstructive pulmonary disease (COPD), the presence of comorbidities is associated with increased mortality risk. We wanted to study the association between bone mineral density (BMD) and mortality among individuals with COPD in a population-based cohort study. Participants were recruited from the second (1995–1997) and third (2006–2008) surveys of the HUNT Study and followed until February 2019. Hip and forearm BMD were included as continuous *T*-scores or categorized according to WHO criteria (normal, osteopenia, and osteoporosis). Hazard ratios with 95% confidence intervals were estimated by multivariable Cox regression models. In total, 2076 and 3239 participants were identified as having COPD by FEV_1/FVC below lower limit of normal (LLN) or <0.70, respectively, according to Global Lung Initiative (GLI) and Global Initiative for Chronic Obstructive Lung Disease (GOLD). The prevalence of osteoporosis was 15.7% vs. 16.6% in the GLI-COPD vs. GOLD-COPD cohorts. Mean follow-up was 12.7 and 11.9 years. Lower *T*-scores were associated with a 5% (95% [Cc]onfidence [Ii]nterval (CI) 1.01–1.09) and 4% (95% CI 1.00–1.08) increased mortality in the GLI-COPD and GOLD-COPD cohorts, respectively. However, the presence of osteoporosis ($T < -2.5$), compared to normal BMD, was not associated with mortality in neither GLI-COPD (HR 1.13, 95% CI 0.91–1.41) nor GOLD-COPD cohorts (HR 1.22, 95% CI 0.99–1.51). Thus, a small positive association was found between decreasing BMD *T*-score and mortality in both GLI-COPD and GOLD-COPD. However, osteoporosis as defined by WHO was not associated with mortality, probably due to loss of power upon categorization.

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Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by progressive airflow obstruction and contributes to morbidity and mortality for individuals, and high costs for the society [1]. Increasing life span, lifestyle and environmental factors have contributed to increasing incidence and prevalence of COPD worldwide, with an estimated global prevalence of 11.7% in adults >30 years in 2010 [2] and a prevalence of 14.8% in Norwegians >40 years in 2006–2008 [3].

Comorbidities contribute to symptom burden and prognosis in persons with COPD [4]. A common comorbidity is osteoporosis [5], which is characterized by reduced bone strength and increased fracture risk. Due to methodological differences, the worldwide prevalence of osteoporosis is difficult to determine, but the overall prevalence in the United States among adults 50 years and older was 10.3% in 2010 [6]. Individuals with COPD have a 1.5–2-fold increased risk of osteoporosis [7], and a recent meta-analysis found a pooled global prevalence of osteoporosis in COPD to be 38% (95% CI, 34–43%) [8]. Shared risk factors and an interplay between compounding elements of the two diseases may partly explain the high prevalence [9]. Osteoporosis is an important risk factor for fractures, but the fracture risk in osteoporosis is increased beyond measured bone mineral density (BMD) due to reduced bone quality and altered bone metabolism [7]. Vertebral fractures add a restrictive component to COPD [10], and hip fractures increase the risk for subsequent fractures and mortality [7]. In women, but not in men, low BMD was found to account for a large proportion of post fracture mortality risk [11]. Few studies have addressed potential associations between BMD and all-

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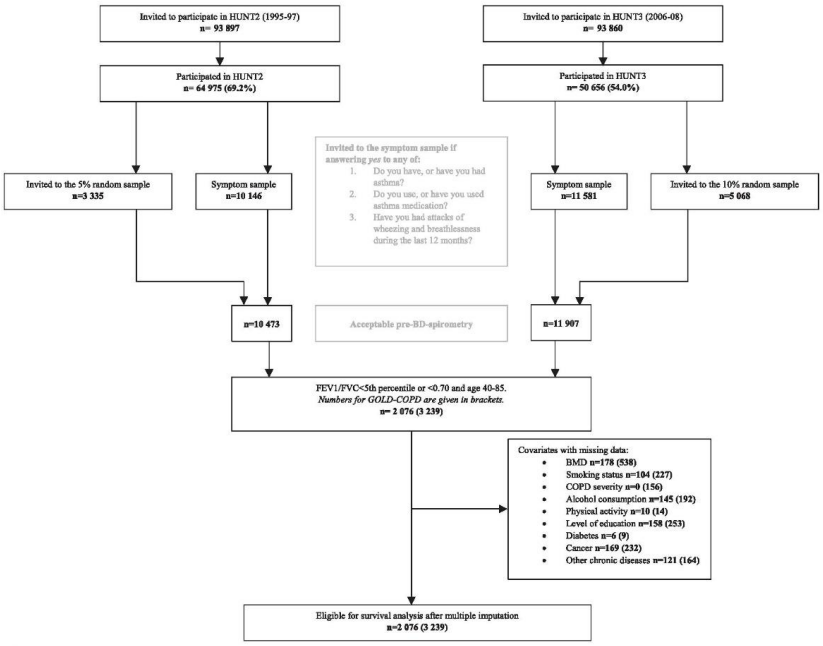

Figure 1. Flow chart – recruitment of the HUNT COPD cohort (follow-up until Feb. 01. 2019).

cause mortality in COPD, but an association between BMD and COPD-specific mortality has been reported by Campos-Obando et al. and Looker [12, 13]. Thus, we aimed to examine the association of BMD and osteoporosis with all-cause mortality in individuals with COPD recruited in a population-based study.

Materials and methods

Design

This cohort study includes participants with COPD from the second (HUNT2, 1995–1997) and third survey (HUNT3, 2006–2008) of the population-based HUNT Study in Norway. Comprehensive data on lifestyle and diseases were collected through questionnaires, interviews and clinical measurement [14].

Study population

Among 78,962 individuals participating in HUNT2 and/or HUNT3 (69.2% and 54.0% of those invited, respectively), random samples of all participants (5% in HUNT2 and 10% in HUNT3), in addition to participants reporting attacks of wheezing or breathlessness during the last 12 months, use of "asthma" medication or ever having had asthma, COPD, emphysema or chronic bronchitis (symptom sample), were invited to the HUNT Lung Study (Figure 1) [14]. In total, 7282 and 8812 individuals aged 40–85 years had spirometry measured in HUNT2 and HUNT3, respectively. A subset of 1387 participants also performed post-bronchodilator (post-BD) spirometry in HUNT2 20–30 min after inhalation of 1 mg terbutaline. Among those reporting respiratory symptoms 2076 participants had forced expiratory volume in one second divided by forced vital capacity (FEV_1/FVC) < fifth percentile ($z < -1.645$) estimated by the Global Lung Function Initiative (GLI) software (GLI-COPD) [15], and 3239 participants had FEV1/FVC < 0.70 (GOLD-COPD) [4]. Spirometry was performed according to the 1994 ATS recommendations in HUNT2 [16] and the 2005 ATS-ERS recommendations in HUNT3 [17], and quality control of measurements were performed [18, 19]. We defined baseline as the earliest participation date in either HUNT2 or HUNT3. Data on covariates were assessed at the time of participation. For those participating in both HUNT2 and HUNT3, only data from HUNT2 were used.

Osteoporosis

In HUNT3 bone densitometry of the total hip was performed by dual-energy X-ray absorptiometry (DXA) (Lunar

Prodigy GE Healthcare, Little Chalfont, UK). BMD in g/cm^2 was also measured at the distal part of the non-dominant forearm by DTX100 or DTX200 (Osteometer MediTech, Inc., Hawthorne, CA) in both HUNT2 and HUNT3. The distal forearm region was defined as the area 24 mm proximal to the site of 8 mm distance between distal radius and ulna. Methods of calibration and quality control are described elsewhere [20].

In both sexes, BMD was standardized as *T*-scores, calculated as observed minus mean BMD divided by standard deviation (SD) from a healthy female reference population aged 20–39 years from the HUNT Study [21]. BMD *T*-score was included in analyses as a continuous variable, but was also categorized according to the WHO criteria into *T*-score $\geq$ −1.0 (normal), −1.0 to −2.5 (osteopenia) and $\leq$ −2.5 (osteoporosis) [21]. In this study we primarily used total hip DXA measurements, but without a measurement at this site, forearm BMD was used as suggested by The International Society for Clinical Densitometry (ISCD) [22].

All-cause mortality

Data on registry status including mortality and migration, was provided by the National Registry. We defined time at risk as from the date of entry into the study until date of death or end of observation (February 1, 2019), whichever came first. There was no loss to follow-up.

Confounders

Based on prior knowledge, potential confounders were identified using directed acyclic graphs (DAGs). This included age at participation, sex, COPD severity, BMI, tobacco smoking, alcohol consumption, physical activity, socioeconomic status and comorbidities including ever cancer, chronic musculoskeletal diseases, chronic diseases affecting activities of daily living (ADL), diabetes mellitus and cardiovascular disorders. Data on these covariates were collected at baseline, either by questionnaires or by clinical measurements. Severity of COPD by GLI criteria was graded using FEV1 in percent of predicted (ppFEV1) calculated by the GLI-2012 software, using cutoff values by *z*-scores of > -2 (mild), < -2.5 (moderate), < -3 (severe) and < -4 (very severe) [15, 23]. Severity of COPD by GOLD criteria was graded using; ppFEV$_1 \geq 80$ (GOLD1/mild), $80 >$ ppFEV1 ≥ 50 (GOLD2/moderate), $50 >$ ppFEV$_1 \geq 30$ (GOLD3/severe) or $30 >$ ppFEV$_1$ (GOLD4/very severe) [17]. BMI was calculated in kg/m^2 and categorized according to the WHO classification: <18.5 (underweight), 18.5–24.9 (normal range), 25.0–29.9 (overweight) and ≥30.0 (obese) [24]. Tobacco smoking was categorized as: never-, former- or current smoker. The latter two categories were subdivided into <10, 10–20 and >20 smoking pack-years. Alcohol consumption was categorized by weekly consumption: low (<14 alcohol units (U.A.) for men and <10 U.A. for women), intermediate (14–21 U.A. for men and 10–14 U.A. for women) and high intake (>21 U.A. for men and >14 U.A. for women). One U.A. was defined as 13 g of alcohol. Physical activity was categorized according to hours of self-reported physical activity per week: inactive (≤1 h light physical activity and no hard activity), low (>1 h light and <1 h hard), medium (1–2 h of hard regardless of light activity) and high (≥3 h of hard regardless of light activity). Level of education was used as a proxy measure for socioeconomic status and was categorized into low (≤9 years), medium (10–12 years) and high (≥13 years) based on the question: "What is your highest level of education?" from HUNT2. Level of education in HUNT3 was assessed using the occupational classification in HUNT according to the Erikson Goldthorpe Portocarero (EGP) social class scheme, based on the question "What kind of paid work do you do?" [25]. Self-reported cardiovascular disorders included previous myocardial infarction, angina pectoris and ischemic-/hemorrhagic brain infarction. We identified participants with hypertension as those answering yes to prior or current use of antihypertensive medication or having hypertension (defined as ≥140 and/or ≥90 mmHg) upon blood pressure measurements in HUNT2 or HUNT3. Chronic musculoskeletal diseases included rheumatoid arthritis, spondyloarthritis, osteoarthritis and "other long-term skeletal or muscular diseases". Chronic diseases affecting ADL was assessed by the following question: "Do you suffer from any long-term illness or injury of a physical or psychological nature that impairs your functioning in your everyday life?". This variable was included to assess the impact of unmeasured comorbidities that were not included separately in the model. All variables on comorbidities were dichotomized to yes/no and included as separate variables in the final models. The final models were: (1) Baseline model with BMD categorized according to *T*-score adjusted for age and sex; (2) Main model with BMD categorized according to *T*-score adjusted for all confounders at baseline and (3) Models in (1) and (2) using BMD as a continuous measure.

Statistical analysis

We presented baseline characteristics for categorical variables as proportions and for continuous variables as means with standard deviation using non-imputed data. All models were run separately for the GLI and GOLD cohorts. Proportional hazard assumptions were evaluated by estimation of Schoenfeld residuals and proportional hazards assumption tests. Survival functions were graphed using restricted cubic splines with three knots, showing change in hazard ratio (HR) as a function of BMD *T*-score (Figure 2). Number of knots were determined using Akaike's- and Bayesian information criterion. Statistical interactions between main exposure and confounders were tested, but no significant interactions were identified. Missing data on covariates were completed using multiple imputation with chained equations, $M = 20$. HRs and 95% confidence intervals (CI) were calculated comparing all-cause mortality in participants with osteopenia and osteoporosis to those with normal BMD. When including BMD *T*-score as a continuous variable, linearity assumptions of BMD were assessed plotting a Lowess smooth of the Martingale residuals. Furthermore, the fully adjusted models with and without

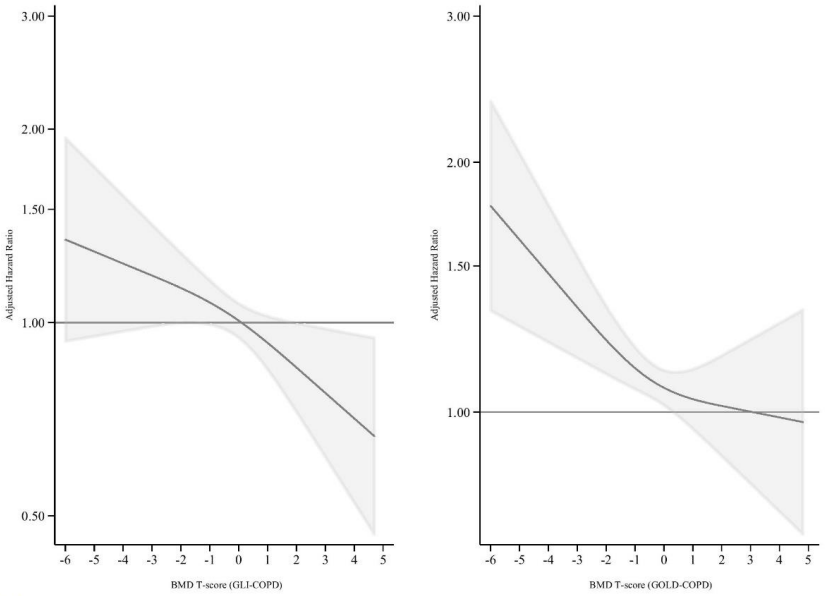

Figure 2. Age-adjusted HR with 95% CI for the association of BMD *T*-score with all-cause mortality in GLI-COPD and GOLD-COPD.

cubic spline terms were compared by Wald- and likelihood ratio tests. None of the tests showed deviation from linearity. In additional sensitivity analyses, cohorts were defined by post-BD spirometry GLI and GOLD cutoff values. Time of follow-up was used as time-scale, defining the starting point as the participation date in either HUNT2 or 3. To assess a potential cohort effect, participation date (HUNT2 or 3) was added in the models. Statistical analyses were performed using Stata 15.1 (StataCorp LCC, College Station, TX, USA).

Ethics

This study was approved by the Regional Committee for Medical and Health Research Ethics (REC) Central Norway (project no. 2014/1114-1). Participants in the HUNT Study have given informed, written consent for research on their data and linkage to specific registries.

Results

Descriptive characteristics

In GLI-COPD 66% of the participants were recruited from HUNT2, and 34% from HUNT3. The corresponding numbers for GOLD-COPD were 54% and 46%, respectively. No cohort effect was found when adjusting for survey recruitment.

GLI LLN criteria identified 2076 participants with COPD with a mean age of 62.1 years, contributing to 26,370 person-years of follow-up (median 12.7, maximum 23.4 years). The severity distribution was as follows: 40.4% had mild, 34.8% moderate, 19.8% severe and 5.1% very severe airway obstruction. In total, 64.2% had a normal BMD *T*-score, 20.1% had osteopenia and 15.7% had osteoporosis. A higher proportion of females had osteopenia or osteoporosis compared to males. Overall, 24.9% had no comorbidities, 45.6% had one comorbidity, and 29.5% had two or more comorbidities. The distribution of confounders and comorbidities resembled that of GOLD-COPD across *T*-score categories (Table 1). Totally, 3239 participants with a mean age of 64.4 years were included in GOLD-COPD, contributing to 38,406 person-years of follow-up (median 11.9, maximum 23.4 years). There were a slightly higher proportion of men in the sample (56.3%), while there were more women with osteopenia and osteoporosis. The distribution of severity was 30.6% in GOLD1, 52.8% in GOLD2, 14.3% in GOLD3 and 2.4% in GOLD4. With GOLD-COPD definitions there was a slight shift toward a higher prevalence of more severe airway obstruction compared to GLI-COPD. In GOLD-COPD 61.1% had a normal BMD *T*-score, 22.4% had osteopenia and 16.6% had osteoporosis. Overall, 21.1% had no comorbidities, 44.0% had one comorbidity, and 34.9% had two or more comorbidities.

Table 1. Descriptive characteristics of the HUNT COPD cohort 40–85 years defined by GLI[a] and GOLD[b].

	FEV1/FVC < GLI-LLN ($n = 2076$)				FEV1/FVC <0.70 ($n = 3239$)			
	Normal BMD *T*-score > 1.0) $n = 1209$	Osteopenia (*T*-score −1.0 to −2.5) $n = 383$	Osteoporosis (*T*-score ≤ −2.5) $n = 306$	Missing BMD $n = 178$	Normal BMD (*T*-score >1.0) $n = 1634$	Osteopenia (*T*-score −1.0 to −2.5) $n = 623$	Osteoporosis (*T*-score≤−2.5) $n = 444$	Missing BMD $n = 538$
Age (years)	59.1 ± 11.2	64.9 ± 10.0	70.2 ± 7.9	60.6 ± 11.1	61.7 ± 11.0	67.0 ± 9.6	71.6 ± 7.5	63.9 ± 9.9
Women	413 (32.1)	246 (61.2)	263 (82.5)	86 (45.3)	526 (29.4)	397 (58.0)	381 (78.6)	262 (44.7)
COPD grade								
Mild	577 (44.8)	152 (37.8)	96 (30.1)	89 (46.8)	599 (33.5)	218 (31.8)	106 (21.9)	195 (33.3)
Moderate	434 (33.7)	148 (36.8)	121 (37.9)	58 (30.5)	944 (52.8)	330 (48.2)	250 (51.6)	215 (36.7)
Severe	225 (17.5)	75 (18.7)	84 (26.3)	34 (17.9)	197 (11.0)	99 (14.5)	109 (22.5)	53 (9.0)
Very severe	51 (4.0)	27 (6.7)	18 (5.6)	9 (4.7)	34 (1.9)	18 (2.6)	15 (3.1)	5 (0.9)
Missing	*0*	*0*	*0*	*0*	*13 (0.7)*	*20 (2.9)*	*5 (1.0)*	*118 (20.1)*
ppFEV1	66.6 (±18.0)	62.4 (±18.6)	57.3 (±16.9)	67.3 (±19.3)	71.7 (±18.9)	70.1 (±20.7)	64.4 (±19.6)	74.9 (±20.7)
Missing	*0*	*0*	*0*	*0*	*13 (0.7)*	*20 (2.9)*	*5 (1.0)*	*118 (20.1)*
Smoking								
Never	159 (12.4)	56 (13.9)	71 (22.3)	21 (11.1)	262 (14.7)	128 (18.7)	128 (26.4)	78 (13.3)
Former								
<10 pack years	79 (6.1)	15 (3.7)	21 (6.6)	8 (4.2)	117 (6.6)	29 (4.2)	35 (7.2)	28 (4.8)
10–20 pack years	100 (7.8)	32 (8.0)	29 (9.1)	17 (9.0)	153 (8.6)	55 (8.0)	42 (8.7)	56 (9.6)
>20 pack years	249 (19.4)	82 (20.4)	46 (14.4)	38 (20.0)	398 (22.3)	144 (21.0)	75 (15.5)	127 (21.7)
Current								
<10 pack years	41 (3.2)	10 (2.5)	9 (2.8)	4 (2.1)	40 (2.2)	12 (1.8)	16 (3.3)	5 (0.9)
10–20 pack years	146 (11.3)	41 (10.2)	32 (10.0)	12 (6.3)	171 (9.6)	56 (8.2)	39 (8.0)	33 (5.6)
>20 pack years	460 (35.7)	133 (33.1)	90 (28.2)	76 (40.0)	549 (30.7)	198 (28.9)	117 (24.1)	186 (31.7)
Missing	*53 (4.1)*	*33 (8.2)*	*21 (6.6)*	*14 (7.4)*	*97 (5.4)*	*63 (9.2)*	*33 (6.8)*	*73 (12.5)*
BMI (kg/m^2)								
18.5	11 (0.9)	14 (3.5)	14 (4.4)	5 (2.6)	13 (0.7)	17 (2.5)	16 (3.3)	7 (1.2)
18.5–24.9	455 (35.4)	175 (43.5)	163 (51.1)	64 (33.7)	541 (30.3)	270 (39.4)	231 (47.6)	177 (30.2)
25–30	577 (44.8)	140 (34.8)	97 (30.4)	78 (41.1)	822 (46.0)	270 (39.4)	159 (32.8)	242 (41.3)
>30	240 (18.7)	72 (17.9)	42 (13.2)	42 (22.1)	404 (22.6)	124 (18.1)	73 (15.1)	154 (26.3)
Missing	*4 (0.3)*	*1 (0.3)*	*3 (0.9)*	*1 (0.5)*	*7 (0.4)*	*4 (0.6)*	*6 (1.2)*	*6 (1.0)*
Physical activity								
Inactive	342 (26.6)	126 (31.3)	155 (48.6)	48 (25.3)	439 (24.6)	204 (29.8)	227 (46.8)	146 (24.9)
Low	487 (37.8)	141 (35.1)	98 (30.7)	71 (37.4)	644 (36.0)	235 (34.3)	154 (31.8)	197 (33.6)
Medium	362 (28.1)	117 (29.1)	61 (19.1)	60 (31.6)	551 (30.8)	205 (29.9)	94 (19.4)	195 (33.3)
High	89 (6.9)	16 (4.0)	5 (1.6)	9 (4.7)	144 (8.1)	38 (5.6)	9 (1.9)	44 (7.5)
Missing	*7 (0.5)*	*2 (0.5)*	*0*	*2 (1.0)*	*9 (0.5)*	*3 (0.4)*	*1 (0.2)*	*4 (0.7)*
Alcohol intake								
Non-drinker	223 (17.3)	110 (27.4)	108 (33.9)	69 (36.3)	361 (20.2)	208 (30.4)	190 (39.2)	218 (37.2)
Low	533 (41.4)	143 (35.6)	116 (36.4)	54 (28.4)	709 (39.7)	227 (33.1)	168 (34.6)	160 (27.3)
Intermediate	38 (3.0)	9 (2.2)	6 (1.9)	5 (2.6)	57 (3.2)	19 (2.8)	8 (1.7)	19 (3.2)
High	393 (30.5)	115 (28.6)	64 (20.1)	56 (29.5)	533 (29.8)	195 (28.5)	90 (18.6)	171 (29.2)
Missing	*100 (7.8)*	*25 (6.2)*	*25 (7.8)*	*6 (3.2)*	*127 (7.1)*	*36 (5.3)*	*29 (6.0)*	*18 (3.1)*
Education								
Low (≤9 years)	505 (39.2)	172 (42.8)	185 (58.0)	39 (20.5)	662 (37.1)	248 (36.2)	264 (54.4)	101 (17.2)
Medium (9–13 years)	523 (40.6)	144 (35.8)	77 (24.1)	118 (62.1)	742 (41.5)	282 (41.2)	131 (27.0)	389 (66.4)
High (≥13 years)	180 (14.0)	52 (12.9)	11 (3.5)	25 (13.2)	258 (14.4)	97 (14.2)	27 (5.6)	66 (11.3)
Missing	*79 (6.1)*	*34 (8.5)*	*46 (14.4)*	*8 (4.2)*	*125 (7.0)*	*58 (8.5)*	*63 (13.0)*	*30 (5.1)*
Comorbidities ever								
CVD	788 (61.2)	247 (61.4)	229 (71.8)	106 (55.8)	1169 (65.4)	440 (64.2)	367 (75.7)	352 (60.7)
Malignancies	72 (5.6)	48 (11.9)	28 (8.8)	4 (2.1)	119 (6.7)	83 (12.1)	53 (10.9)	54 (9.2)
Musculoskeletal diseases	303 (23.5)	119 (29.6)	108 (33.9)	43 (22.6)	468 (26.0)	210 (30.7)	174 (35.9)	167 (28.5)
Diabetes mellitus	65 (5.0)	29 (7.2)	11 (3.5)	15 (7.9)	133 (7.4)	51 (7.5)	22 (4.5)	66 (11.3)

(*continued*)

Continued.

	FEV1/FVC < GLI-LLN ($n = 2076$)				FEV1/FVC <0.70 ($n = 3239$)			
	Normal BMD *T*-score > 1.0) $n = 1209$	Osteopenia (*T*-score −1.0 to −2.5) $n = 383$	Osteoporosis (*T*-score ≤ −2.5) $n = 306$	Missing BMD $n = 178$	Normal BMD (*T*-score >1.0) $n = 1634$	Osteopenia (*T*-score −1.0 to −2.5) $n = 623$	Osteoporosis (*T*-score≤−2.5) $n = 444$	Missing BMD $n = 538$
Chronic disease affecting ADL	728 (56.6)	666 (61.3)	277 (68.9)	231 (72.4)	1 078 (60.3)	463 (67.6)	347 (71.6)	406 (69.3)
Number of comorbidities								
0–1	992 (77.1)	292 (72.6)	214 (67.1)	152 (80.0)	1302 (72.9)	484 (70.7)	302 (62.3)	412 (70.3)
2–3	291 (22.6)	109 (27.1)	105 (32.9)	38 (20.0)	478 (26.8)	199 (29.1)	181 (37.3)	171 (29.2)
>3	4 (0.3)	1 (0.3)	0	0	7 (0.4)	2 (0.3)	2 (0.4)	3 (0.5)

Values are mean ± SD or *n* (%). Missing data were completed using multiple imputation. Continuous variables were measured upon entry date into the cohort.

Abbreviations: GLI, Global Lung Initiative; LLN, lower limit of normal; GOLD, Global Initiative of Lung Disease; COPD, chronic obstructive lung disease; BMI, body mass index; BMD, bone mineral density; SD, standard deviation; ppFEV1, FEV1 percent of predicted.

[a]GLI criteria defined as FEV1/FVC < fifth percentile ($z < -1.645$), as well as presence of respiratory symptoms. Severity of COPD by GLI criteria was graded using ppFEV1 calculated by the GLI-2012 software, using cutoff values by *z*-scores of > -2 (mild), < -2.5 (moderate) and < -4 (very severe).

[b]GOLD fixed ratio criteria defined as forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) <0.70, as well as presence of respiratory symptoms. GOLD categories are defined as GOLD1/mild (ppFEV1) ≥80; GOLD2/moderate (≤50 ppFEV1 < 80); GOLD3/severe (≤30 ppFEV1 < 50) GOLD4/very severe (ppFEV1 < 30).

There were more never-smokers among those with osteoporosis compared to those with *T*-score > −2.5, but a larger proportion of participants with osteoporosis had severe or very severe airway obstruction. Furthermore, participants with osteoporosis had lower BMI, were less physically active and had shorter education compared to the other groups (Table 1). There were 282 participants (92.2%) defined as having osteoporosis by forearm BMD in the GLI cohort, and 416 (93.7%) in the GOLD cohort.

Osteoporosis in association with all-cause mortality

During follow-up 1164 (56%) and 1610 (52%) participants in GLI-COPD and GOLD-COPD died, including 258 (12.4%) and 393 (12.1%) among those with osteoporosis. Analyses using BMD *T*-score as a continuous exposure showed 5% increased mortality per one unit decrease in BMD *T*-score (HR 1.05, 95% CI 1.01–1.09) in GLI-COPD, and 4% in the GOLD-COPD (HR 1.04, 95% CI 1.00–1.08) (Table 2). Adjusted survival graphs illustrate the increasing HRs at decreasing levels of BMD *T*-score (Figure 2). Sensitivity analyses using HUNT2 post-BD spirometry values showed similar results as the main analysis (Supplementary File).

In the cohort based on the GLI-COPD definitions for caseness and severity of COPD, the baseline model gave a HR of 1.24 (95% CI 1.04–1.48) for osteopenia, and a HR of 1.23 (95% CI 1.01–1.50) for osteoporosis compared to normal BMD. For the adjusted model, HRs were 1.12 (95% CI 0.92–1.35) for osteopenia and 1.13 (95% CI 0.91–1.41) for osteoporosis (Table 2). In both GLI-COPD and GOLD-COPD, smoking, COPD severity and age seemed to be the strongest confounders.

In the corresponding analysis using the GOLD-COPD criteria for caseness and severity of COPD we found a HR of 1.08 (95% CI 0.94–1.24) for participants with osteopenia, and a HR of 1.33 (95% CI 1.15–1.53) for participants with osteoporosis compared to those with normal BMD in the baseline model (Table 1). For the adjusted model, HRs were 1.12 (95% CI 0.93–1.34) for osteopenia and 1.22 (95% CI 0.99–1.51) for osteoporosis (Table 2).

There was no statistical interaction found between the main exposure, sex and mortality, hence combined models adjusted for sex were chosen. Results from baseline and adjusted models stratified by sex were calculated and showed the same direction and similar magnitude of effect as the combined analyses (data not shown).

Discussion

Key results

This prospective cohort study showed a small but significant positive association between decreasing BMD *T*-score and mortality in both GLI-COPD and GOLD-COPD, with a clear dose–response relationship between each unit decrease in *T*-score and mortality. The association remained after adjusting for known confounders, including other chronic

Table 2. Mortality rates and adjusted HRs with 95% CIs for the association between bone mineral density and all-cause mortality within the COPD cohort of the HUNT Lung Study.

COPD classification	Person-years	Observed deaths	Death rate[a]	n^b	Baseline HR[c]	95% CI	Adjusted HR[d]	95% CI
FEV1/FVC < GLI LLN[e]								
Normal BMD (*T*-score ≥−1.0)	18,137	666	36.7	1332			1.00 (*Ref.*)	
Osteopenia (*T*-score −1.0 to −2.5)	4600	240	52.2	418	1.24	1.04–1.48	1.12	0.92–1.35
Osteoporosis (*T*-score ≤-2.5)	3633	258	71.0	326	1.23	1.01–1.50	1.13	0.91–1.41
p-value for trend					*0.015*		*0.152*	
BMD (per one unit decrease in *T*-score)	26,370	1164	44.1	2076	1.07	1.03–1.11	1.05	1.01–1.09
FEV1/FVC <0.70[f]								
Normal BMD (*T*-score ≥−1.0)	24,725	943	38.1	1979			1.00 *(Ref.)*	
Osteopenia (*T*-score −1.0 to −2.5)	7883	353	44.8	724	1.08	0.94–1.24	1.12	0.93–1.34
Osteoporosis (*T*-score ≤-2.5)	5 798	393	67.8	536	1.33	1.15–1.53	1.22	0.99–1.51
p-value for trend					*0.000*		*0.056*	
BMD (per one unit decrease in *T*-score)	38,406	1689	45.6	3239	1.06	1.03–1.09	1.04	1.00–1.08

[a]Per 1000 person-years.
[b]Count after completing missing data by multiple imputation.
[c]Adjusted for sex and continuous age upon entering the cohort.
[d]Adjusted for all confounders including sex, continuous age, COPD severity (mild, moderate, severe, very severe), BMI (underweight, normal weight, overweight, obesity), tobacco smoking (never, former, current, with the latter two categories stratified by <10, 10–20 and >20 pack-years), alcohol use (low, intermediate, high), physical activity (inactive, low, medium, high), level of education (low, medium, high) and ever cardiovascular disease, diabetes, cancer, musculoskeletal or rheumatic disorders and chronic diseases affecting ADL (yes/no).
[e]GLI-COPD defined by FEV1/FVC *z*-score < −1.64, as well as having symptoms of wheeze, sputum production and/or chronic cough.
[f]GOLD-COPD defined by FEV1/FVC ratio <0.70, as well as having symptoms of wheeze, sputum production and/or chronic cough.

diseases that might affect mortality. However, osteoporosis, defined by a *T*-score below -2.5, was not associated with mortality.

Categorizing *T*-score according to pre-defined cutoffs, although clinically meaningful, may have led to loss of information, obscuring the real association. The risk gradient of fractures increases continuously as BMD decreases, and in the femoral neck the risk increase is 2.6 times per SD decrease in total hip BMD [26]. Furthermore, categorization could lead to loss of power, leading to wider confidence intervals [26]. Minimizing within-category risk variation by using BMD *T*-score on a continuous scale could reduce this power loss. To overcome a potential power problem, we did spline analysis of the fully adjusted models, which largely confirmed this assumption as it showed an increase in HR with decreasing BMD (Figure 2). There were minor differences in mortality between the GOLD-COPD and GLI-COPD cohorts. As these are different models with a potential healthier reference sample in the GOLD cohort, the estimates cannot be directly compared.

Comparison with other studies

Among previous studies on mortality in individuals with COPD and osteoporosis [12, 13, 27–32], only two studies included BMD measurements in the analyses [12, 13]. The remaining studies examined the association between other measures of bone strength and mortality, and included participants with moderate-to-severe airway limitation [27, 28, 31], several of them on patients hospitalized for either fractures or acute COPD exacerbations [27, 29, 30]. Assessment of bone quality was done with a range of methods, spanning from CT-measured bone attenuation to information obtained retrospectively from health records.

Campos-Obando et al. included 5779 and 2055 subjects from two study cycles of the Rotterdam study [26]. They found a strong association between BMD and chronic lung disease related mortality, with 170–200% increased mortality in men, and around 70% increased mortality in women [12]. In a similar study from NHANESIII including 3275 participants, Looker reported a 38% increased risk of COPD-specific mortality in participants with osteoporosis [13]. All-cause mortality was not assessed in either of these studies, and both were done on general populations not restricted to persons with COPD, making them less comparable to our study.

Two studies reported twice the mortality in participants with COPD and vertebral compression fractures (VCF) compared to those without such fractures [27, 28]. The higher mortality found in these studies compared to the current study likely reflects the inclusion of milder COPD in the present population-based study, with a mean ppFEV$_1$ of 70% (GOLD) compared to 33.5% [27] and 41% [28] in the previous studies. The association between VCF and mortality supports the theory of the negative biomechanical effects of VCF on the thoracic cavity and a resulting restriction of lung movement. De Luise et al. found that in a cohort of 11,985 patients having sustained a hip fracture, having COPD increased the risk of mortality by 70% [29].

In a study comparing 1634 COPD subjects from the ECLIPSE cohort with smoking- and nonsmoking controls, Romme et al. did not find an association between decreased bone attenuation of thoracic vertebrae and all-cause mortality [31]. From the same cohort Miller et al. studied the association between comorbidities and clinical outcome measures in 2164 COPD patients compared to smoking- and nonsmoking controls. They found that a cumulative number of comorbidities, including osteoporosis, increased mortality in individuals with COPD, but did not find osteoporosis itself to contribute to higher mortality [32]. In contrast to the present study, these studies did not include participants with GOLD grade I [32] or excluded participants with inflammatory disease, a recent cancer diagnosis or long-term corticosteroid use [31].

Strengths and limitations

The HUNT Study is a large study with a relatively high participation rate and long follow-up time. The percentage of participation in HUNT2 and HUNT3 for the age group 40–85 years was higher than for other age groups, constituting 78% and 64% [14]. A nonparticipation study after HUNT3 found higher prevalence of several chronic diseases among non-responders, including COPD [33]. For the biological association between COPD and osteoporosis, however, attendance should not affect the estimates.

The distribution among GOLD grades found in this study corresponds well to prevalence reported by other studies [3], indicating that the study sample is representative to a general COPD population. GOLD guidelines recommend the use of post-BD spirometry for the diagnosis of COPD [4]. As we had post-BD spirometry data from only half of the participants in the HUNT2 Lung Study, we included pre-BD spirometry measurements to obtain higher statistical power for the analyses. Use of pre-BD spirometry has been reported to give an overestimation of around 26% in COPD cases compared to use of post-BD results [34]. However, individuals with more severe disease are still identified by pre-BD spirometry, and only persons with mild airway obstruction would potentially be misclassified. Using the presence of respiratory symptoms in addition to spirometry for diagnosis, as we did in the current study, would reduce the number of misclassified participants. Furthermore, sensitivity analyses using post-BD spirometry gave HRs similar to the estimates in the main analysis (Supplementary table), and a clear dose–response relationship was found when using BMD as a continuous measure, even when adjusting for known confounders, indicating that for each SD decrease in BMD, a statistically significant increase in mortality was seen (Table 2).

Several studies have shown that the use of fixed-ratio criteria recommended by GOLD for diagnosis of COPD underestimates the prevalence in younger age groups, and overestimates the prevalence in the older age groups [35]. ATS and ERS have therefore recommended that LLN criteria should be used in the diagnosis of COPD [36]. In this study COPD cohorts have been defined according to both diagnostic criteria to increase knowledge of influence by comorbidities for COPD defined by different criteria.

Scandinavian countries have a high prevalence of osteoporosis and osteoporotic fractures [37]. The reason for this is debated but could rely on genetic factors, in addition to lack of sun exposure during the autumn and winter season. This could decrease the external validity of our findings toward other ethnic groups. The WHO international reference standard for osteoporosis is based on total hip *T*-scores. In this study forearm BMD was used in participants without hip densitometry data. Some studies show that the use of forearm densitometry tends to overestimate the prevalence of osteoporosis [38]. To adjust for this, site of measurement was included in the model, and this did not change the estimates (data not shown).

Due to practical and geographical purposes, participants selected for spirometry in small municipalities had to return to do the measurements some weeks later, resulting in a loss of around 20%. This might lead to selection bias but with small magnitude, as this is a pre-selected group largely constituted by individuals already reporting chronic airway symptoms or a known diagnosis of obstructive lung disease. A relatively high level of nonparticipation in the bone densitometry sub-study was due to lack of personnel resources at the field stations rather than factors related to participants.

Conclusion

Individuals with COPD are at increased risk of osteoporosis due to shared risk factors and disease compounding. Both conditions benefit from secondary preventive strategies such as smoking cessation and increased physical activity, and medical treatment exist for established disease.

We found a small positive association between decreasing BMD *T*-score and mortality in both GLI-COPD and GOLD-COPD. However, osteoporosis as defined by WHO was not associated with mortality, probably due to loss of power upon categorization. Future studies including patient-centered outcomes could add further value to these findings.

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This work was previously presented at the 2018 European Respiratory Society (ERS) International Congress, and a preliminary version of the abstract was published in the ERS Abstract Book [39].

Declaration of interests

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Paper II

Paper II

The association of anxiety and depression with mortality in a COPD cohort. The HUNT Study, Norway.

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Short summary: In a 20-year follow-up study of a general COPD cohort, symptoms of anxiety and depression at baseline were associated with increased all-cause mortality.

Abstract

Background

Anxiety and depression are prevalent among individuals with chronic obstructive pulmonary disease (COPD), but the impact of these comorbidities on long-term mortality is unknown.

Aims

This study aims to compare mortality in individuals with COPD who had or did not have symptoms of anxiety or depression as well as the impact of a change in these symptoms on mortality.

Methods

Individuals with COPD according to the Global Lung Initiative (GLI) criteria (n=2076) were recruited from the second (1995–97) and third (2006–08) surveys of the HUNT Study and followed until January 2019 for mortality. We assessed baseline status of anxiety or depression using the Hospital Anxiety and Depression Scale (HADS), and caseness was defined as a score ≥8. We used Cox regression to calculate hazard ratios (HR) with 95% confidence intervals (CI). Change in HADS score over time was assessed using joint models.

Results

Among the individuals with COPD, 16.2% were defined as caseness of anxiety and 15.9% as caseness of depression. Compared to those with HADS-A and -D score <8, caseness of anxiety or depression increased mortality by 21% (95% CI 05-47%) and 21% (2-44%), respectively. Over the approximately 11-year period between surveys, change of HADS-A from ≥8 to <8 was associated with a decrease in mortality (HR 0.97 [95% CI 0.94-1.00]), but not in HADS-D (0.97 [95% CI 0.93-1.18]).

Conclusions

Individuals with COPD and caseness of anxiety or depression have increased mortality, and improved HADS-A score with time is associated with lower mortality.

Introduction

A large proportion of individuals with COPD suffer from concurrent diseases, contributing to increased disease burden and mortality [1]. Anxiety and depression often co-exist, and are two of the most common comorbidities in COPD, with an estimated prevalence of up to 40% and 25%, respectively, in a clinical population [2]. Despite the high prevalence in COPD, these conditions are under-recognized and undertreated [3,4]. Symptoms of anxiety and depression impact on quality of life, coping strategies and adherence to treatment of both COPD and comorbid diseases [2,3]. Changes in breathing patterns, hyperventilation and dynamic hyperinflation associated with anxiety also contribute to a downward spiral of disease progression [5].

The Hospital Anxiety and Depression Scale (HADS) is a self-administered questionnaire with two 7-item subscales [6]. It was developed as a screening tool to assess possible and probable caseness of anxiety and depression in non-psychiatric hospital outpatients, excluding physical symptoms of anxiety and depression from its structure [6]. Psychometric properties of HADS have been thoroughly tested both in the general population [7,8] and in COPD patients [9], and it is widely used in epidemiological studies.

Despite a large body of evidence confirming the impact of anxiety and depression on adverse outcomes in COPD, most studies have been either cross-sectional or with short observation time (< 5 years) [2,10]. Furthermore, these associations have primarily been examined in individuals with moderate to severe COPD, excluding individuals with mild symptoms [2]. We aimed to examine all-cause mortality among individuals with COPD with mild to severe airflow limitation in relation to HADS score, over a long follow-up. Further, we aimed to assess the impact of longitudinal change in HADS score on mortality.

Methods

Study sample

Participants were recruited in the second (HUNT2, 1995-97) and third (HUNT3, 2006-08) surveys of the HUNT Study, a longitudinal population-based study, and followed until February 01, 2019 [11]. Participants reporting asthma related symptoms during the last 12 months, use of asthma or COPD medication or a diagnosis of obstructive lung disease were invited to the Lung Study, as well as random samples of all participants: 5% in HUNT2 and 10% in HUNT3.

MasterScope spirometers (Erich Jaeger GmbH, Wuerzburg, Germany) were used. Measurement technique and quality control have been described previously [12]. All participants aged 40-85 years with acceptable pre-bronchodilator spirometry were recruited to the study cohort. Participants were identified as having COPD if they had 1) a z-score of forced expiratory volume in 1 second (FEV_1) divided by forced vital capacity (FVC) < –1.64 according to Global Lung Initiative (GLI-2012) [13]; and 2) respiratory symptoms (chronic cough, dyspnea, wheezing) [14].

Assessment of symptoms of anxiety and depression

HADS was used to assess caseness of anxiety and depression and was part of the baseline assessment of all participants in HUNT. Of 14 items, seven relate to symptoms of anxiety (HADS-A), and seven to symptoms of depression (HADS-D). Each item was scaled by 0–3, resulting in a total score between 0–21. Caseness was defined if the participant scored above the chosen clinical-cut-off of either HADS-A or -D, classifying it as a clinical case. We chose a cut-off of ≥8 for caseness of anxiety or depression [15,16] which has been shown to have the most optimal sensitivity and specificity with both around 0.80 [16]. Analyses were also performed using continuous HADS subscores. The HADS variables were reported as missing if some or all items were left unchecked in the questionnaires.

Other covariates

Severity of COPD was graded using FEV_1 z-scores, using cut-offs of –2, –2.5, –3 and –4, corresponding to mild, moderate, severe and very severe airflow obstruction [17]. Level of education (years) was categorized into low (≤9), medium (10–12) and high (≥ 13). Body mass index (BMI) was calculated in kg/m^2 and categorized as <18.5 (underweight), 18.5–24.9 (normal), 25.0–29.9 (overweight) and ≥30.0 (obese) [18]. Physical activity (PA) was categorized according to hours of self-reported PA per week: inactive (≤1 h light and no hard PA), low (>1 h light and <1 h hard), medium (1–2 h of hard regardless of light PA) and high (≥3 h of hard regardless of light PA). Functional limitation was assessed by the question: "Do you suffer from any long-term illness or injury of a physical or psychological nature that impairs your functioning in your everyday life?". Self-reported cardiovascular disorders included previous myocardial infarction, angina pectoris and ischemic-/ hemorrhagic brain infarction. We identified participants with

hypertension as those answering yes to prior or current use of antihypertensive medication or having hypertension (defined as ≥140 and/or ≥90) upon blood pressure measurements in HUNT2 or HUNT3. Chronic musculoskeletal diseases included rheumatoid arthritis, spondyloarthritis, osteoarthritis and "other long-term skeletal or muscular diseases". All variables on comorbidities were dichotomized to yes/no.

Mortality data

Data on all-cause mortality was provided by the National Registry (https://www.skatteetaten.no/en/person/national-registry/). We defined time at risk as from the date of entry into the study until date of death, emigration or end of observation, whichever came first. There was no loss to follow-up.

Ethical considerations

This study was approved by the Regional Committee for Medical and Health Research Ethics (REC) Central Norway (project no. 2014/1114-1). Participants in the HUNT Study have given informed, written consent for research on their data and linkage to specific registries.

Statistical analysis

Baseline characteristics were presented using means (± standard deviations, SD) for continuous variables and counts (percentages) for categorical variables by categories of HADS-A and –D. Confounders were identified using directed acyclic graphs. Self-reported comorbidities were regarded as mediators, and thus not adjusted for. Model 1 was adjusted for age and sex. Model 2 (main model) was adjusted for age, sex, COPD severity, education, BMI, PA and functional limitation at baseline.

We used Cox proportional hazard (PH) models to estimate hazard ratios (HR) with 95% confidence intervals (CI) for the association between HADS subscores and all-cause mortality. Age was used as the time scale. The three first years of follow-up time were removed due to non-PH. We tested PH assumptions using Schoenfield's residuals. Linearity assumptions was tested by plotting Martingale residuals against continuous covariates. Interactions terms between main exposure and covariates were tested using likelihood ratio tests. Age- and sex-djusted HRs (hazard functions for Model 1) were plotted as a function of continuous HADS score using restricted cubic

splines with three knots. The number of knots were determined using Akaike's- and Bayesian information criterion. Further analyses were performed based on the following mutually exclusive groups: i) non-symptomatic (HADS-A and HADS-D <8); ii) anxiety symptoms only (HADS-A ≥8 and HADS-D<8); iii) depression symptoms only (HADS-A<8 and HADS-D≥8); and iv) both anxiety and depression symptoms (HADS-A and HADS-D ≥8).

To assess the impact of change in symptoms of anxiety or depression over time on mortality, we used a joint model of longitudinal and survival data by the Stata suite *stjm* [19], combining linear mixed effects models for longitudinal measurements of HADS scores (cut-off ≥8) and Weibull survival submodels. All participants were included to avoid survivor bias. The models were fully adjusted using time-dependent covariates including age, COPD severity, education, BMI and PA, as well as sex. Individuals with change in HADS score were compared to those without change. Complete case analysis was used. In sensitivity analyses, missing data of all variables were imputed using multiple imputation with chained models ($M = 5$) (Figure 1). Statistical analyses were done using Stata 15.1 (StataCorp LCC, College Station, TX, USA).

Results

Descriptive characteristics

A total of 2076 individuals were identified as having COPD. 500 (24.1%) and 302 (14.6%) of these had missing data on the HADS-A and –D, respectively. They were followed for an average of 13 years (3.0-23.4 years) during which 1164 individuals (56%) died. At baseline, mean age was 62 years (±11.2) and 45% were women. Mean ppFEV1 was 64% (±18.3). In total, 40% had mild; 35% moderate; 20% severe and 5% very severe airflow limitation.

Prevalence and characteristics of subjects with anxiety or depression

Both caseness of anxiety (HADS-A ≥8) (16.2%) and -depression (HADS-D ≥8) (15.9%) was present in around one out of six subjects. Thirty percent of participants had either caseness of anxiety or depression, and 10% had both. There was no difference in age between individuals with caseness of anxiety or depression. More women had caseness of anxiety (57.6%), and more men had caseness of depression (53.2%). There were more active smokers among those having caseness of anxiety, they were more physically active and had higher education than those with caseness of depression. Alcohol intake was slightly higher in those with caseness of depression

compared to -anxiety. The mean ppFEV1 was lower in those with caseness of depression than -anxiety, and there were more individuals with severe and very severe airflow limitation in this group. More individuals with caseness of depression also had cardiovascular diseases and musculoskeletal disorders. However, more individuals with caseness of anxiety reported having functional limitations (Table 1).

Participants with missing data on HADS were older, less likely to be heavy smokers, were more inactive, had lower education and lower ppFEV1 than the other groups. The distribution of severity grades (mild to very severe COPD) resembled that of the non-missing groups (Table 1). Non-responders on HADS had a slightly increased mortality compared to responders, possibly associated with the higher mean age.

Association between symptoms of anxiety/depression and all-cause mortality

We observed a positive association between HADS scores as a continuous measure and all-cause mortality (Figure 2). The age and sex adjusted risk of death increased by 3% (95% CI 1.01-1.05) and 4% (95% CI 1.02-1.06) per one unit increase in HADS-A and D, respectively. When adjusting for severity of airflow limitation, levels of education, PA, BMI and functional limitation (Model 2), the association between both caseness of anxiety and depression with all-cause mortality was slightly attenuated (HR 1.02, 95% CI 1.00-1.04) and (HR 1.02 95% CI 1.00-1.04), respectively. For HADS-A and -D dichotomized by ≥8, the corresponding results were 1.30 (95% CI 1.09-1.55) and 1.28 (95% CI 1.10-1.50) in Model 1, and 1.21 (95% CI 1.00-1.47), and 1.21 (95% CI 1.02-1.44) in Model 2 (Table 3).

Having caseness of depression alone increased mortality with 31% (4-68%), and both caseness of anxiety and depression gave a 37% (7-75%) increase. Only caseness of anxiety was not significantly associated with mortality (HR 1.13 [95% CI 0.87-1.46]) (Model 2, Table 4).

For participants with a repeated measures of HADS-A (n=741) and HADS-D (n=826), we found a reduced risk of mortality in those who went from HADS-A ≥8 in HUNT2 to <8 in HUNT3 (HR 0.97 [95% CI 0.94-1.00]), and the same reduction was found using HADS-D, although the estimate was less precise (HR 0.97 [95% CI 0.93-1.18]). A HADS score change from <8 to ≥8 was associated with an increase in mortality for HADS-A (HR 1.03 [95% CI 1.01-1.05]). A

similar association was observed for HADS-D, but the estimate was less precise (HR 1.03 [95% CI 0.99-1.18]) (Table 5).

No interaction between sex and HADS score with mortality was found. Sensitivity analyses using multiple imputation did not materially change the estimates (data not shown).

Discussion

In this longitudinal, population-based study following individuals with COPD up to 23 years, we found that comorbid symptoms of anxiety or depression were associated with a minor increase in mortality per unit increase in HADS-A and -D. Similarly, caseness of anxiety as well as caseness of depression based on validated and accepted thresholds showed significantly increased mortality. When comparing combinations of HADS subscores, the risk of mortality was more pronounced for depression. Further, there appeared to be an association of change in HADS-A or –D scores between the two surveys with mortality.

Comparison with other studies

Participants with symptoms of anxiety or depression differed in several characteristics, including exposure and comorbidity profile. More individuals with caseness of anxiety had higher cumulative smoking exposure, higher levels of education and physical activity. On the contrary, more in this group reported more functional limitation. A current systematic review found no clear associations between smoking and anxiety [20], but other studies have pointed to increased smoking as a form of self-medication [21]. The restlessness often experienced in individuals with anxiety could explain the higher physical activity levels, as could the less severe COPD in the anxiety group. More individuals with caseness of depression had higher alcohol intake, severe COPD, and reported more cardiovascular and musculoskeletal disorders compared to those with caseness of anxiety. Correspondingly, there has been reported an association with depression and alcohol in the general population [22], and the association between male gender and alcohol use in depression may play a role [23], as there were more men with depressive symptoms in our sample. Both anxiety and depression are known to be associated with cardiovascular disease [24], and a recent systematic review found that stress reactivity was associated with a range of long-term health outcomes including cardiovascular diseases, musculoskeletal symptoms, poorer self-reported health and physical disability [25].

Several factors may explain why individuals with symptoms of anxiety or depression have increased mortality. Depression is associated with increased mortality in the general population [26]. This has partly been explained with lower adherence to treatment and self-care, but also dysregulation of biological stress pathways, including hyperactivity of the hypothalamic-pituitary-adrenal axis and chronic activation of the somatic nervous system [26]. In a comprehensive meta-analysis from 2014, excess mortality associated with depression was only found in studies of COPD patients, and not in other disease groups or general community samples [26]. It was theorized that these findings could relate to known risk factors for mortality in COPD, including airflow limitation, hypercapnia, hypoxemia, dyspnea, and nutritional status [26,27]. The association of anxiety with mortality in the general population is equivocal. A recent meta-analysis did not find increased mortality in anxious individuals derived from community-based samples [28], but other studies have shown both favorable and unfavorable associations [29] as well as U-shaped association curves [15], which could be explained by heightened awareness of somatic symptoms at moderate levels of anxiety. A seemingly J-shaped curve identified in our sample using the cubic spline model may support these findings.

Changes in respiratory patterns, as seen in both anxiety and depression, could also be part of the explanation. In COPD, anxiety may lead to hyperventilation, reducing expiration time and thereby increasing dynamic hyperinflation and increased dyspnea sensation [30]. The vicious circle of worsening dyspnea and anxiety may lead to exacerbations, which in itself is a significant predictor of disease progression and death [14]. Indeed, from a previous study conducted on our cohort, it was reported that anxiety symptoms were strongly associated with reporting dyspnea [31]. Symptoms of depression have also been identified as a major determinant of dyspnea scores [31,32], related through complex causal pathways [33].

An increase in HADS subscore between the two surveys was associated with increased mortality, although the estimates were less precise in HADS-D. This could potentially indicate that treating symptoms of anxiety and depression could lead to improved outcomes in COPD. On the other hand, anxiety and depression could just be a marker of more severe disease. We adjusted for COPD severity to account for confounding by disease but cannot rule out some residual confounding to influence our estimates.

There were sex-related differences in the prevalence of anxiety and depression in women and men, with women having more symptoms of anxiety, and men having more symptoms of depression. However, we found no interactions between HADS score and sex with mortality. This contrasts with several large studies finding higher mortality among women with comorbid COPD and anxiety [1]. As HADS does not cover somatic symptoms [34], metric invariance between men and women is avoided [35], as it is claimed that a higher prevalence of depression in women is caused by women reporting higher levels of vegetative and somatic symptoms than men [36]. The prevalence of anxiety and depression symptoms were high in our sample. In the source population of this study cohort the prevalence of anxiety and depression was substantially lower; 9.6% and 4.9% [15].

This study has a large sample size and a long follow-up time. It consists of a COPD population with external generalizability. However, due to extensive adjustment within the models, some significance of the associations is lost, probably due to low power.

Strengths and limitations with the use of HADS

We found diverging results using HADS subscores alone or in combination. Symptoms of anxiety was associated with mortality only when the HADS-D scores were high, or not included in the analysis. Symptoms of depression was associated with mortality in all analyses. This discrepancy is probably caused by the difference in reference groups in the analyses, and illustrates the symptom overlap of anxiety and depression, as well as the high correlation between the HADS-A and -D subscores. The bifactorial structure of HADS measures a general factor of negative affect common for both anxiety and depression, as well as two orthogonal group factors accounting for specific anxiety and depression variance [35]. Item response theory analysis has shown problems with the item structure, with anxiety items loading on depression factors, and depression factors loading on the general distress factor, suggesting that HADS fail to discriminate between anxiety and depression [37]. Recent systematic reviews suggest that HADS is a better measure of general psychological distress [35,38]. Although the factor structure of HADS is debated, its validity and reliability in the general population and COPD samples are shown to be satisfactory [16,38]. Snaith et al suggested three categories for HADS scoring [6], but various cut-off levels have been reported from different study populations. We chose to use a cut-off of 8, which has been shown to have good discriminatory effect between

caseness and noncase [16], and also analyzed the HADS subscales on a continuous scale as well as with the use of restricted cubic splines to avoid loss of information.

There was a relatively high number of missing values of HADS in our study, partly because those lacking data on one or more HADS items were registered as missing. Since the mechanism of missingness was unclear, we chose to present complete case results, and did sensitivity analyses where all missing values were imputed. As there were no significant differences between complete case and imputed results, the former should give us unbiased estimates.

HADS lacks psychometric evaluation in older individuals, which constitutes a large proportion of our sample. It is unclear how this influences our results; however, a recent study suggests that HADS could be applied on an older general population, despite pronounced ceiling effects [39]. The functional form of the association of HADS-A with mortality indicated non-linearity when HADS-A was <5. Although formal tests did not support deviation from linearity of the association, caution should be taken when interpreting the association between low HADS-A scores and mortality.

Conclusion

In this observational study with long follow-up time, individuals with mild to very severe COPD and symptoms of anxiety or depression had a minor increased risk of mortality. Improved HADS scores over time appeared to be associated with reduced mortality. Further studies assessing longitudinal outcomes like exacerbation rates, symptom burden and quality of life would add further value to these findings.

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Declaration of interests

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Table 1 Descriptive characteristics of the HUNT COPD cohort 40-85 years defined by GLI LLN* (n=2 076).

	All subjects	**HADS-D**		**HADS-A**	
		Normal (0-7)	Anxiety (8-21)	Normal (0-7)	Depression (8-21)
	n=2 076	**n=1 239(59.7)**	**n=337(16.2)**	**n=1 443(69.5)**	**n=331(15.9)**
Age (years)	62.1±11.2	61.3±11.1	60.2±11.1	61.5±11.1	62.7±11.3
Sex (women)	943(45.4)	508(41.0)	194(57.6)	645(44.7)	155(46.8)
BMI (kg/m^2)					
<18.5	43(2.1)	19(1.5)	9(2.7)	25(1.7)	10(3.0)
18.5-24.9	795(38.3)	458(37.0)	130(38.6)	544(37.7)	132(39.9)
25-30	849(40.9)	536(43.3)	124(36.8)	613(42.5)	123(37.2)
>30	381(18.4)	222(17.9)	73(21.7)	257(17.8)	65(19.6)
Missing	*8(0.4)*	*4(0.3)*	*1(0.3)*	*4(0.3)*	*1(0.3)*
Current smoking					
<10 pack years	71(3.4)	48(3.9)	7(2.1)	50(3.5)	9(2.7)
10-20 pack years	236(11.4)	137(11.1)	47(14.0)	177(12.3)	31(9.4)
>20 pack years	624(30.1)	365(29.5)	133(39.5)	429(29.7)	116(35.0)
Missing	*296(14.3)*	*136(11.0)*	*43(12.8)*	*168(11.6)*	*47(14.2)*
Education					
Low (≤9 years)	905(43.6)	516(41.7)	151(44.8)	610(44.6)	170(51.4)
Medium (9-13 years)	733(35.3)	472(38.1)	124(36.8)	533(38.9)	117(35.3)
High (≥ 13 years)	280(13.5)	206(16.6)	34(10.1)	226(16.5)	20(6.0)
Missing	*158(7.6)*	*45(3.6)*	*28(8.3)*	*74(5.1)*	*24(7.3)*
ppFEV1	63.9±18.3	65.1±18.3	64.2±17.4	65.0±18.4	61.4±17.3
COPD grade					
Mild	838(40.4)	523(42.2)	138(40.9)	607(42.1)	118(28.9)
Moderate	723(34.8)	434(35.0)	113(33.5)	503(34.9)	122(34.1)
Severe	410(19.8)	235(19.0)	61(18.1)	267(18.5)	71(25.8)
Very severe	105(5.1)	47(3.8)	25(7.4)	66(4.6)	20(11.3)
Missing	*0(0.0)*	*0(0.0)*	*0(0.0)*	*0(0.0)*	*0(0.0)*

GLI COPD defined by FEV1/FVC z-score <–1.64.
** BMI, body mass index; ppFEV1, FEV1 percent of predicted;

Table 2 Mortality rates and adjusted HRs with 95% CIs for the association between continuous HADS subscores (0–21) and all-cause mortality within the COPD± cohort of the HUNT Lung Study (n=2076).

HADS subscore	**Person-years**	**Observed deaths**	**Death rate***	**Mean HADS score**	***n***	**Model 1 (HR, 95% CI) [a]**	***n***	**Model 2 (HR, 95% CI)[b]**
HADS-A	20686	810	39.2	4.90	*1576*	1.03(1.01-1.05)	*1442*	1.02 (1.00-1.04)
HADS-D	23092	965	41.8	4.36	*1774*	1.04 (1.02-1.06)	*1593*	1.02 (1.00-1.04)

* Per 1000 person-years.
a Adjusted for sex and continuous age at baseline; **b** Adjusted for sex, continuous age, COPD severity (mild, moderate, severe, very severe), BMI (underweight, normal weight, overweight, obesity, physical activity (inactive, low, medium, high), level of education (low, medium, high) and functional limitation (yes/no).
± GLI COPD defined by FEV1/FVC z-score <–1.64.

Table 3 Mortality rates and adjusted HRs with 95% CIs for the association between categorical HADS subscores (dichotomized at cut-off 8) and all-cause mortality within the COPD cohort of the HUNT Lung Study.

HADS score	Mean HADS-A score	Mean HADS-D score	*n*	Model 1 (HR, 95% CI)[a]	*n*	Model 2 (HR, 95% CI)[b]
HADS-A						
Normal (0-7)	3.18	3.76	*1239*	*Ref.*	*1141*	*Ref.*
Caseness of anxiety (8-21)	10.51	7.56	*337*	1.30 (1.09-1.55)	*301*	1.21 (1.00-1.47)
HADS-D						
Normal (0-7)	3.90	3.33	*1443*	*Ref.*	*1304*	*Ref.*
Caseness of depression (8-21)	8.11	9.89	*331*	1.28 (1.10-1.50)	*289*	1.21 (1.02-1.44)

a Adjusted for sex and continuous age at baseline. b Adjusted for sex, continuous age, COPD severity (mild, moderate, severe, very severe), BMI (underweight, normal weight, overweight, obesity, physical activity (inactive, low, medium, high) and level of education (low, medium, high). c Adjusted for sex, continuous age, COPD severity (mild, moderate, severe, very severe), BMI (underweight, normal weight, overweight, obesity, physical activity (inactive, low, medium, high), level of education (low, medium, high) and functional limitation (yes/no).
± GLI COPD defined by FEV1/FVC z-score <–1.64.

Table 4 Mortality rates and adjusted HRs with 95% CIs for the association between combination of HADS subscores and all-cause mortality within the COPD cohort of the HUNT Lung Study (n=1526).

HADS score	n	Mean HADS score		Model 1 (HR, 95% CI)[a]	Model 2 (HR, 95% CI)[b]
		HADS-A	*HADS-D*		
HADS A+D <8	989	*3.18*	*2.91*	*Reference*	
Only HADS-A ≥8	147	*9.77*	*4.66*	1.09 (0.85-1.38)	1.13 (0.87-1.46)
Only HADS-D ≥8	119	*4.26*	*9.35*	1.34 (1.07-1.68)	1.34 (1.05-1.71)
HADS-A+D ≥8	140	*11.51*	*10.40*	1.56 (1.24-1.95)	1.29 (1.00-1.67)

* Count after removal of missing values by complete case (Model 2 and 3).
a Adjusted for sex and continuous age at baseline. b Adjusted for sex, continuous age, COPD severity (mild, moderate, severe, very severe), BMI (underweight, normal weight, overweight, obesity, physical activity (inactive, low, medium, high), level of education (low, medium, high) and functional limitation (yes/no).
± GLI COPD defined by FEV1/FVC z-score <–1.64.

Table 5 Mortality rates and adjusted HRs with 95% CIs for the association of change in HADS score (cut-off ≥8) from HUNT2 to HUNT3 with mortality.

HADS subscore	*n*	HR, 95% CI[a]
HADS-A no change	*609*	*Reference*
HADS-A high to low	*68*	0.97 (0.94-1.00)
HADS-A low to high	*64*	1.03 (1.01-1.05)
IIADS-D no change	*692*	*Reference*
HADS-D high to low	*53*	0.97 (0.93-1.18)
HADS-D low to high	*81*	1.03 (0.99-1.18)

a Adjusted for sex, continuous age, COPD severity (mild, moderate, severe, very severe), BMI (underweight, normal weight, overweight, obesity, physical activity (inactive, low, medium, high) and level of education (low, medium, high ± GLI COPD defined by FEV1/FVC z-score <−1.64.

Figure 1 Flowchart of recruitment into the HUNT COPD cohort (follow-up until February 1. 2019)

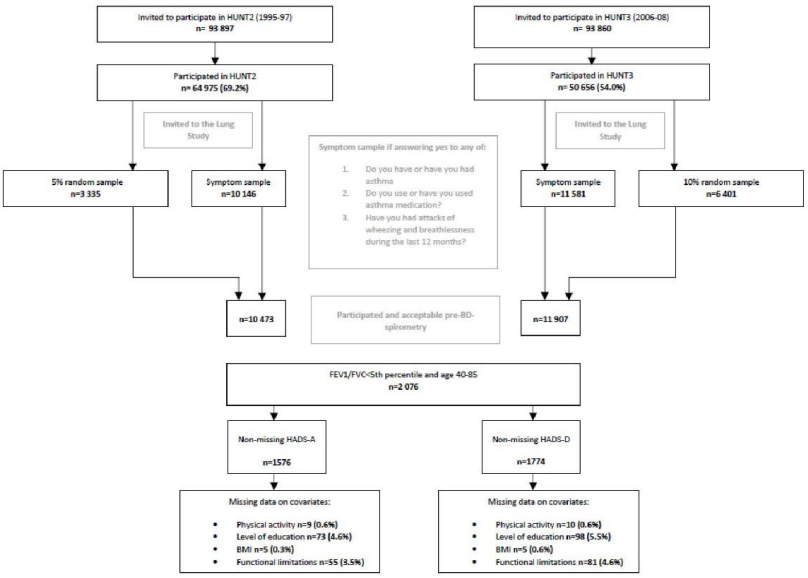
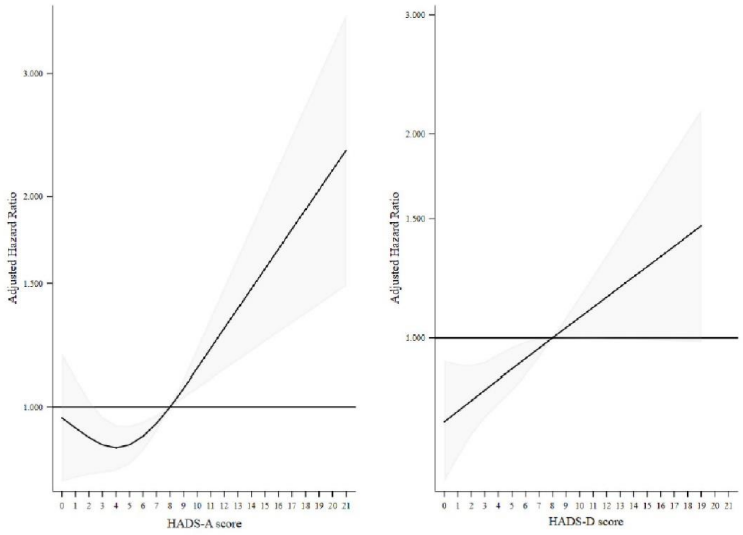


Figure 2 Age- and sex-adjusted HR with 95% CI for the association of HADS-A and HADS-D subscores with all-cause mortality.



Paper III

Paper III

The association of comorbidity clusters with long-term survival and incidence of exacerbation in a COPD cohort. The HUNT Study, Norway.

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ABSTRACT

Background

COPD is a heterogeneous disease often viewed as part of a multimorbidity complex. There is a need for better phenotyping of the disease, characterization of its interplay with other comorbidities and its association to long-term outcomes.

Aims

This study aims to examine how clusters of comorbidities are associated with severe exacerbations and mortality in COPD.

Methods

Participants with potential COPD were recruited from the second (1995–97) and third (2006–08) survey of the HUNT Study and followed until April 2020. Ten objectively identified comorbidities were clustered using self-organizing maps (Viscovery SOMine). COPD exacerbations requiring hospitalization was assessed using hospital registry data. All-cause mortality was collected from registries. Multivariable Cox regression was used to calculate hazard ratios (HR) with 95% confidence intervals (CI) for the association between comorbidity clusters and all-cause mortality, and Poisson regression was used to calculate incidence rate ratios (IRR) with 95% CI for the cumulative number of severe exacerbations for each cluster.

Results

Five distinct clusters were identified, including "less comorbidity", "psychological", "cardiorenal", "metabolic" and "cachectic" clusters. Using the less comorbidity cluster as reference, the psychological and cachectic clusters were associated with all-cause mortality; HR 1.23 (1.04-1.45) and HR 1.83 (1.52-2.20). The same clusters also had an increased risk of severe exacerbations, with an unadjusted IRR of 1.24 (95% CI 1.04-1.48) and 1.50 (95% CI 1.23-1.83), correspondingly.

Conclusions

With up to 25 years follow-up, individuals belonging to the psychological and cachectic clusters had increased all-cause mortality. Furthermore, these clusters had an increased risk of severe COPD exacerbations requiring hospitalization. Evidence of such associations was not found with the other clusters.

Introduction

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide, making it an important public health challenge (1). Coexisting chronic diseases, also called comorbidities, are highly prevalent in COPD, and might influence disease burden, mortality and health care expenditure (2). However, comorbidities in COPD are often overlooked and undertreated, increasing the risk of exacerbations and premature death (3, 4). Comorbidities in COPD have been shown to appear in distinct clusters, showing the heterogeneity of COPD phenotypes (5-7). However, few studies have examined the association of such clusters with important long-term outcomes like severe COPD exacerbations and death. Exacerbations are important adverse events in COPD, and are important indicators of disease progression, quality of life and mortality (1, 8).

We aimed to 1) Identify clusters of objectively identified comorbidities in a COPD cohort recruited from a population-based study and 2) Examine the association between these clusters and COPD exacerbations requiring hospitalization and 3) all-cause mortality.

Methods

Study design

Participants were recruited in the second (HUNT2) and third (HUNT3) surveys of the HUNT Study, a longitudinal population-based study, and followed until April 01, 2020 (9). Participants reporting respiratory symptoms (attacks of wheezing or breathlessness) during the last 12 months, use of asthma or COPD medication or a diagnosis of asthma, COPD, emphysema or chronic bronchitis, as well as random samples of all participant (5% in HUNT2 and 10% in HUNT3), were invited to the HUNT Lung Study. All participants aged 40-85 years in HUNT2 or HUNT3 with acceptable pre-bronchodilator spirometry measurements were recruited to the study cohort. Participants were identified as having COPD if they had forced expiratory volume first 1 second (FEV1) divided by forced vital capacity (FVC) z-score < -1.64 according to Global Lung Initiative (GLI-2012) (10); and 2) respiratory symptoms.

Assessments

Data on covariates including demographics, smoking status and respiratory symptoms were collected at baseline in either HUNT2 or -3. The following assessments were performed: pre-bronchodilator spirometry; dual-energy X-ray absorptiometry (DXA) of distal forearm (HUNT2) and total hip and lumbar spine (HUNT3); weight and height; waist circumference;

blood pressure, symptoms of psychological distress (Hospital Anxiety and Depression Scale [HADS]); and dyspnoea (modified Medical Research Council [mMRC] scale). Venous blood samples were taken in non-fasting state, and serum creatinine, glucose and lipid status (triglyceride and high-density lipoprotein [HDL]) were analysed. Estimated GFR (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) formula. A diagnosis of diabetes was determined by questionnaires and blood glucose (11), and diabetes types were further defined by glutamic acid decarboxylase antibodies (anti-GAD) and protein tyrosine phosphatase-like protein IA-2 (anti-IA-2) status, fasting C-peptide levels and self-reported insulin treatment. Albuminuria was analysed in three different urine samples from the same individual using morning urine from three consecutive days (12, 13).

Definitions of comorbidities

Ten objectively measured comorbidities were identified among the participants using established cut-offs (see online supplement): hypertension (systolic pressure >140 mm Hg or diastolic pressure >90 mm Hg); chronic renal failure (eGFR 45-60 ml/min/1.73 m^2 + presence of moderately increased albuminuria or eGFR<45 ml/min/1.73 m^2); hyperlipidemia (triglyceride level >1.7 mmol/L or HDL <1.03 mmol/L [males] or <1.29 mmol/L [females]); obesity (body mass index [BMI] ≥30.0 kg/m^2); underweight (BMI <21.0 kg/m^2); osteoporosis (T-score <–2.5 at distal forearm or total hip); anxiety (HADS-A ≥8); depression (HADS-D ≥8), any diabetes mellitus and myocardial infarction (MI) (ECG changes, dynamic changes in blood levels of cardiac enzymes and coronary symptoms). Moderately increased albuminuria was defined as a urine albumin creatinine ratio (ACR) 3.0- 30.0 mg/mmol in at least two out of three urine samples (14).

Outcome variables

Data on registry status and all-cause mortality was provided by the National Registry. We defined time at risk as from the date of entry into the study until date of death, emigration or end of observation, whichever came first. There was no loss to follow-up. Data on COPD exacerbations requiring hospitalizations (severe exacerbations) were collected from one regional and two local hospitals in the county, covering the entire catchment area of the HUNT cohort population. ICD-9 and -10 codes combined with admission data were linked to our datafile using national personal identification numbers (see online supplement). Outliers >2 SD from the mean were deleted.

Other variables

Potential confounders were identified using directed acyclic graphs and included sex and age. Severity of COPD was graded using z-scores according to GLI-2012: >−2.0 (mild), −2.0 - −2.4 (moderate), −2,5 - −2,9 (severe) and <−3 (very severe) (15). Tobacco smoking was categorized as: never-, former- or current smoker. Smoking pack-years were calculated for ever-smokers. Physical activity was categorized according to hours of self-reported physical activity per week: inactive (≤1 hour light physical activity and no hard activity), low (>1 hour light and <1 hour hard), medium (1-2 hour of hard regardless of light activity) and high (≥3 hour of hard regardless of light activity). Level of education was categorized into low (≤9 years), medium (10–12 years) and high (≥ 13 years) based on the question: "What is your highest level of education?" from HUNT2. Level of education in HUNT3 was assessed using the occupational classification in HUNT according to the Erikson Goldthorpe Portocarero (EGP) social class scheme, based on the question "What kind of paid work do you do?" (16). Self-reported health status was assessed by the following question: "How is your health at the moment?". The scale is rated from 1-4 (poor, not so good, good, very good), and has been shown to predict mortality and use of health care services well (17).

Statistics

All analyses were performed using Viscovery SOMine version 7.2 (Viscovery Software GmbH, Vienna, Austria) and Stata version 16.0 (StataCorp LCC, College Station, TX, USA). Summary variables were presented as mean ± standard deviations (SD) for quantitative variables, and as percentages for discrete variables. Clustering is a method of grouping similar data, where unlabelled data are grouped together in clusters of similar input. To achieve this, self-organizing maps were generated using machine learning algorithms using unsupervised, competitive learning through neighbourhood functions. It used dimensionality reduction to produce a two-dimensional representation of complex input. Cox proportional hazards regression was used to assess the association of comorbidity clusters with all-cause mortality using a dummy variable indicating membership of a given cluster as the independent variable. Furthermore, in supplementary analyses, Cox regression was used to assess the association of each separate comorbidity with mortality. Hazard ratios (HR) with 95% confidence intervals (CI) were estimated. Time of follow-up was used as the time scale. Proportional hazards assumptions were tested using Schoenfield's residuals. Multivariable Poisson regression was used to assess the association between comorbidity clusters and the cumulative number of severe COPD exacerbations requiring hospitalization. Incidence rate ratios (IRR) with 95%

CIs were estimated. Interactions terms between main exposure and covariates were tested using likelihood ratio tests. Model 1 was unadjusted, and Model 2 was adjusted for age and sex. Missing data (summarized in Table S1) were handled by SOMine, using information from complete cases to inform the learning process. This approach requires missingness to be non-informative, which is our assumption of the data. We performed Chi-square tests for categorized variables and independent t-tests for continuous variables to analyse differences between the clusters.

Results

General cohort characteristics

In our population, 2076 individuals had COPD according to GLI criteria. Of these, 949 (45.7%) had missing data on one or more comorbidities (Table S1). The sample included individuals with mild to very severe COPD, 45.4% were females, and the mean age was 62.1 years. Totally, 51.0% were current smokers with a mean cumulative exposure of 24.7 pack-years (±15.4 SD) (Table 2). The frequencies of comorbidities ranged from 2-48% (Table S1). 371 individuals (17.9%) had no comorbidities, and 1016 (48.9%) had two or more comorbidities (Figure 1). The most prevalent comorbidities were obesity, hypertension and dyslipidaemia (Figure 2).

Comorbidity clusters

We identified five distinct, heterogeneous clusters of comorbidities (Figure 4). The "less comorbidity" cluster had fewer comorbidities compared to the other clusters. Dyslipidaemia, the most prevalent comorbidity, had the lowest prevalence in this cluster. The "psychological" cluster had the highest prevalence of depression and anxiety, but also a high burden of other comorbidities including obesity, hypertension, dyslipidaemia and osteoporosis. The "cardiorenal" cluster had higher prevalence of myocardial infarction and kidney disease than the remaining clusters, and the highest prevalence of dyslipidaemia next to the metabolic cluster. The prevalence of hypertension, obesity and diabetes mellitus was low in this cluster. The "metabolic" cluster had high prevalence of hypertension, dyslipidaemia, diabetes mellitus and obesity, but also the highest prevalence of other comorbidities. The "cachectic" cluster had high prevalence of underweight and osteoporosis, but also high levels of anxiety, depression and hypertension. None of the individuals in this cluster had diabetes mellitus or obesity. The differences are summarized in Table 2.

Difference of individual characteristics between clusters

No differences in $pFEV_1$% were found across the clusters, except in the cachectic cluster where it was significantly lower (Table 2). The less comorbidity cluster had the highest self-reported health status, the lowest mean mMRC dyspnoea score, the lowest number of pack-years, and the second lowest proportion of smokers. Furthermore, it had the lowest proportion of physically inactive, and the second highest level of education. The psychological cluster had the highest mean mMRC dyspnoea score, the lowest health status, a high proportion of active smokers, the highest cumulative tobacco exposure and the lowest proportion of individuals with high education. The cardiorenal cluster had the lowest proportion of females and never smokers, as well as a proportion of physical inactive individuals similar to the less comorbidity cluster. The metabolic cluster resembled the less comorbidity cluster in several ways, with a high proportion of never smokers, low proportion of current smokers, and a high level of education. This clusters also had the highest mean $pFEV_1$%. Finally, the cachectic cluster had the highest mean age, the highest proportion of current smokers, high level of physical inactivity, and the highest proportion of individuals with low education.

Association between comorbidity clusters and all-cause mortality

In a median of 15.7 years of follow-up time (maximum 24.6 years), 1213 individuals (58.4%) died. Time at risk was 27 443 person-years, and the incidence rate per 1000 person-years was 44.2. In crude analyses, only the psychological and cachectic clusters were significantly associated with mortality, with a HR of 1.23 (95% CI 1.04-1.45) and HR 1.83 (95% CI 1.52-2.20), respectively (Table 3). Adjusting for age and sex, the associations were attenuated, with corresponding figures of HR 1.19 (95% CI 1.00-1.40) and HR 1.42 (95% CI 1.17-1.71), respectively. The metabolic and cardiorenal clusters were not associated with mortality.

Association between comorbidity clusters and severe COPD exacerbations

There was a significant variability in the cumulative number of COPD exacerbations between the clusters. Within the entire cohort the number of severe exacerbations ranged from 0-118, where 825 individuals (39.7) had never had a severe exacerbation. The mean number of severe exacerbations was 6.7 (±11.6). Outliers > 2 SD from the mean (>30 admissions) were excluded from the analyses. The lowest mean frequency of severe exacerbations was found in the metabolic cluster (4.3±7.3), whereas the cachectic cluster had the highest mean frequency (8.0±9.6) (Table 4). The pattern was the same as with all-cause mortality: the psychological and cachectic clusters had the highest risk of severe exacerbation, with an unadjusted IRR of

1.24 (95% CI 1.04-1.48) and 1.50 (95% CI 1.23-1.83), correspondingly (Table 4). The association between severe COPD exacerbations and the metabolic and cardiorenal clusters were non-significant. The associations remained unchanged when adjusting for sex and age.

Discussion

In a cohort of individuals with mild to very severe COPD recruited from a general population, we objectively identified ten different comorbidities. All comorbidities were present although highly variable in prevalence, and five distinct comorbidity clusters were identified. Although the degree of airflow limitation was similar among the clusters, we found notable differences in health-related quality of life and long-term outcomes. Compared to a COPD cluster characterized by less comorbidities, patients that clustered together based on psychological disorders, and the patients that cluster based on a cachectic phenotype, had higher mortality risk and a higher exacerbation incidence, while the metabolic cluster of patients had opposite outcomes.

Comorbidities are more prevalent in COPD than in non-COPD controls (18). This may be due to aging, shared risk factors and disease compounding, or it may have an underlying common causative mechanism. The complex network made up of risk factors and premorbid conditions like obesity, dyslipidaemia, atherosclerosis and arterial stiffness, hypertension and prediabetic states makes it difficult to investigate the causative mechanisms behind COPD and comorbidities (19). However, it is clear that COPD often occurs as part of a multimorbidity complex (i.e. two or more diseases (20)), but it is unclear whether COPD independently predisposes to the development of other diseases (19).

Anxiety and depression often co-exist and are two of the most common comorbidities in COPD, with an estimated prevalence of up to 40% and 25%, respectively (21). Symptoms of anxiety and depression impact on quality of life, coping strategies and adherence to treatment of both COPD and comorbid diseases (21). Changes in breathing patterns, hyperventilation and dynamic hyperinflation associated with anxiety compound on this factors, might contribute to disease progression (22). Further, biological pathways including hyperactivity of the hypothalamic-pituitary-adrenal axis and chronic activation of the somatic nervous system is suggested to increase the vulnerability for lower respiratory tract infections (23), thereby increasing the risk for exacerbations. Divo et al showed that out of 79 comorbidities, anxiety was closest associated with death in individuals with COPD, however most impactful in

women (3). The psychological cluster was one of the two clusters associated with mortality and exacerbation risk and underline the need for routine assessment and treatment of mood disorders in COPD care. Further, this cluster had the highest cumulative smoking exposure, the highest dyspnoea score and the lowest self-reported health status. Our results illustrate well that the cachectic and psychological clusters are specific phenotypes of COPD. This is in line with findings by Triest et al (6) comparing individuals with COPD with non-COPD controls. Here the psychologic and cachectic cluster were specifically related to individuals with COPD, due to the lower prevalence of the relevant disorders in the control population (6).

BMI, both low and high, are important predictors of outcomes in COPD. In the general population there is a dose-response relationship between BMI and mortality (24), and a large proportion of the global burden of disease is attributable to overweight and obesity (24). However, in several meta-analyses overweight and obesity were associated with lower risk of all-cause mortality in COPD, the so-called "obesity paradox" (25, 26), which is also seen in other chronic diseases like diabetes mellitus type II and chronic kidney disease (26). This could partly explain the lack of association between the metabolic cluster and mortality, which had the highest prevalence of obesity. This cluster also had the lowest prevalence of current smokers and the highest level of education, known predictors of mortality.
Low BMI (<21.0) is a known predictor of mortality in COPD, independent of disease severity (27, 28). The reason is probably multifactorial, with appetite loss due to reduced physical activity, dyspnoea interfering with eating, enhanced energy expenditure due to increased work of breathing, and the effect of humoral factors like inflammatory cytokines, adipokines and different hormones (29). Low fat-free mass has been found to be an independent predictor of mortality in COPD (30, 31), and is associated with osteoporosis and low BMI (5). Furthermore, it deteriorates quality of life and exercise capacity in these individuals, and increases the risk of acute exacerbations (29). The cachectic cluster had the highest mortality and the highest risk of exacerbations and could reflect the influence of these factors. The proportion of females were significantly higher compared to the other clusters, and the highest mean age and the lowest FEV_1 percent predicted was found in this group. Contrary to previous studies (5, 6), the cachectic cluster had the lowest prevalence of chronic kidney disease.

Dys- and hyperlipidaemia are common in COPD, and a major risk factor for cardiovascular disease (32). However, the presence of these conditions in COPD has not uniformly been found to be negative. In a multicentre study, hyperlipidaemia was associated with less hyperinflation

and airway obstruction in individuals with COPD (33). Interestingly, individuals with COPD and hyperlipidaemia have been found to have decreased incidence of pneumonia and reduced mortality (3, 34). Furthermore, a retrospective database study found that the incidence of cardiovascular disease was not increased in individuals with COPD and hyperlipidaemia compared to COPD with normal lipid levels (34). It is not clear whether this is mediated by better nutrition and higher BMI. Dyslipidaemia was the most prevalent comorbidity in our cohort, and was highly prevalent in the psychological, cardiorenal and metabolic clusters. In univariable analysis, dyslipidaemia seemed to decrease mortality in our cohort (Supplementary table S1). However, this could be confounded by the use of statins. No definitive conclusions of the role of dyslipidaemia can be drawn from these findings, and it remains an represents an interesting field for future studies.

Moderately increased albuminuria (previously known as microalbuminuria) which was used as marker of renal damage in our definition of CKD, is also found to be a biomarker of cardiovascular disease (12). It is thought to reflect damage to the microvascular system, and is a common denominator between CKD and MI. This could explain the coexistence of these diseases in the cardiorenal cluster. The lack of association between this cluster and long-term mortality was surprising, as MI and CKD in themselves was strongly associated with mortality (Supplementary table S1). However, this was probably caused by the dispersion of the attributes among the clusters, reducing the power of the associations. This is supported by the low prevalence of obesity, hypertension and diabetes in this cluster, conditions that are otherwise risk factors of both kidney and heart disease, and which could not be explained by confounding by indication, as all conditions were assessed at baseline.

Among the 79 comorbidities included in the network analysis of the COPD "comorbidome", only 12 diseases were significantly associated with mortality (3). Interestingly, depression, a comorbidity found to be associated with mortality in both COPD patients (21) and in the general population (35), was not among the 12 diseases contributing to mortality in the comorbidome (3). In univariate analyses of our study cohort, the association between single comorbidities and mortality did not reflect the mortality of the different clusters. This points towards the complex dynamics among coexisting diseases, and the lack of predicting ability of single comorbidities on adverse outcomes.

In a large primary care database study from the Netherlands, several comorbidities were associated with increased COPD exacerbation risk, including depression, osteoporosis and peripheral vascular disease (36). On the other hand, individuals with comorbid diabetes had decreased risk of exacerbations (36). In a multicentre study, depression was found to be independently associated with COPD exacerbations and hospitalizations (37), whereas the relationship between anxiety and exacerbations was more complex (38).

Strengths and limitations

The study sample was recruited from a general population, with no exclusion criteria, including individuals with mild to very severe COPD. The external validity may therefore be higher than previous studies (5, 6). Comorbidities was objectively measured and defined according to international consensus guidelines, further strengthening the validity.

We aimed to use objective parameters to identify comorbidities. However, this limited the inclusion of comorbidities to those having been assessed at the time of the study cycles. We aimed to include prevalent diseases known to coexist in COPD (3), representing important disease groups including cardiovascular, metabolic and psychiatric illnesses. However, impactful and prevalent diseases including lung cancer, chronic heart failure, obstructive sleep apnoea and loss of skeletal muscle was not assessed but could add further value to the findings. Other lung function measurements including lung diffusion capacity and whole-body plethysmography was only available for a subset of individuals and was not included.

Some of the comorbidities, including anxiety, depression and osteoporosis, had a quite high number of missing values. However, cluster analysis is less impacted by missing values compared to e.g. regression techniques, since the iterative process works as an imputation procedure, allowing complete cases to inform the learning process.

Conclusions

We confirmed five distinct comorbidity clusters in a COPD cohort recruited from a general population. Two of these, a psychological and a cachectic cluster, were significantly associated with mortality and the risk of severe COPD exacerbations. Further exploration of these clusters could add to our understanding of progression and severe outcomes in COPD, inform early intervention, and possible identify future targets for treatment.

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Declaration of interests

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Table 1 Distribution of comorbidities within clusters of 2076 persons with COPD.

	Less comorbidity (n=608)	Psychological (n=410)	Cardiorenal (n=506)	Metabolic (n=325)	Cachectic (n=227)
Mean number of comorbidities	0.68±1.00	2.32±1.13	1.51±0.75	2.70±0.86	1.64±1.02
Hypertension %	27.9	11.1	6.4	65.3	10.6
Dyslipidemia %	1.0	49.0	92.3	100	12.0
Myocardial infarction %	2.8	9.0	23.5	8.3	6.2
Diabetes mellitus %	3.3	1.6	0.8	13.8	0
Obesity %	21.4	14.4	0	61.5	0
Underweight %	0	5.1	3.6	2.5	68.3
Anxiety %	6.0	71.5	3.6	11.9	16.9
Depression %	4.1	73.5	0.2	9.2	10.0
Chronic kidney disease %	4.8	6.4	10.3	13.0	3.2
Osteoporosis %	6.7	14.8	13.5	9.6	57.8

Table 2 Characteristics of the comorbidity clusters in 2076 persons with COPD.

	All individuals (n=2076)	Less comorbidity (n=608)	Psychological (n=410)	Cardiorenal (n=506)	Metabolic (n=325)	Cachectic (n=227)
Female (%)	943 (45.4)	280 (46.1)	199 (48.5)	171 (33.8)	146 (44.9)	147 (64.8)
Age (years)	62.1±11.2	60.8±11.1	62.0±11.5	62.2±11.3	61.5±11.1	65.9±10.0
$pFEV_1$%	63.9±18.3	64.8±18.6	62.3±18.0	65.7±18.1	66.2±16.9	56.9±18.4
FEV_1/FVC	0.57±0.1	0.58±0.1	0.57±0.1	0.58±0.1	0.59±0.1	0.54±0.1
mMRC score	1.6±1.3	1.5±1.3	1.9±1.4	1.5±1.2	1.6±1.4	1.7±1.3
Self-reported health status	2.3±0.6	2.5±0.6	2.1±0.6	2.4±0.6	2.4±0.6	2.3±0.6
Smoking status						
Never	281 (13.9)	89 (17.5)	47 (14.0)	49 (12.0)	49 (18.5)	29 (15.0)
Former	722 (35.6)	175 (34.4)	102 (30.3)	143 (35.0)	106 (40.0)	50 (25.8)
Current	874 (50.5)	244 (48.0)	188 (55.8)	217 (53.1)	110 (41.5)	115 (59.3)
Pack-years	24.7±15.4	23.6±14.8	26.2±14.0	24.6±16.1	25.8±16.6	24.1±15.6
Physical inactivity	636 (30.8)	169 (27.9)	134 (33.0)	143 (28.4)	101 (31.2)	89 (39.4)
Education						
Low (≤9 years)	905 (47.2)	222 (39.2)	195 (53.4)	260 (54.7)	109 (35.6)	119 (58.1)
Medium (10-12 years)	733 (38.2)	237 (41.8)	139 (38.1)	160 (33.7)	132 (43.1)	65 (31.7)
High (≥13 years)	280 (14.6)	108 (19.1)	31 (8.5)	55 (11.6)	65 (21.2)	21 (10.2)

Blue highlight: lowest mean or percentage among clusters. Yellow highlight: highest mean or percentage among clusters.
Summary variables are presented as mean ± standard deviations for quantitative variables, and as percentages for discrete variables.
Definitions: Pack-years: number of packs of cigarettes smoked per day x number of years the patient has smoked. 1 pack = 20 cigarettes. Physical inactivity: ≤1 hour light physical activity and no hard activity (self-reported). Self-reported health status: rated from 1-4 (poor, not so good, good, very good). Low education: ≤9 years.
Abbreviations: BMI; body mass index, FEV1; forced expiratory volume in 1 second, FVC; forced vital capacity, mMRC; modified Medical Research Council dyspnoea scale.

Figure 1 Percentage of different comorbidities in the HUNT COPD cohort (n=2976).

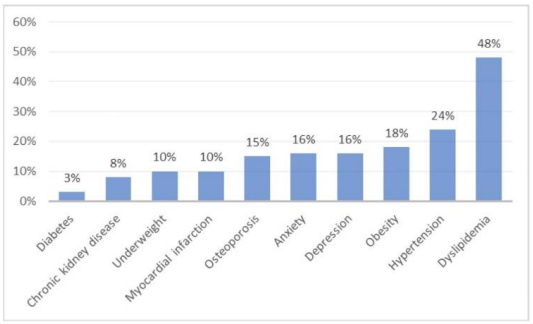


Figure 2 Distribution of comorbidities in the HUNT COPD cohort (n=2076).

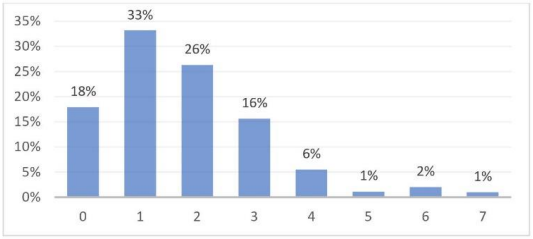


Table 3 Association between comorbidity clusters and all-cause mortality in the HUNT COPD cohort.

	n	Person-years (py)	Observed deaths	Death rate/ 1000 py	Crude HR*	95% CI	Adjusted HR**	95% CI
Less comorbidity	608	8300.0	321	38.7				
Metabolic	325	3956.9	150	37.9	1.02	0.84-1.24	0.95	0.78-1.16
Cardiorenal	506	7221.9	312	43.2	1.09	0.93-1.27	0.99	0.85-1.16
Psychological	410	5393.3	256	47.5	1.23	1.04-1.45	1.19	1.00-1.40
Cachectic	227	2570.8	174	67.7	1.83	1.52-2.20	1.42	1.17-1.71

* Unadjusted ** Adjusted for age and sex.

Table 4 Association between comorbidity clusters and cumulative number of severe COPD exacerbations in the HUNT COPD cohort.

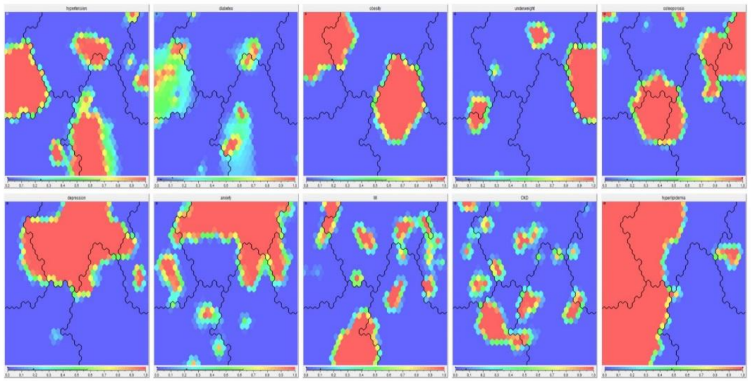
Cluster	n	# of severe exacerbations (±SD)	Crude IRR*	95% CI	Adjusted IRR**	95% CI
Less comorbidity	608	5.3±8.0			*Reference*	
Metabolic	325	4.3±7.4	0.81	0.65-1.02	0.81	0.65-1.01
Cardiorenal	506	6.2±8.9	1.17	0.98-1.39	1.15	0.97-1.37
Psychological	410	6.6±9.1	1.24	1.04-1.49	1.24	1.04-1.48
Cachectic	227	8.0±9.6	1.50	1.23-1.83	1.50	1.23-1.84

Table S1 Prevalence of comorbidities, frequency of missing variables and associations of single comorbidities with all-cause mortality.

Comorbidity	Prevalence	Missing	HR*	95% CI
Osteoporosis	306 (14.7)	178 (8.6)	1.97	1.72-2.27
Anxiety	337 (16.2)	500 (24.1)	1.05	0.90-1.24
Depression	331 (15.9)	302 (14.6)	1.36	1.17-1.58
Hypertension	409 (23.9)	367 (17.7)	0.86	0.71-1.04
Chronic kidney disease	143 (7.5)	47 (2.3)	2.69	2.18-3.31
Diabetes	37 (1.8)	0	1.43	0.91-2.26
Myocardal infarction	214 (10.3)	0	1.37	1.16-1.62
Hyperlipidemia	1006 (48.5)	0	0.92	0.82-1.03
Obesity	389 (18.7)	0	0.92	0.79-1.07
Underweight	202 (9.7)	0	1.69	1.42-2.00

Prevalence and missing values are given in n (%). Abbreviations: HR; hazard ratio, CI; confidence interval.
* Adjusted for sex and age.

Figure 4 Comorbidity clusters in the HUNT COPD cohort.



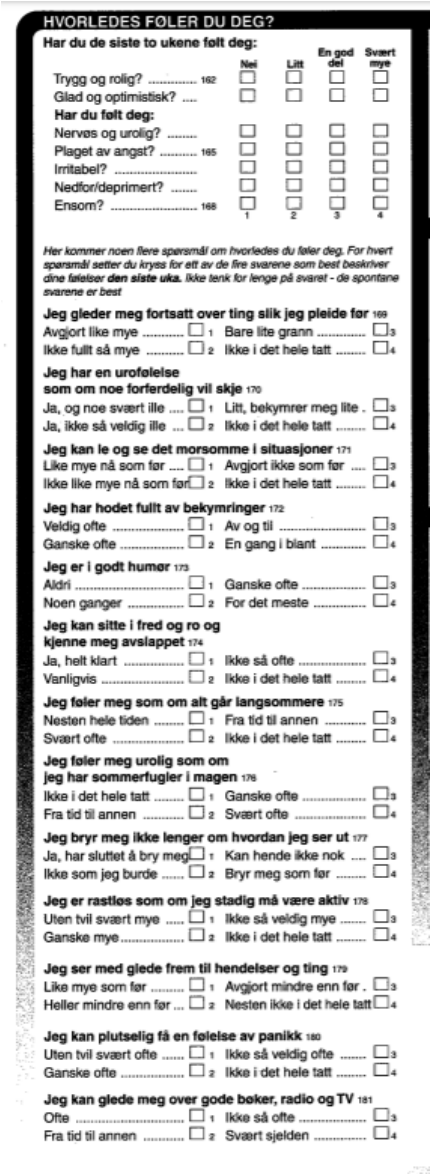
Attribute maps created with Viscovery SOMine (www.viscovery.net). Subjects are placed on the map based on how closely they resemble in comorbidity profiles. Red color represents a high prevalence, and blue color represents a low prevalence. Each attribute (=comorbidity) has a separate map. The black lines represent the clusters identified by the software.

Appendices 1-5

1. HADS
    1.1. Norwegian version (HUNT2 Q1)
    1.2. Norwegian version (HUNT3 Q2)
    1.3. Standardized English version
2. mMRC
    2.1. Norwegian version
    2.2. English version
3. HUNT2 Lung Study Q3
4. HUNT3 Lung Study interview
5. Table of instruments

Appendix 1

HADS questionnaires (Norwegian and English versions)



HVORLEDES FØLER DU DEG?

Har du de siste to ukene følt deg:

	Nei	Litt	En god del	Svært mye
Trygg og rolig? 162	☐	☐	☐	☐
Glad og optimistisk?	☐	☐	☐	☐
Har du følt deg:				
Nervøs og urolig?	☐	☐	☐	☐
Plaget av angst? 165	☐	☐	☐	☐
Irritabel?	☐	☐	☐	☐
Nedfor/deprimert?	☐	☐	☐	☐
Ensom? 168	☐ 1	☐ 2	☐ 3	☐ 4

*Her kommer noen flere spørsmål om hvorledes du føler deg. For hvert spørsmål setter du kryss for ett av de fire svarene som best beskriver dine følelser **den siste uka.** Ikke tenk for lenge på svaret - de spontane svarene er best*

Jeg gleder meg fortsatt over ting slik jeg pleide før 169
Avgjort like mye ☐ 1 — Bare lite grann ☐ 3
Ikke fullt så mye ☐ 2 — Ikke i det hele tatt ☐ 4

Jeg har en urofølelse som om noe forferdelig vil skje 170
Ja, og noe svært ille ☐ 1 — Litt, bekymrer meg lite ☐ 3
Ja, ikke så veldig ille ☐ 2 — Ikke i det hele tatt ☐ 4

Jeg kan le og se det morsomme i situasjoner 171
Like mye nå som før ☐ 1 — Avgjort ikke som før ☐ 3
Ikke like mye nå som før ☐ 2 — Ikke i det hele tatt ☐ 4

Jeg har hodet fullt av bekymringer 172
Veldig ofte ☐ 1 — Av og til ☐ 3
Ganske ofte ☐ 2 — En gang i blant ☐ 4

Jeg er i godt humør 173
Aldri ☐ 1 — Ganske ofte ☐ 3
Noen ganger ☐ 2 — For det meste ☐ 4

Jeg kan sitte i fred og ro og kjenne meg avslappet 174
Ja, helt klart ☐ 1 — Ikke så ofte ☐ 3
Vanligvis ☐ 2 — Ikke i det hele tatt ☐ 4

Jeg føler meg som om alt går langsommere 175
Nesten hele tiden ☐ 1 — Fra tid til annen ☐ 3
Svært ofte ☐ 2 — Ikke i det hele tatt ☐ 4

Jeg føler meg urolig som om jeg har sommerfugler i magen 176
Ikke i det hele tatt ☐ 1 — Ganske ofte ☐ 3
Fra tid til annen ☐ 2 — Svært ofte ☐ 4

Jeg bryr meg ikke lenger om hvordan jeg ser ut 177
Ja, har sluttet å bry meg ☐ 1 — Kan hende ikke nok ☐ 3
Ikke som jeg burde ☐ 2 — Bryr meg som før ☐ 4

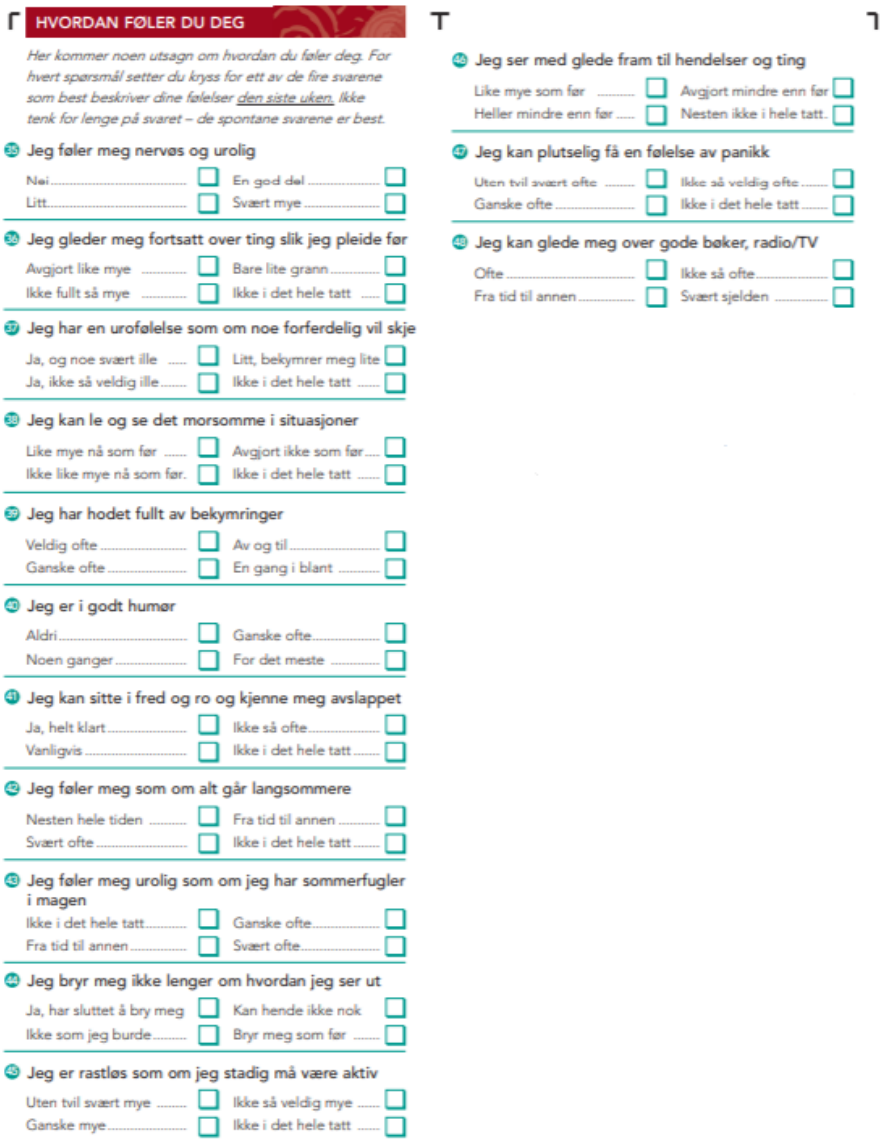
Jeg er rastløs som om jeg stadig må være aktiv 178
Uten tvil svært mye ☐ 1 — Ikke så veldig mye ☐ 3
Ganske mye ☐ 2 — Ikke i det hele tatt ☐ 4

Jeg ser med glede frem til hendelser og ting 179
Like mye som før ☐ 1 — Avgjort mindre enn før ☐ 3
Heller mindre enn før ☐ 2 — Nesten ikke i det hele tatt ☐ 4

Jeg kan plutselig få en følelse av panikk 180
Uten tvil svært ofte ☐ 1 — Ikke så veldig ofte ☐ 3
Ganske ofte ☐ 2 — Ikke i det hele tatt ☐ 4

Jeg kan glede meg over gode bøker, radio og TV 181
Ofte ☐ 1 — Ikke så ofte ☐ 3
Fra tid til annen ☐ 2 — Svært sjelden ☐ 4

1.1. HADS Norwegian version (HUNT2 Q1)



HVORDAN FØLER DU DEG

Her kommer noen utsagn om hvordan du føler deg. For hvert spørsmål setter du kryss for ett av de fire svarene som best beskriver dine følelser den siste uken. Ikke tenk for lenge på svaret – de spontane svarene er best.

35 Jeg føler meg nervøs og urolig

- Nei
- En god del
- Litt
- Svært mye

36 Jeg gleder meg fortsatt over ting slik jeg pleide før

- Avgjort like mye
- Bare lite grann
- Ikke fullt så mye
- Ikke i det hele tatt

37 Jeg har en urofølelse som om noe forferdelig vil skje

- Ja, og noe svært ille
- Litt, bekymrer meg lite
- Ja, ikke så veldig ille
- Ikke i det hele tatt

38 Jeg kan le og se det morsomme i situasjoner

- Like mye nå som før
- Avgjort ikke som før
- Ikke like mye nå som før.
- Ikke i det hele tatt

39 Jeg har hodet fullt av bekymringer

- Veldig ofte
- Av og til
- Ganske ofte
- En gang i blant

40 Jeg er i godt humør

- Aldri
- Ganske ofte
- Noen ganger
- For det meste

41 Jeg kan sitte i fred og ro og kjenne meg avslappet

- Ja, helt klart
- Ikke så ofte
- Vanligvis
- Ikke i det hele tatt

42 Jeg føler meg som om alt går langsommere

- Nesten hele tiden
- Fra tid til annen
- Svært ofte
- Ikke i det hele tatt

43 Jeg føler meg urolig som om jeg har sommerfugler i magen

- Ikke i det hele tatt
- Ganske ofte
- Fra tid til annen
- Svært ofte

44 Jeg bryr meg ikke lenger om hvordan jeg ser ut

- Ja, har sluttet å bry meg
- Kan hende ikke nok
- Ikke som jeg burde
- Bryr meg som før

45 Jeg er rastløs som om jeg stadig må være aktiv

- Uten tvil svært mye
- Ikke så veldig mye
- Ganske mye
- Ikke i det hele tatt

46 Jeg ser med glede fram til hendelser og ting

- Like mye som før
- Avgjort mindre enn før
- Heller mindre enn før
- Nesten ikke i hele tatt

47 Jeg kan plutselig få en følelse av panikk

- Uten tvil svært ofte
- Ikke så veldig ofte
- Ganske ofte
- Ikke i det hele tatt

48 Jeg kan glede meg over gode bøker, radio/TV

- Ofte
- Ikke så ofte
- Fra tid til annen
- Svært sjelden

1.2 HADS Norwegian version (HUNT3 Q2)

Hospital Anxiety and Depression Scale (HADS)

Tick the box beside the reply that is closest to how you have been feeling in the past week.
Don't take too long over you replies: your immediate is best.

D	A		D	A	
		I feel tense or 'wound up':			**I feel as if I am slowed down:**
	3	Most of the time	3		Nearly all the time
	2	A lot of the time	2		Very often
	1	From time to time, occasionally	1		Sometimes
	0	Not at all	0		Not at all
		I still enjoy the things I used to enjoy:			**I get a sort of frightened feeling like 'butterflies' in the stomach:**
0		Definitely as much		0	Not at all
1		Not quite so much		1	Occasionally
2		Only a little		2	Quite Often
3		Hardly at all		3	Very Often
		I get a sort of frightened feeling as if something awful is about to happen:			**I have lost interest in my appearance:**
	3	Very definitely and quite badly	3		Definitely
	2	Yes, but not too badly	2		I don't take as much care as I should
	1	A little, but it doesn't worry me	1		I may not take quite as much care
	0	Not at all	0		I take just as much care as ever
		I can laugh and see the funny side of things:			**I feel restless as I have to be on the move:**
0		As much as I always could		3	Very much indeed
1		Not quite so much now		2	Quite a lot
2		Definitely not so much now		1	Not very much
3		Not at all		0	Not at all
		Worrying thoughts go through my mind:			**I look forward with enjoyment to things:**
	3	A great deal of the time	0		As much as I ever did
	2	A lot of the time	1		Rather less than I used to
	1	From time to time, but not too often	2		Definitely less than I used to
	0	Only occasionally	3		Hardly at all
		I feel cheerful:			**I get sudden feelings of panic:**
3		Not at all		3	Very often indeed
2		Not often		2	Quite often
1		Sometimes		1	Not very often
0		Most of the time		0	Not at all
		I can sit at ease and feel relaxed:			**I can enjoy a good book or radio or TV program:**
	0	Definitely	0		Often
	1	Usually	1		Sometimes
	2	Not Often	2		Not often
	3	Not at all	3		Very seldom

Please check you have answered all the questions

Scoring:
Total score: Depression (D) _____ Anxiety (A) _____
0-7 = Normal
8-10 = Borderline abnormal (borderline case)
11-21 = Abnormal (case)

Appendix 2

mMRC dyspnea scale (Norwegian and English versions)

7 Hvor mye, og i hvilke situasjoner blir du plaget av pustebesvær?

(Velg ett alternativ)

Jeg blir bare tungpustet når jeg virkelig anstrenger meg, ikke når jeg tar en rask spasertur eller går i motbakke	☐	(Lite plaget)
Jeg blir tungpustet når jeg tar en rask spasertur eller går i motbakke.....	☐	
Jeg blir tungpustet når jeg går på flat mark i forhold til andre på min alder	☐	(Plaget)
Jeg blir så tungpustet når jeg går på flat mark at jeg må stoppe opp selv om jeg bestemmer farten	☐	
Jeg blir tungpustet når jeg vasker meg eller kler på meg.....	☐	(Mye plaget)

2.1 mMRC – Norwegian version

Grade	Description of Breathlessness
0	I only get breathless with strenuous exercise.
1	I get short of breath when hurrying on level ground or walking up a slight hill.
2	On level ground, I walk slower than people of the same age because of breathlessness, or have to stop for breath when walking at my own pace.
3	I stop for breath after walking about 100 yards or after a few minutes on level ground.
4	I am too breathless to leave the house or I am breathless when dressing.

2.2 mMRC – English version

Appendix 3

HUNT2 Lung Study questionnaire (Q3)

hunt-lunge

Helseundersøkelsen i Nord-Trøndelag

Hvis du ikke ønsker å besvare spørreskjemaet: Sett kryss her og returner skjemaet. Da slipper du purring.
Jeg ønsker ikke å besvare skjemaet ☐

Ved at du fyller ut dette skjemaet hjelper du oss i arbeidet med å forbedre behandlingen av lungesykdommer i Les forøvrig brosjyren «hunt - spesial» som du fikk ved Helseundersøkelsen.

Lykke til !

Blir du vanligvis tungpust, får piping i brystet eller uttelt hoste i disse situasjoner:

	Ja	Nei
* Ved fysisk aktivitet utendørs i kaldt vær?	☐	☐ 01
* I støvete eller røykfylte omgivelser?	☐	☐ 02
* Ved mye bilekspos eller andre former for luftforurensning?	☐	☐ 03
* Ved sterke lukter, parfymer, krydder, såper, trykksverte osv?	☐	☐ 04

Når du er i nærheten av dyr, fjær eller i en støvfylt del av huset, har du da noen gang opplevd at du:

	Ja	Nei
* Starter å hoste?	☐	☐ 05
* Får piping i brystet?	☐	☐ 06
* Føler deg tett i brystet?	☐	☐ 07
* Føler deg tungpust?	☐	☐ 08
* Får tett eller rennende nese eller begynner å nyse?	☐	☐ 09
* Får kløe eller renning fra øyne?	☐	☐ 10

Når du er i nærheten av trær, gress, blomster eller når det er mye pollen, har du da noen gang opplevd at du:

	Ja	Nei
* Starter å hoste?	☐	☐ 11
* Får piping i brystet?	☐	☐ 12
* Føler deg tett i brystet?	☐	☐ 13
* Føler deg tungpust?	☐	☐ 14
* Får tett eller rennende nese eller begynner å nyse?	☐	☐ 15
* Får kløe eller renning fra øyne?	☐	☐ 16

	Ja	Nei
Får du tung pust eller pipelyder i brystet under arbeid?	☐	☐ 17
Har du noen gang skiftet eller sluttet i arbeid fordi du ble plaget med pusten?	☐	☐ 18

Hvis «Ja» på forrige spørsmål, hvilket yrke var det?

..... 19
(Skriv hvilke yrke her)

	Ja	Nei
Blir du mer tungpust (andpusten) enn jevnaldrende når du går i motbakker?	☐	☐ 20
Blir du tungpust når du går opp 2 etasjer i vanlig fart?	☐	☐ 21
Blir du tungpust når du går med vanlig fart på flat mark?	☐	☐ 22

	Ja	Nei
Er du tungpust når du sitter i ro?	☐	☐ 23

I hvor stor grad vil du si at pusteplager hemmer dine daglige aktiviteter? (sett ett kryss)

Ikke i det hele tatt	Litt	I stor grad	I svært stor grad	
☐	☐	☐	☐	24

Da du var barn, hadde dere noen av følgende kjæledyr:

	Ja	Nei
* Katter ?	☐	☐ 25
* Hunder ?	☐	☐ 26
* Hester ?	☐	☐ 27
* Fugler ?	☐	☐ 28
* Andre pelskledde dyr?	☐	☐ 29

	Ja	Nei
Har du fått diagnosen astma av lege?	☐	☐ 30
Har du fått diagnosen kronisk bronkitt eller emfysem av lege?	☐	☐ 31
Har du vært til undersøkelse hos allmennpraktiserende lege pga pusteplager?	☐	☐ 32
Hvis «Ja», er det tatt pusteprøve av deg der?	☐	☐ 33
Har du vært til undersøkelse hos barnelege, lungelege eller annen sykehuslege pga pusteplager?	☐	☐ 34
Hvis «Ja», har du vært til undersøkelse hos slik lege siste 12 mnd?	☐	☐ 35

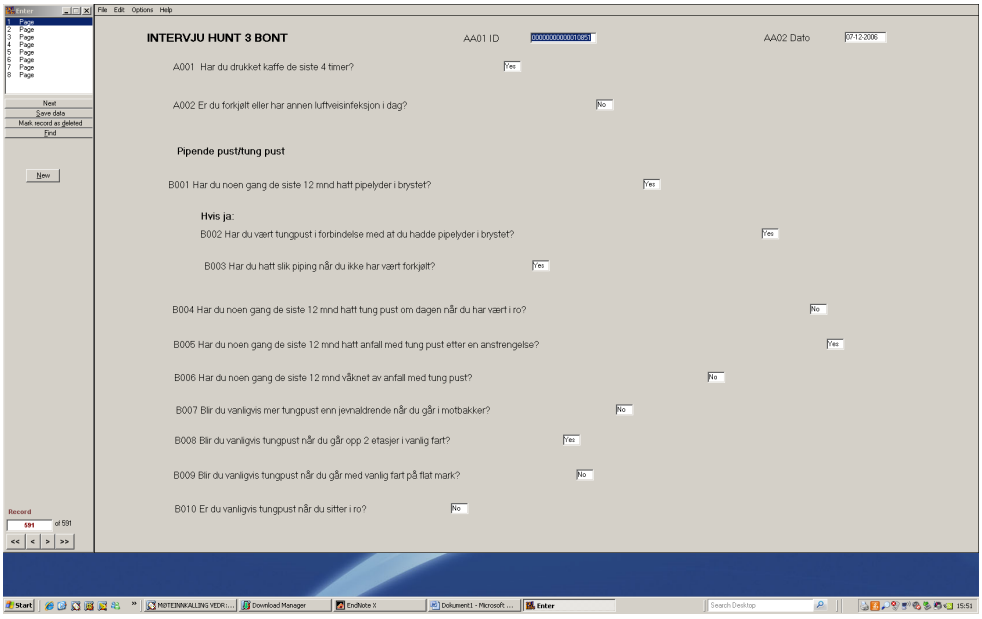
Vennligst legg skjemaet i samme konvolutt som det andre skjemaet du fikk ved Helseundersøkelsen og postlegg den snarest.

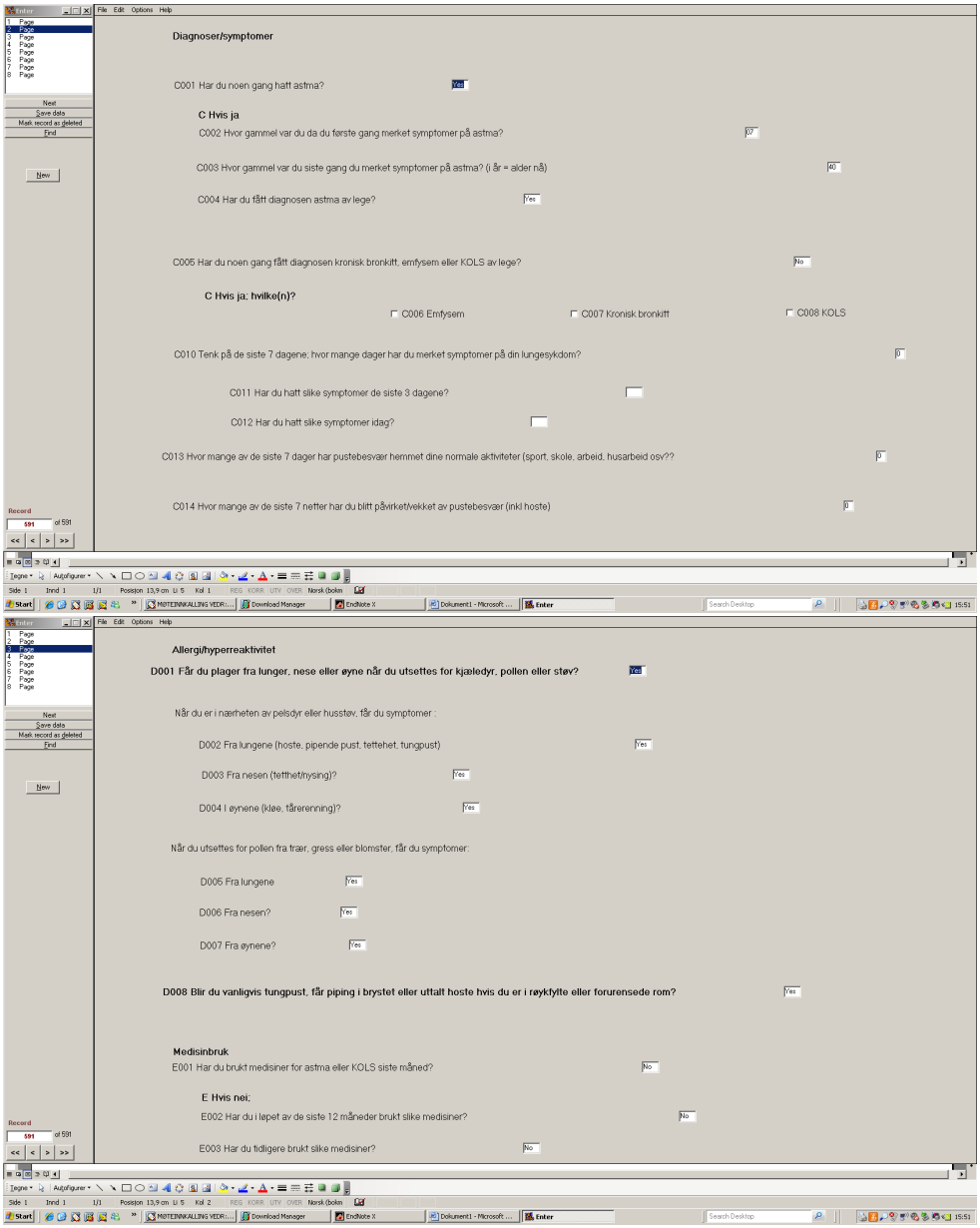
Porto er betalt.

Hjertelig takk for hjelpen!

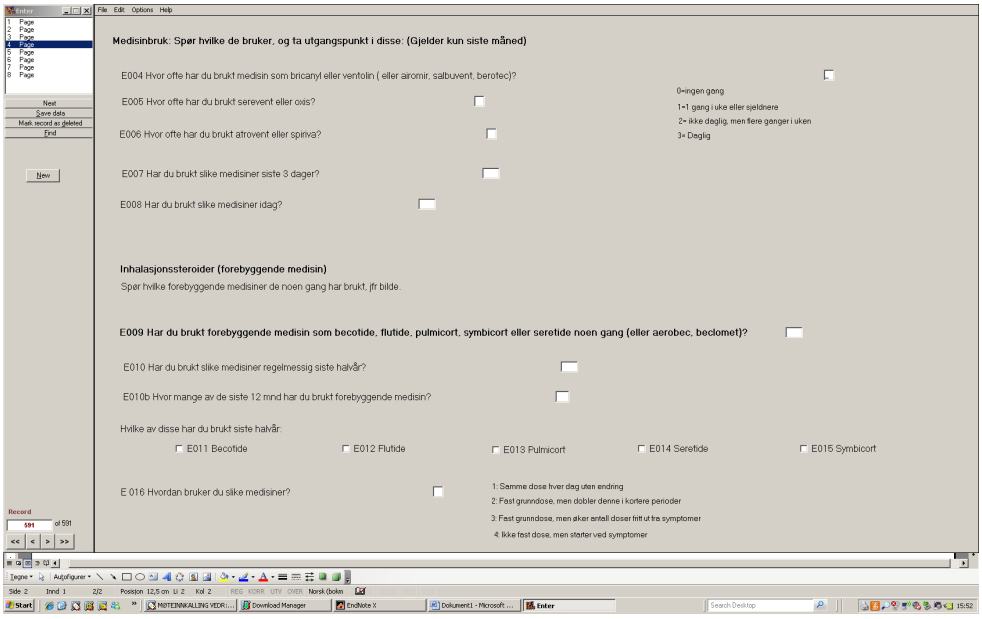
Appendix 4

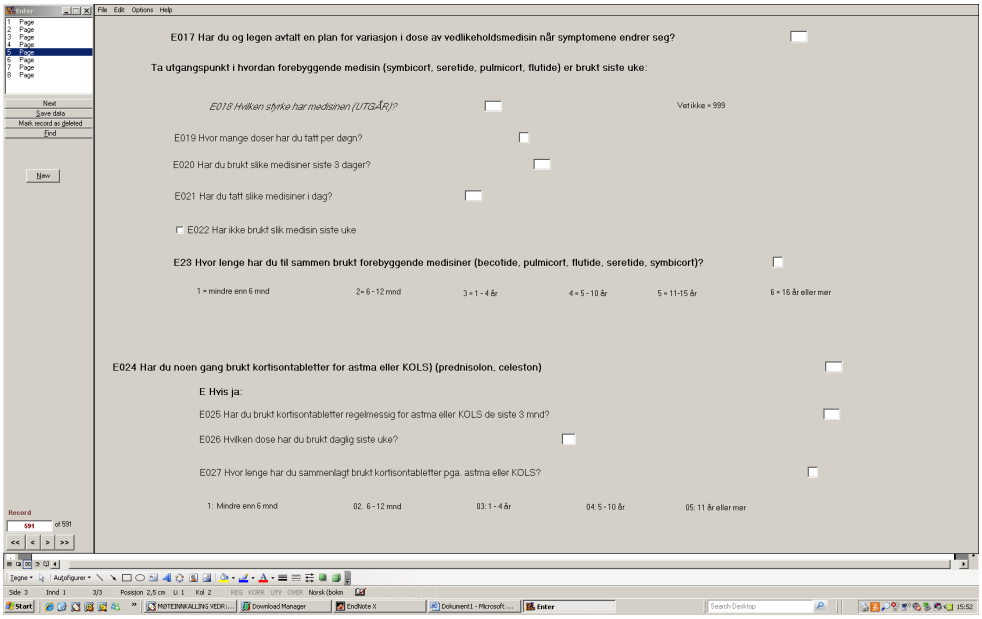
HUNT3 Lung Study Interview

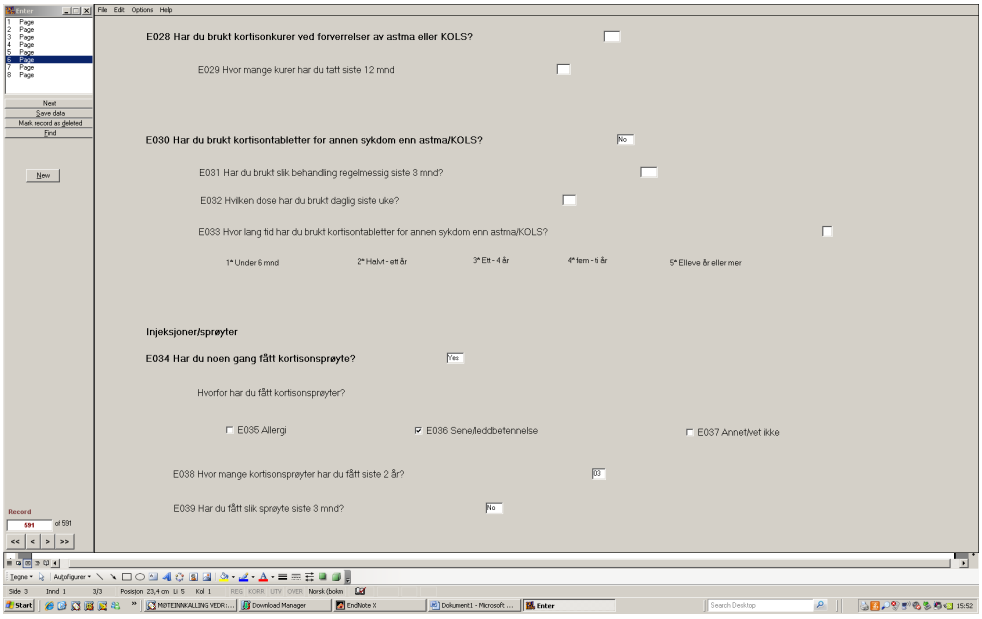

File Edit Options Help
INTERVJU HUNT 3 BONT
AA01 ID
AA02 Dato
07-12-2006
A001 Har du drukket kaffe de siste 4 timer?
A002 Er du forkjølt eller har annen luftveisinfeksjon i dag?
Pipende pust/tung pust
B001 Har du noen gang de siste 12 mnd hatt pipelyder i brystet?
Hvis ja:
B002 Har du vært tungpust i forbindelse med at du hadde pipelyder i brystet?
B003 Har du hatt slik piping når du ikke har vært forkjølt?
B004 Har du noen gang de siste 12 mnd hatt tung pust om dagen når du har vært i ro?
B005 Har du noen gang de siste 12 mnd hatt anfall med tung pust etter en anstrengelse?
B006 Har du noen gang de siste 12 mnd våknet av anfall med tung pust?
B007 Blir du vanligvis mer tungpust enn jevnaldrende når du går i motbakker?
B008 Blir du vanligvis tungpust når du går opp 2 etasjer i vanlig fart?
B009 Blir du vanligvis tungpust når du går med vanlig fart på flat mark?
B010 Er du vanligvis tungpust når du sitter i ro?
Next
Save data
Mark record as deleted
Find
New
Record
of 591


Diagnoser/symptomer
C001 Har du noen gang hatt astma?
C Hvis ja
C002 Hvor gammel var du da du første gang merket symptomer på astma?
C003 Hvor gammel var du siste gang du merket symptomer på astma? (i år = alder nå)
C004 Har du fått diagnosen astma av lege?
C005 Har du noen gang fått diagnosen kronisk bronkitt, emfysem eller KOLS av lege?
C Hvis ja; hvilke(n)?
C006 Emfysem
C007 Kronisk bronkitt
C008 KOLS
C010 Tenk på de siste 7 dagene; hvor mange dager har du merket symptomer på din lungesykdom?
C011 Har du hatt slike symptomer de siste 3 dagene?
C012 Har du hatt slike symptomer idag?
C013 Hvor mange av de siste 7 dager har pustebesvær hemmet dine normale aktiviteter (sport, skole, arbeid, husarbeid osv??
C014 Hvor mange av de siste 7 netter har du blitt påvirket/vekket av pustebesvær (inkl hoste)

Allergi/hyperreaktivitet
D001 Får du plager fra lunger, nese eller øyne når du utsettes for kjæledyr, pollen eller støv?
Når du er i nærheten av pelsdyr eller husstøv, får du symptomer :
D002 Fra lungene (hoste, pipende pust, tetthet, tungpust)
D003 Fra nesen (tetthet/nysing)?
D004 I øynene (kløe, tårerenning)?
Når du utsettes for pollen fra trær, gress eller blomster, får du symptomer:
D005 Fra lungene
D006 Fra nesen?
D007 Fra øynene?
D008 Blir du vanligvis tungpust, får piping i brystet eller uttalt hoste hvis du er i røykfylte eller forurensede rom?
Medisinbruk
E001 Har du brukt medisiner for astma eller KOLS siste måned?
E Hvis nei;
E002 Har du i løpet av de siste 12 måneder brukt slike medisiner?
E003 Har du tidligere brukt slike medisiner?


Medisinbruk: Spør hvilke de bruker, og ta utgangspunkt i disse: (Gjelder kun siste måned)
E004 Hvor ofte har du brukt medisin som bricanyl eller ventolin (eller airomir, salbuvent, berotec)?
E005 Hvor ofte har du brukt serevent eller oxis?
E006 Hvor ofte har du brukt atrovent eller spiriva?
0=ingen gang
1=1 gang i uke eller sjeldnere
2= ikke daglig, men flere ganger i uken
3= Daglig
E007 Har du brukt slike medisiner siste 3 dager?
E008 Har du brukt slike medisiner idag?
Inhalasjonssteroider (forebyggende medisin)
Spør hvilke forebyggende medisiner de noen gang har brukt, jfr bilde.
E009 Har du brukt forebyggende medisin som becotide, flutide, pulmicort, symbicort eller seretide noen gang (eller aerobec, beclomet)?
E010 Har du brukt slike medisiner regelmessig siste halvår?
E010b Hvor mange av de siste 12 mnd har du brukt forebyggende medisin?
Hvilke av disse har du brukt siste halvår:
E011 Becotide
E012 Flutide
E013 Pulmicort
E014 Seretide
E015 Symbicort
E 016 Hvordan bruker du slike medisiner?
1: Samme dose hver dag uten endring
2: Fast grunndose, men dobler denne i kortere perioder
3: Fast grunndose, men øker antall doser hvit ut tre symptomer
4: Ikke fast dose, men starter ved symptomer


E017 Har du og legen avtalt en plan for variasjon i dose av vedlikeholdsmedisin når symptomene endrer seg?
Ta utgangspunkt i hvordan forebyggende medisin (symbicort, seretide, pulmicort, flutide) er brukt siste uke:
E018 Hvilken styrke har medisinen (UTGÅR)?
Vet ikke = 999
E019 Hvor mange doser har du tatt per døgn?
E020 Har du brukt slike medisiner siste 3 dager?
E021 Har du tatt slike medisiner i dag?
E022 Har ikke brukt slik medisin siste uke
E23 Hvor lenge har du til sammen brukt forebyggende medisiner (becotide, pulmicort, flutide, seretide, symbicort)?
1 = mindre enn 6 mnd
2= 6 - 12 mnd
3 = 1 - 4 år
4 = 5 - 10 år
5 = 11-15 år
6 = 16 år eller mer
E024 Har du noen gang brukt kortisontabletter for astma eller KOLS) (prednisolon, celeston)
E Hvis ja:
E025 Har du brukt kortisontabletter regelmessig for astma eller KOLS de siste 3 mnd?
E026 Hvilken dose har du brukt daglig siste uke?
E027 Hvor lenge har du sammenlagt brukt kortisontabletter pga. astma eller KOLS?
1: Mindre enn 6 mnd
02. 6 - 12 mnd
03: 1 - 4 år
04: 5 - 10 år
05: 11 år eller mer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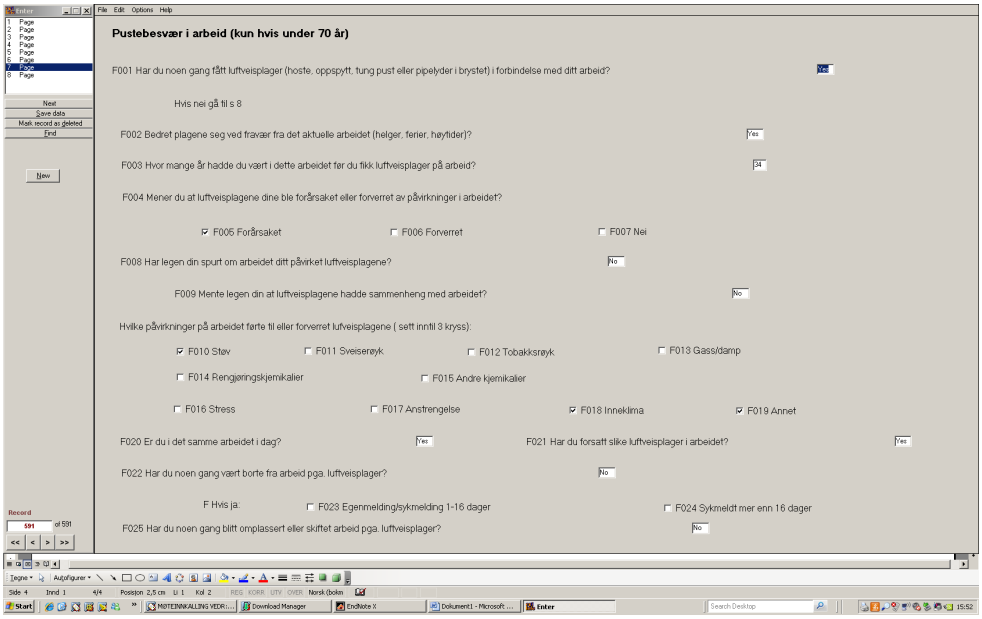
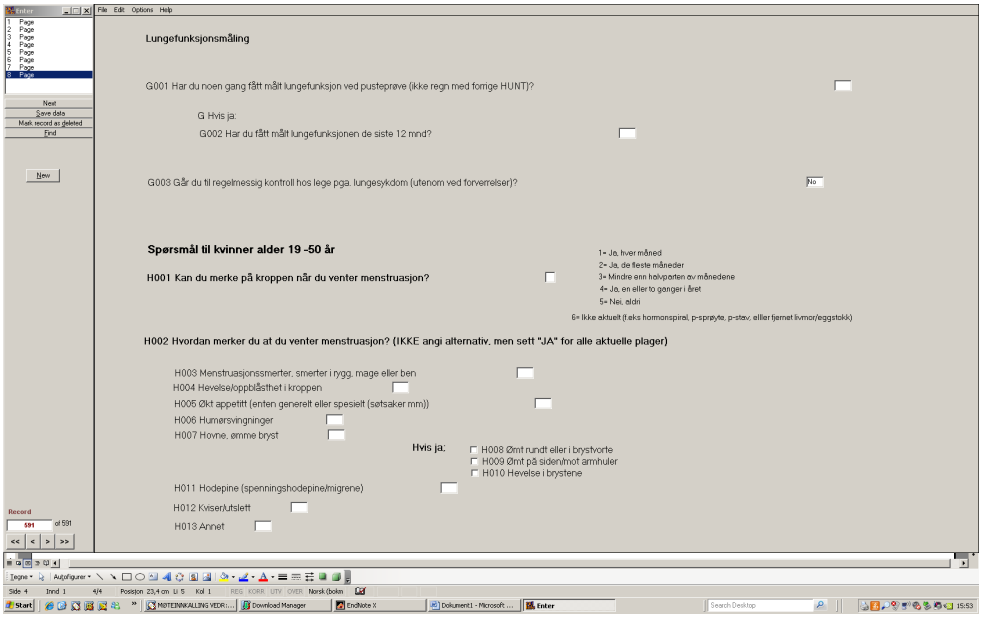
E028 Har du brukt kortisonkurer ved forverrelser av astma eller KOLS?
E029 Hvor mange kurer har du tatt siste 12 mnd
E030 Har du brukt kortisontabletter for annen sykdom enn astma/KOLS?
No
E031 Har du brukt slik behandling regelmessig siste 3 mnd?
E032 Hvilken dose har du brukt daglig siste uke?
E033 Hvor lang tid har du brukt kortisontabletter for annen sykdom enn astma/KOLS?
1* Under 6 mnd
2* Halvt - ett år
3* Ett - 4 år
4* fem - ti år
5* Elleve år eller mer
Injeksjoner/sprøyter
E034 Har du noen gang fått kortisonsprøyte?
Yes
Hvorfor har du fått kortisonsprøyter?
E035 Allergi
E036 Sene/leddbetennelse
E037 Annet/vet ikke
E038 Hvor mange kortisonsprøyter har du fått siste 2 år?
03
E039 Har du fått slik sprøyte siste 3 mnd?
No
New
Save data
Mark record as deleted
Find
Record
591
of 591


File Edit Options Help
Pustebesvær i arbeid (kun hvis under 70 år)
F001 Har du noen gang fått luftveisplager (hoste, oppspytt, tung pust eller pipelyder i brystet) i forbindelse med ditt arbeid?
Yes
Hvis nei gå til s 8
F002 Bedret plagene seg ved fravær fra det aktuelle arbeidet (helger, ferier, høytider)?
Yes
F003 Hvor mange år hadde du vært i dette arbeidet før du fikk luftveisplager på arbeid?
34
F004 Mener du at luftveisplagene dine ble forårsaket eller forverret av påvirkninger i arbeidet?
F005 Forårsaket
F006 Forverret
F007 Nei
F008 Har legen din spurt om arbeidet ditt påvirket luftveisplagene?
No
F009 Mente legen din at luftveisplagene hadde sammenheng med arbeidet?
No
Hvilke påvirkninger på arbeidet førte til eller forverret lufveisplagene (sett inntil 3 kryss):
F010 Støv
F011 Sveiserøyk
F012 Tobakksrøyk
F013 Gass/damp
F014 Rengjøringskjemikalier
F015 Andre kjemikalier
F016 Stress
F017 Anstrengelse
F018 Inneklima
F019 Annet
F020 Er du i det samme arbeidet i dag?
Yes
F021 Har du forsatt slike luftveisplager i arbeidet?
Yes
F022 Har du noen gang vært borte fra arbeid pga. luftveisplager?
No
F Hvis ja.
F023 Egenmelding/sykmelding 1-16 dager
F024 Sykmeldt mer enn 16 dager
F025 Har du noen gang blitt omplassert eller skiftet arbeid pga. luftveisplager?
No
New
Save data
Mark record as deleted
Find
Record
591
of 591



Interview version dated December 10. 2007

Appendix 5

Table of instruments

Name of instrument variable	Object	Target group	Type	Name of scales & ways of scoring	Properties	Commentary	References
Hospital Anxiety and Depression Scale (HADS)	Symptoms of anxiety and depression	Adult out-patients with chronic diseases	Inventory with two subscales (anxiety/depression), each with 7 items. Likert scale 0-3 on each item.	0-3 for each item, summarized as a total score on each subscale (HADS-A, HADS-D), as well as a total score (HADS-T). Clinical cut-offs: 0-7 (normal); 8-10 (borderline); >10 (caseness) OR ≥8 (caseness).	**Reliability** 0.80 **Validity** 0.80	Licensing: https://eprovide.mapi-trust.org/instruments/hospital-anxiety-and-depression-scale HADS copyright © R.P. Snaith and A.S. Zigmond, 1983, 1992, 1994. Record form items originally published in Acta Psychiatrica Scandinavica 67, 361–70, copyright © Munksgaard International Publishers Ltd, Copenhagen, 1983. Published by GL Assessment Limited, 1st Floor Vantage London, Great West Road, London TW8 9AG, UK. All rights reserved. GL Assessment is part of the GL Education Group.	Snaith RP. The Hospital Anxiety and Depression Scale. Health Qual Life Outcomes 2003; 1: 29. Mykletun, A. Hospital Anxiety and Depression (HAD) scale: factor structure, item analyses and internal consistency in a large population. Br J Psychiatry, 2001.
Modified Medical Research Council (mMRC) test	Symptoms of dyspnea	Adults with COPD	Self-report. 4 items – respondent chooses the most fitting statement.	Scored on a continuous scale from 0-4. Clinical cut-off at 2.	**Reliability** 0.72		Mahler DA, Wells CK. Evaluation of clinical methods for rating dyspnea. Chest. 1988;93(3):580-6. Mahler DA. Patient-Reported Dyspnea in COPD Reliability and Association With Stage of Disease. Chest. 2009.
DTX-100	SXA of distal forearm.	All.	Bone densitometry.	According to WHO criteria: T-score > –1.0 (normal), –1.0 to –2.5 (osteopenia) and < –2.5 (osteoporosis).	No information available.		Hans, DB. Peripheral Dual-Energy X-ray Absorptiometry in the Management of Osteoporosis: The 2007 ISCD Official Positions. J Clin
DTX-200	DXA of distal forearm.	All.			**Precision:** In vivo precision in measurement of 15 volunteers 5 times each with repositioning		

					between all replicate measurements; Distal BMC 0.68%, distal BMD 0.87, nROI BMD 1.11% **Accuracy:** Measured with repeated reading using a phantom: within ±0.5%.		Densitometry 2008.
GE Lunar Prodigy	DXA of total hip and lumbar columna L1-L4.	All.			**Precision:** <1.5 **Accuracy:** >90% (hip)		