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Peter Hatlen

Lung cancer - influence of comorbidity on incidence and survival

The Nord-Trøndelag Health study

Doctoral Thesis

Peter Hatlen

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The role and contribution of the PhD candidate

a) PhD education: completed all courses, passed exams, achieved the score needed

b) Scientific work - the three papers

He has performed continuously literature research, worked independently under supervision, and coordinated the involved cooperators and registry applications

He has contributed to the study ideas, designs and performed applications.

He has been the main contributor working with the data files, analyzing data, including statistical calculations, sorting out and working with the results

He has been the main contributor writing the manuscripts and responded to review

He has taken part in inclusion of participants in the PEG study and NLCG registry study

He has planned, organized and lead the project meetings

He has prepared and presented abstract presentations, on international congresses (first and second paper)

c) Compilation: the PhD candidate has been the main contributor to the compilation, put the PhD work in the current context of this research field

d) Given topic / lecture on the day of dissertation: he will independently within the given time limits prepare and present the given lecture before the dissertation

Abstracts presented at international congresses

1. ERS 2011 Amsterdam, Netherland Sept 2011 - Poster discussion. Prolonged survival in lung cancer patients with diabetes mellitus: a large cohort study. Peter Hatlen, Bjørn Henning Grønberg, Arnulf Langhammer, Sven M. Carlsen, Tore Amundsen

2. ERS, Vienna, Austria, Sept 2012 – Poster discussion. Osteoporosis and risk of NSCLC, the HUNT study. Peter Hatlen, Arnulf Langhammer, Siri Forsmo, BH Grønberg, Sven M. Carlsen, Tore Amundsen

3. ERS, Barcelona, Spain Sept 2013 – Oral presentation. Cardio vascular disease and the risk of lung cancer, the HUNT study. Hatlen P, Carlsen SM, Salvesen Ø, Langhammer A. Amundsen T

Trondheim, October 2013

Lunge kreft og samtidig opptredende sykdommer

I Norge ble det i 2010 diagnostisert 2826 lungekrefttilfeller. Ved utgangen av 2010 levde 16% av kvinner og 11% av men som fikk diagnosen lungekreft lenger enn 5 år. Som oftest diagnostiseres lungekreft i et såpass langt kommet stadium at helbredende behandling ikke lenger er mulig. Tidlig diagnostikk av lungekreft er derfor viktig for å kunne tilby flere pasienter en behandling som kan gjøre dem friske eller gi langtidsoverlevelse.
Gjennomsnittlig alder ved diagnosetidspunkt er ca. 70 år, og derfor har mange av pasientene med lungekreft andre sykdommer i tillegg. Noen sykdommer opptrer hyppig hos pasienter med lungekreft. Man kan spørre seg om det er ren tilfeldighet eller om det kan være en direkte sammenheng. Videre er det uavklart om samtidig opptredende sykdom kan påvirke utvikling av og overlevelse ved lungekreft.
Denne avhandlingen har som målsetning å besvare følgende spørsmål:

- Påvirker andre sykdommer overlevelsen hos pasienter med lungekreft?
- Påvirker andre sykdommer risikoen for å utvikle lungekreft?

Det første forskningsspørsmålet ble undersøkt hos pasienter med lungekreft med og uten diabetes mellitus. Vi fant at pasienter med lungekreft og diabetes mellitus lever lenger enn pasienter med lungekreft som ikke har diabetes mellitus.
Det andre forskningsspørsmålet ble undersøkt ved å studere en mulig sammenheng mellom beintetthet og opptreden av lungekreft, og videre ved å undersøke en eventuell sammenheng mellom hjerte-kar-sykdom og opptreden av lungekreft. Personer med lav bentetthet hadde en høyere risiko for lungekreft i forhold til personer med normal bentetthet.
Vi fant videre at personer som røyker og har hjerte-kar-sykdom har en høyere risiko for lungekreft uavhengig av røykemengde.
Avhandlingen viser at samtidige sykdommer kan ha betydning for risiko for å utvikle lungekreft og for overlevelse hos pasienter med lungekreft.

Kandidatens navn: *Peter Hatlen*
Institutt: *Institutt for sirkulasjon og bildediagnostikk*
Veiledere: *Tore Amundsen, Arnulf Langhammer, Bjørn Henning Grønberg, Sven Magnus Carlsen*

Table of content

1. Acknowledgements

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I would like to thank all the participants in the HUNT studies. Without them it would not be possible for me to carry out the present thesis.

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Finally, I want to thank my family for their patience and support.

2. List of papers

2.1. 1st paper

Prolonged Survival in Patients with Lung Cancer with Diabetes Mellitus

Hatlen P, Grønberg BH, Langhammer A, Carlsen SM, Amundsen Tore

J Thorac Oncol. 2011 Nov; 6(11):1810-1817

PMID: 21964531

2.2. 2nd paper

Bone mineral density, fracture history, self-reported osteoporosis as proxy variables for estrogen and the risk of non-small-cell lung cancer — A population based cohort study, the HUNT study: Are proxy variables friends or faults?

Hatlen P, Langhammer A, Forsmo S, Carlsen SM, Amundsen T

Lung Cancer 2013 Jul; 81(1):39-46. doi: 10.1016/j.lungcan.2013.04.001. Epub 2013 Apr 22.

2.3. 3rd paper

Cardio vascular disease and the risk of lung cancer, the HUNT study

Hatlen P, Langhammer A, Carlsen SM, Salvesen Ø, Amundsen T

Abstract 4636: oral presentation ERS, Barcelona 2013.

3. Abbreviations and terms

ASCO	American Society of Clinical Oncology
ANOVA	Analysis of variance
BMI	Body mass index
BMD	Bone mineral density
CRN	Cancer registry of Norway
CT	Computer tomography
CI	Confidence interval
COPD	Chronical obstructive Pulmonary disease
DM	Diabetes mellitus
Eml4-alk	echinoderm microtubule associated protein like 4 anaplastic lymphoma kinase
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
FISH	Fluorescence in situ hybridization
FEV1	Forced expiratory volume in one second
FVC	Forced vital capacity
HR	Hazard ratio
HRT	Hormone replacement therapy
IHC	Immunohistochemistry
IGF-1R	Insulin like growth factor 1 receptor
IASLC	International Association for the Study of Lung Cancer
ICD	International Classification of Diseases
Kras	Kirsten rat sarcoma viral oncogene homolog
MR	Magnetic resonance tomography
NSCLC	Non small cell lung cancer
HUNT	Nord-Trøndelag Health Study
NLCB	Norsk lunge kreft biobank
N	Number
OR	Odds Ratio
OS	Overall survival
PEG	Pemetrexed-Gemcitabine Study
PS	Performance status
PY	Pack years
PET-CT	Positron emission tomography - CT
PASW	Predictive Analytics Soft Ware

REK	Regional Committee for Medical and Health Research Ethics
SXA	Single Energy X-ray Absorptiometry
SCLC	Small cell lung cancer
SD	Standard deviation
SSB	Statistic Norway
SBRT	Stereotactic body radiation therapy
US	United States
VDR	Vitamin D receptor
WHO	World Health Organization
y	Years

4. Background

4.1. Lung cancer

4.1.1. Epidemiology and etiology

4.1.1.2. Epidemiology

Lung cancer has the second highest incidence and the highest mortality of all malignant diseases.

Incidence

In the western world male lung cancer incidence rate has increased until 1996, has been stable to 2006, and is now decreasing. In women the incidence rate is still increasing. Women started smoking later compared with men and the proportion of men who smokes has decreased from 1960, while the proportion of smoking women was increasing until 1970 and is now stable.

Today lung cancer has the second highest cancer incidence in men after prostate cancer and the second highest in women after breast cancer, Figure 1 (table 1).

Before 2010 squamous cell carcinoma (SQCC) was the dominating histology of lung tumors, but thereafter adenocarcinoma became the most common histology. In the last ten years more women and more never-smokers developed lung cancer [1]. The reasons for the changes are not clear. However, the incidence of lung cancer in men is decreasing and plateauing in women is most probably related to the smoking habit, with less daily smokers, in the western world. Another fact that is supporting the smoking theory is that the smoking related SCLC is also decreasing. The rise in adenocarcinoma is absolute and relative, it is less smoking related

than SQCC and SCLC, but other explanations are to date not revealed. In contrast, in other countries like China, Korea or several countries in Africa we see increasing portions of daily smokers and lung cancer shows an increasing incidence [2]. Also the tobacco-products have changed, now more filtered cigarettes that causes lower nicotine content and deeper inhalation are used [3].

The reduction in prevalence of smoking and change in tobacco products can therefore be expected to lead to a further decrease in the incidence of lung cancer over time.

Figure 1: Time trends in age-standardized rates in Norway for selected cancers (semi-log scale), Source Cancer registry of Norway (CRN)

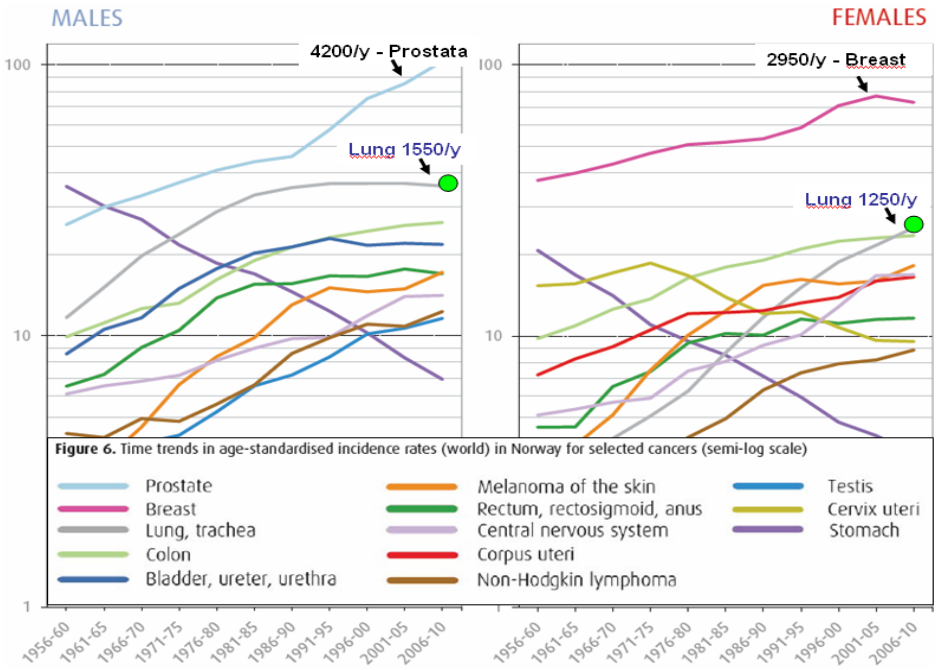


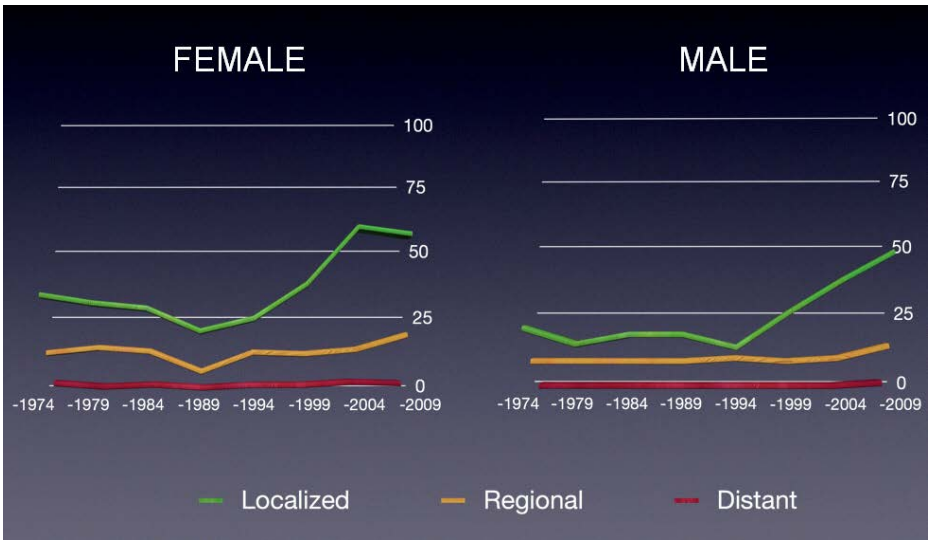
Table 1: Average annual numbers of new cases of lung cancer and age-adjusted incidence rates for lung cancer in the period 1971 to 2010, separately for males and women (Norway), Source: CRN.

Average annual number of new cases of lung cancer in the period 1971 to 2010 for males							
1971-75	**1976-80**	**1981-85**	**1986-90**	**1991-95**	**1996-2000**	**2001-05**	**2006-2010**
679	876	1056	1160	1229	1288	1397	1507
Average annual number of new cases of lung cancer in the period 1971 to 2010 for females							
1971-75	**1976-80**	**1981-85**	**1986-90**	**1991-95**	**1996-2000**	**2001-05**	**2006-2010**
165	213	303	423	548	702	880	1139
Age-adjusted (world) incidence tares per 100000 person-years for lung cancer in the period 1971 to 2010 for males							
1971-75	**1976-80**	**1981-85**	**1986-90**	**1991-95**	**1996-2000**	**2001-05**	**2006-2010**
23.7	28.8	33.2	35.3	36.5	36.6	36.6	35.8
Age-adjusted (world) incidence tares per 100000 person-years for lung cancer in the period 1971 to 2010 for females							
1971-75	**1976-80**	**1981-85**	**1986-90**	**1991-95**	**1996-2000**	**2001-05**	**2006-2010**
5.1	6.3	8.7	11.7	15.1	18.8	21.6	25.1

Survival

Data from the Cancer Registry of Norway (CRN) show that the overall survival in lung cancer patients is rather short and has changed only marginally the last three decades, five years survival after diagnosis was 16% in women and 11% in men in the end of 2010 in Norway. The overall survival in men has changed from 1971 – 1975 to 2006 – 2010 from 8% to 11%. In women it has improved from 10% to 16% [4]. The highest improvement in survival has been observed in cases where the disease has been detected and treated in early stages. In men the survival has increased from 19.2% to 41.4% for localized disease, in female from 27.2% to 50.5% in the period 1971 to 2010, respectively [2].

Figure 2: Survival rates, stratified by stage, for men and women from 1974 to 2009.



Survival rates varied more among the histological sub groups. The poorest survival is seen in patients with SCLC (5% 5 years survival rate and 2% 10 years survival rate). In contrast, the 10-year relative survival rate for patients with lung sarcomas was 18%. Patients with adenocarcinoma of the lung had a better 5 years survival rate (10%) than patients with other sub groups of histology. Surgical candidates diagnosed with Stage I squamous cell carcinoma, adenocarcinoma, and large cell carcinoma had 5-year relative survival rates of 55– 63%. The prognosis for patients with SCLC was poor under any conditions, but patients treated with both chemotherapy and radiation therapy had longer survival than patients treated with chemotherapy alone at every clinical stage of disease.

4.1.1.3. Etiology of lung cancer

1: *Tobacco smoking* is regarded as the main cause of lung cancer. 90% of all patients with lung cancer have a positive smoking history [5]. On the contrary, of smoking persons

there are rather few that develop lung cancer. Smoking is probably not alone sufficient to develop lung cancer. In Norway there has been a decrease in the prevalence of daily smokers; in 1973 51% of males and 32% of women were daily smokers, whilst in 2010 17% of both sexes were daily smokers (figure 3) [6].

2: But also *secondhand smoking* is an important cause of lung cancer. Approximately 15% of lung cancers in never smokers are accounted to passive smoking [7, 8].

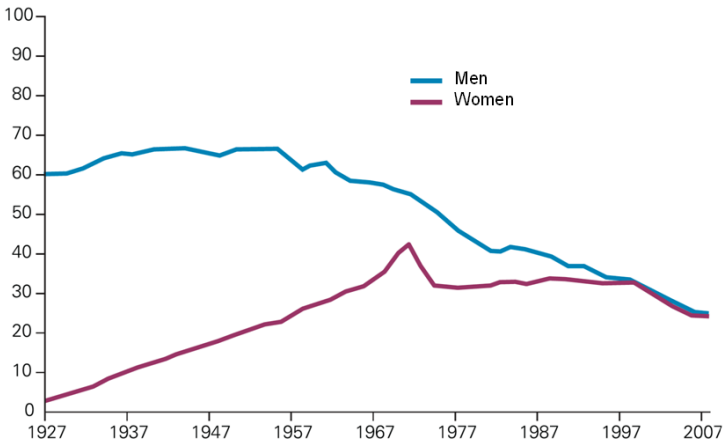
3: *Occupational and every-day exposure to radon* (in our environment) is regarded as a risk factor for lung cancer [7]. Occupational exposure to asbestos increases the risk of lung cancer, especially in case of concomitant tobacco smoking, where the risk increases in a multiplicative way [7, 9, 10].

4: A *family history* of lung cancer in a first-degree relative is associated with a higher risk of lung cancer, independent of smoking [11].

5: People who received *radiotherapy* for other diseases are at a higher risk for lung cancer [12].

6: *COPD* is also known as an independent risk factor for lung cancer.

Figure 3: Fraction of daily smokers among men and women in Norway from 1927 to 2007, Source: CRN.



Chronic inflammation has been documented to influence risk for LC through increased cell turnover with the potential of generating genetic errors, as well as stimulating angio-neogenesis and apoptosis [13, 14]. The fact that the use of inhaled corticosteroid reduces the risk of lung cancer support this theory [15, 16].

Some factors seem to protect against lung cancer. Higher physical activity reduces the risk of lung cancer [17] and aspirin may reduce the risk of lung cancer [18].

4.1.2. Histological classification of lung cancer

The World Health Organisation (WHO) has published the current recommendation for the classification of malignant lung tumours [19].

Lung cancer has the last decades primarily been divided in NSCLC and SCLC. NSCLC is further sub-classified into adeno-, squamous cell-, adenosquamous and large cell carcinomas, and NOS (not otherwise specified). The last category has the poorest 5-year survival estimates (5.8%) as well as a median overall survival (OS) of 5 months [20]. SCLC is sub-classified into oat cell cancer and combined small-cell cancer. For more detailed classification se (table 2).

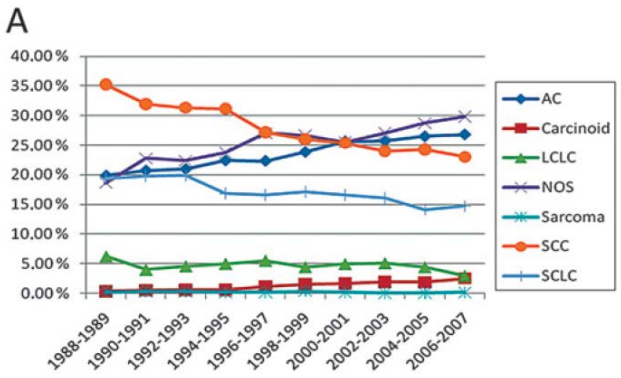
However, many lung cancers consist of a mixture of histological subtypes. The portion that is most differentiated defines the final histological diagnosis, except for tumors containing SCLC which is described as SCLC independent of the other histological components. The most important classification is however the division in SCLC and NSCLC with it sub-types (table 1), because it is a premise for choosing therapy, and for individualized treatment.

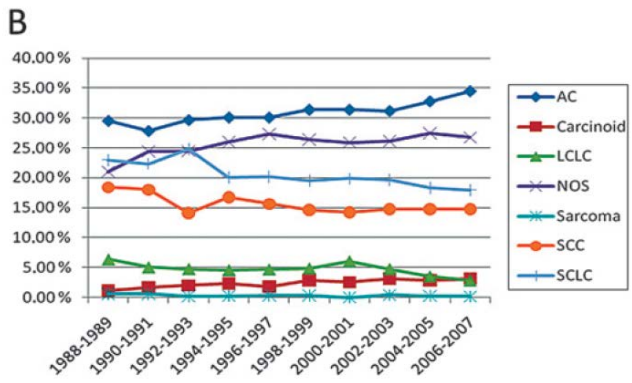
Table 2: WHO guidelines for histologic classification of lung cancer

Tumour type	Tumour subtype
Non-Small-Cell Lung Cancer	Adenocarcinoma
	Squamous cell carcinoma
	Adenosquamous carcinoma
	Carcinomas with pleomorphic, sarcomatous characteristics,
	Carcinoid
	Carcinomas of salivary gland origin
	Not otherwise specified
Small-Cell Lung Cancer	Oat cell cancer
	Combined small-cell

The different histology subgroups in lung cancer have changed during the last decades. The proportion of SCLC and SQCC (squamous cell carcinoma) is decreasing in both sexes (more prominent in men), probably due to less daily smokers. Adenocarcinoma is increasing in both sexes (more prominent in women) [21]. Figure 4 shows the changes of incidence proportions of the histological subgroups.

Figure 4: Lung cancer incidence proportion of histological subgroups in males (A) and females (B), Source [21].





4.1.3. TNM classification and clinical staging

Like most other malignant diseases, lung cancer distribution is classified by the TNM classification system, based on CT examination. “T” describes the size, invasion in neighbor structures and location of the primary tumor, “N” describes possible lymph node involvement and localization and “M” describes the presence and localization of metastases. This TNM classification was established in the 1940s and for lung cancer the latest is the seventh edition published in 2009 [22]. The clinical staging and histology of NSCLC and SCLC are crucial estimating prognosis and for the choice of therapy, curative versus palliative treatment (table 3 and figure 5).

Stage Ia - IIb are defined as localized, IIIa - IIIb as locoregional and stage IV as advanced disease. All patients with suspected lung cancer should undergo a computer tomography (CT) scan of the thorax and the upper abdomen. In patients with probably limited SCLC and NSCLC based on CT, a CT caput or Magnetic resonance tomography (MR) caput and positron emission tomography CT (PET-CT) are taken to assess if it is really limited or loco regional or advanced disease, before the final evaluation if it is eligible for curative treatment. Based on the imaging results showing probably N2-3 disease, invasive EBUS (endo bronchial ultra sound) or subsequent mediastinoscopy are performed to further differentiate between

tumor positive or negative lymph nodes (for the definition of the final clinical stage) Further description of classification and clinical staging are not the scope of this thesis.

Table 3: Lung cancer stage groups according to TNM [22]

T/M	Subgroup	N0	N1	N2	N3
T1	T1a	Ia	IIa	IIIa	IIIb
	T1b	Ia	IIa	IIIa	IIIb
T2	T2a	Ib	IIa	IIIa	IIIb
	T2b	IIa	IIb	IIIa	IIIb
T3	$T3_{>7}$	IIb	IIIa	IIIa	IIIb
	$T3_{Inv}$	IIb	IIIa	IIIa	IIIb
	$T3_{Satell}$	IIb	IIIa	IIIa	IIIb
T4	$T4_{Inv}$	IIIa	IIIa	IIIb	IIIb
	$T4_{Ipsi\ Nod}$	IIIa	IIIa	IIIb	IIIb
M1	$M1a_{Contra\ Nod}$	IV	IV	IV	IV
	$M1a_{Pl\ Disem}$	IV	IV	IV	IV
	M1b	IV	IV	IV	IV

Stage Ia
Stage Ib
Stage IIa
Stage IIb
Stage IIIa
Stage IIIb
Stage IV

4.1.4. Treatment of lung cancer

Choice of treatment depends on tumor factors like histology, TNM classification, clinical stage and host factors like comorbidity, and heart-lung function, and to a lesser degree on age. In clinical stage I-II, T1aN0M0 to T2bN1M0, curative surgery is recommended for NSCLC; and 30-70% of these patients will live longer than 5 years, depending on the specific clinical stage. If the patient is not medically fit for the surgery, a less traumatic treatment option is stereotactic body radiation therapy (SBRT). This leaves the patients with a lower overall survival rate than surgery. In stage I radical surgery alone is considered sufficient as treatment and in stage II, if no contraindications, adjuvant chemotherapy is indicated, and increases the

5-years overall survival 4-10% in this group [23]. Stage III SCLC and NSCLC stage IIIA-N2 disease, loco-regional disease, is a heterogenic group of lung cancer and should be evaluated for semi-curative treatment, radio-chemo therapy with or without surgery and about 20-30% of the patients will live longer than 5 years. The more advanced locoregional stage IIIB disease may in some cases be treated with radio-chemotherapy leading to a 5 years OS of 10-15%. Even more advanced stage IIIB and stage IV disease are offered chemotherapy alone with palliative intention. These patients do not have a curable disease and the overall survival is 8-10months with therapy, and 4-6 months without therapy. But, the group is heterogeneous both in disease distribution and prognosis and the survival varies substantially.

4.1.4.1. Individualized treatment in patients with lung cancer

During the last years individualized treatment based on histological subgroups and molecular analyses of the receptor structure at the cell membrane has been up-coming. Future lung cancer treatment will probably be even more individualized, based on gene molecular characteristics of the tumor cell involved.

To date, the most therapeutically important mutations in molecular mapping for personalized and optimized lung cancer treatments are:

(1) The Epidermal growth factor receptor (*EGFR) mutation (detected by PCR, IHC or FISH)*, which is more common in adenocarcinomas, never-smokers, women and persons from Asia, and is found in about 8% of NSCLC patients in Norway

(2) The *EML4-ALK fusion (detected by PCR, IHC or FISH)*, which is present in approximately 3 to 6 percent of adenocarcinomas and is also more common in people who have never smoked.

(3) The *KRAS mutation (detected by PCR, IHC or FISH)*, which is more common in past or current smokers.

Epidermal growth factor mutations were among the first biomarkers shown to have therapeutic implications, other biomarkers are being evaluated in clinical trials [24-26]. Three pharmacological agents are registered and approved for use in Norway in patients with detected DNA mutation or particular proteins in the tumor: crizotinib, gefitinib and erlotinib and we are waiting for afitinib.

Individualized treatment is also a challenge for the health care personal, new knowledge, ongoing studies and a good collaboration between pulmonologists and pathologists is required.

4.1.5. Screening for lung cancer

Local lung cancer disease detected more accidentally on imaging for other reasons are often presenting without symptoms, and has a god survival rate when early treated. This fact emphasizes the importance of early detection and treatment of lung cancer, at a localized stage when curative treatment by surgery or stereotactic radiation is possible, Thus, around the world there is a lot of attention and effort made in order to characterize the population at highest risk of getting lung cancer, to find the most cost-effective screening procedure and offering the people with localized disease curative treatment, including surgery and stereotactic radiation.

Up to date, known risk factors like tobacco smoking, age above 50 years and COPD has been studied as relevant factors in screening for lung cancer, especially for inclusion in large trials using CT imaging, but the question about cost-effectiveness has to be settled [27, 28]. Until then, research groups are hunting for new and relevant risk factors for lung cancer, to improve

the characterization of the population at risk. Detecting new risk factors may also open possibilities for early prevention or treatment of the condition of interest.

Screening is defined as a systematic testing of individuals who are asymptomatic but have a higher risk for a specific disease [29] dependent on one or more detectable risk factors, like newborn screening.

In 1968 the WHO published the document "PRINCIPLES AND PRACTICE OF SCREENING FOR DISEASE" written by Wilson and Junger [30] (table 4).

Table 4: The Wilson criteria for screening

The Wilson criteria for screening emphasise the important features of any screening program, as follows:

- the condition should be an important health problem
- the natural history of the condition should be understood
- there should be a recognisable latent or early symptomatic stage
- there should be a test that is easy to perform and interpret, acceptable, accurate, reliable, sensitive and specific
- there should be an accepted treatment recognised for the disease
- treatment should be more effective if started early
- there should be a policy on who should be treated
- diagnosis and treatment should be cost-effective
- case-finding should be a continuous process

The purpose of screening is to prevent, interrupt or delay the development of a certain disease. In cancer disease it is important to detect the disease before metastases have developed. In general, screening must lead to better outcomes regarding lung cancer or overall survival, being easy and gentle to perform for all person defined being at risk, to a cost-effective price.

The screening technique must be sensitive enough to identify most cases and must be specific enough to avoid an excess of unnecessary investigations, treatments and patient's worries.

The most widely used endpoint in studies investigating the benefit of screening is disease-specific mortality [31].

At the moment, in Norway established screening programs are used for breast cancer and cervix cancer. A pilot study for colon cancer screening started in 2012 in Norway.

A discussion about the cost-effectiveness of screening for lung cancer is still ongoing. Most screening tests are non-invasive, however some involve procedures that may cause complications [32]. Because of increased risk for comorbidities among long time and heavy smokers, complications associated with invasive diagnostic procedures and therapy may be more frequent in these groups. Further, screening can cause anxiety and worries as well as further unnecessary procedures to check false positive results [33]. On the other hand, false negative results may lead to ignorance of symptoms and delayed diagnosis.

In lung cancer screening different strategies have been investigated in clinical trails, like Chest X-ray, Chest X-ray with sputum histology and low dose spiral CT.

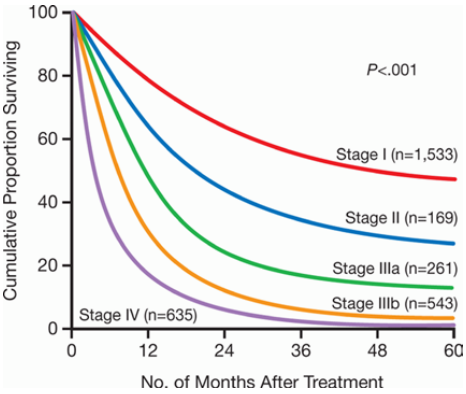
Repeated chest x-ray with and without sputum histology has not been shown to reduce the mortality in lung cancer and is consequently not recommended as screening methods for lung cancer [34, 35]. Therefore CT scan was introduced as a possible screening method in lung cancer [31], like the National Lung cancer Screening Trial (NLST), The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial, Dutch-Belgian Randomized Lung Cancer Screening Trial (NELSON), DANTE trial and the Danish Randomized Lung Cancer CR Screening Trial.

The National Lung cancer Screening Trial (NLST) showed a 20% reduced relative mortality of lung cancer [36] while the PLCO trial did not show any reduction of mortality [37]. The Dutch-Belgian Randomized Lung Cancer Screening Trial (NELSON) showed a reduced mortality, however the DANTE trial and the Danish Randomized Lung Cancer CR Screening Trial did not show any mortality reduction.

At the moment, screening with low dose CT scan is not recommended in lung cancer screening [38, 39] in general, with an exception for US where they follow the NLST criteria in the regions with access to the same clinical expertise as in the study. Also in Norway screening for lung cancer is not recommended at the time.

75% of the patients with lung cancer are not curable at the time of diagnosis [40]. Survival in patients with NSCLC is directly related to the stage of the disease at the time of diagnosis and varies from above 50% (stage IA) to 0% (stage IV) 5 years survival (figure 5). Corresponding estimates are limited for SCLC, but it is reason to expect that survival is related to stage (limited vs. extended disease) for this type as well. Possibly, screening can save lives in selected groups (high risk persons) based on combined risk scores and this shows the importance of finding new risk factors relevant to screen for.

Figure 4: Survival Curves for Patients with Lung Cancer by Stage, source http://www.pet-vghks.com.tw/?p=3057



4.2. Lung cancer and comorbidities

4.2.1. Definition

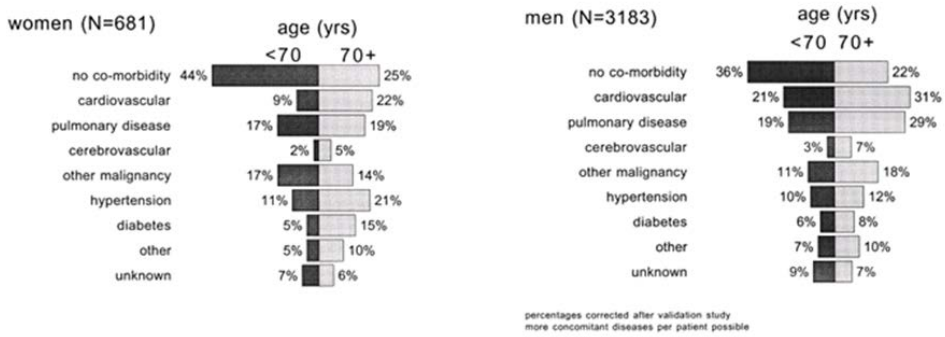
Comorbidity is defined as a coexisting medical conditions or diseases that are additional to a primary diagnosis. Comorbidity is more frequent in older patients, and a risk factor may be associated to different diseases, which makes causal relation, degrees of association and interaction difficult to study. Since the mean age for the debut of lung cancer is 71 years, comorbidity is common in patients with lung cancer [41].

Another term used is multimorbidity. Multimorbidity and comorbidity are often used similarly but by definition comorbidity indicates a condition that coexists with a disease of interest (in our study lung cancer) while multimorbidity does not assume that a patient has a disease of interest (like in our study lung cancer), but the individuals may of course be at higher risk for lung cancer. In one study 99% of women and 97% of men aged 65 years or older have had two or more medical conditions [42]. Persons with multimorbidity are more likely getting additional coexisting medical condition than persons without multimorbidity [43]. Comorbidity, as multimorbidity, can affect treatment choice, prognosis, and survival in older people [44].

4.2.2. Comorbidity in patients with lung cancer

Today in patients with lung cancer, the most frequent concomitant diseases are cardiovascular diseases (23%), compared to 10% in the general population at 65 years or older, chronic obstructive pulmonary diseases, COPD (22%), compared to 11% in the general population at 65 years or older, (figure 6). Comorbidity is higher in men and in patients with squamous-cell carcinoma, probably due to the smoking habit of men [45].

Figure 5: Age-specific prevalence of concomitant diseases for men and women, source [45]



Comorbidity seems to be associated with earlier diagnosis of lung cancer, but it may also leads to less aggressive treatment. Thus the prognosis in patients with lung cancer can be negatively influenced by comorbidity [45].

4.2.3. How to measure comorbidity

Handling of comorbidities is important in the care of cancer patients but there is no definite consensus about how to measure it. The difficulties in measuring comorbidity depend on many factors. The importance of different comorbidities vary with the type of cancer [46]. In patients with lung cancer the Charlson Index is the most common method to measure the existence, burden and consequences of comorbidities [47]. This index was developed by M. E. Charlson in 1987 and is based on a point scoring system (from 0 to 40) for the presence of one or more associated diseases and taking into account the age of the patient. The advantage of the Charlson Index is the capability of evaluating the patient's mortality risk based on age and comorbidity. In the absence of comorbidity the mortality is 12%, at 1–2 points it is 26%

at 3–4 points it is 52% and with the accumulation of more than 5 points it is 85% in all stages of the disease.

4.2.4. The impact of comorbidity on survival and treatment

In general comorbidity in patients with lung cancer is associated with decreased survival [48]. Studies show that 25% - 40% of the lung cancer patients with comorbidity do not die of the cancer itself but because of present comorbidity [49]. The influence of comorbidity on survival is most important in patients with early stage of lung cancer disease and is less important in advanced lung cancer diseases [50].

The impact of comorbidity on choice of treatment and consequences for survival is still unclear. At present there is no consent whether comorbidity in general is a prognostic negative factor on survival. In one study, Janssen-Heijnen et al show no association between comorbidity and survival [51, 52] while Asmis et al show that the presence of comorbidity is associated with a poorer survival [53], and there is at the time no guidelines pointing out the impact of comorbidity on the choice of treatment [54].

Medical decisions should incorporate patient's preferences. Limited evidence for treatment choice in older patients with comorbidity, benefits and prognosis can be different in this patient group [55, 56]. The complexity of treatment can be different in older people with comorbidity and the choice of therapy should be optimize with respect on the quality of life of older patients [57].

5. Diabetes mellitus

Diabetes mellitus (DM) is a common endocrine disorder, a metabolic disease with tendency to hyperglycemia and is a disorder in the insulin activity or function, either lack of insulin or an inadequate effect of insulin on cells, are the two main characterizations. In general two major types of DM are present. Type 1(DM-1) is caused by pancreatic beta cell destruction, which in the end leads to absolute insulin deficiency. Type 2 (DM-2) is characterized by insulin resistance (through changes in the cells' insulin surface receptors as well as intracellular insulin signaling). Initially the body compensate by releasing more insulin to overcome this insulin resistance. However, with time a relative insulin deficiency or an insulin secretory defect (due to insufficient insulin production in the beta- cells in the pancreas) develops and glucose levels start to rise.

In DM-1, insulin is missing in the circulation and it must be substituted. In DM-2 there are in principle two ways of treating the condition. One way is to increase the insulin levels either by stimulating secretion of insulin from the pancreas or inject insulin into the patient; the other way is to increase the sensitivity of the target cells for insulin. Sulfonylureas, introduced in the U.S. in 1955, are a group of pharmaceuticals that increases the insulin secretion from the pancreas, and metformin, introduced as treatment for DM in 1958 in the U.K., decreases the insulin resistance of the target cells.

5.1. Diabetes mellitus and lung cancer

The insulin resistance plays a central role in DM-2. It leads to higher levels of endogenous insulin. Higher insulin levels can leads to an increased mitogen effect in most cells and further, it can leads to insulin resistance and higher blood glucose levels that stimulate the

insulin like growth factor 1 (IGF-1) secretion. Bioactive IGF-1 has antiapoptotic effect. Both mitogen and antiapoptotic effects promote cancer development and cancer growth [58-60]. Several studies have showed an increased risk of cancer (breast, large bowel, endometrial and pancreatic cancer) in patients with DM [61-65]. Further it is known that insulin and insulin analogues have a cell growth promoting activity [66] and may influence the survival in patients with lung cancer.

Some observational studies indicate that metformin used in DM-2 reduces the risk of cancer [67], and it may be initiated through the effect of metformin on the AMP-activated protein kinase (AMPK) which suppresses cell proliferation in non-malignant as well as in tumor cells [68]. An additional effect may be that metformin improves the prognosis in cancer [69]. The mechanism for these effects is still not clear and it is also discussed whether metformin directly acts with or within the cancer cells [70]. Two studies have shown reduced cancer risk associated with use of metformin [71, 72]. There is evidence that insulin may increase the risk of lung cancer [73].

Few studies have investigated the association between incidence and survival in patients with lung cancer and DM. One study showed no increased risk of lung cancer in patient with DM [74]. Another study showed that patients with DM and NSCLC had a lower risk getting a metastatic diseases but there was no reduction in mortality among lung cancer patient with DM [75]. Three studies looked at the survival in patients with lung cancer and DM, independent whether DM-1 or DM-2, and the results were conflicting. Two study showed increased survival in patients with DM and lung cancer [76, 77]. The other one did not find a positive association between DM and survival in lung cancer [78].

5.2. Metformin and lung cancer

Metformin prevents tobacco carcinogen–induced lung tumor genesis in mice [79]. Other studies showed that metformin inhibits the growth of lung cancer cells and induces apoptosis [80]. However may diabetics using metformin develop a more aggressive cancer phenotype [81]. One case-control study from 2013 did not show a decreased risk of lung cancer in patients using metformin [82].

Metformin may also have an indirect effect on lung cancer treatment and may enhance radiation response of NSCLC [83]. Further studies are needed to evaluate whether metformin is a drug that may be used in lung cancer treatment.

6. Osteoporosis

Osteoporosis, a disease of the bone, is characterized by an abnormal interplay between osteoclast and osteoblast function, and leads to fragile bone tissue. Three main factors have a central role in developing osteoporosis: An inadequate peak bone mineral at early adult age, a high bone resorption and third an inadequate formation of new bone [84]. As early as in 1940 it was described that estrogen played a central role in the pathogenesis of osteoporosis, and during the last decades several studies have confirmed the central role of estrogen. Estrogen deficiency increases and estrogen treatment decreases bone remodeling. There are two estrogen receptors, ERα and ERβ, which are important for the impact of estrogen on the bone formation [85, 86].

6.1. Estrogen and lung cancer

Studies have indicated that estrogen has a central role in lung cancer. Estrogen receptors ERα and ERβ have been found in non-small-cell lung cancer tissue [87-89]. The presence of ERα and the absence of ERβ are associated with a poorer prognosis among NSCLC patients [90]. This could imply that estrogen may promote lung cancer carcinogenesis via estrogen receptors. In line with this, studies have found decreased mortality and reduced lung cancer cell proliferation in patients treated with anti-estrogens [91, 92]. There is evidence for a sex difference in expression of estrogenic receptors in lung cancer [93, 94]. The difference may be explained in the different gene location in the tumor cells and the different protein expression in the tumor cells.

6.2. Osteoporosis and cancer

Several studies have been performed to evaluate the impact of osteoporosis and low bone mineral density (BMD) on the risk of cancer. After our knowledge only one study has investigated the association between osteoporosis, defined by the International Classification of Diseases (ICD)-10 coding system, and the incidence of lung cancer. Patient younger than 70 years of age having osteoporosis in general have an increased risk of getting cancer [95]. They explain this tendency with the fact that this population are more likely to smoke.
The association between BMD and other cancer conditions is studied in more details.
Two studies have shown an inverse relation between BMD and risk of colon cancer [96, 97]. In two case-control studies on breast cancer no relation between BMD and the risk of breast cancer were observed [98, 99]. Another study showed that women with osteoporosis have a decreased risk of breast cancer [95].

Patients with a low BMD have a reduced risk developing endometrial cancer [95].
In patients with prostate cancer there are conflicting results. One study shows no association [99], one shows that increasing BMD associates with increased risk getting prostate cancer [100], and one shows that decreasing BMD associates with an increased risk of getting prostate cancer [101].

Osteoporosis is associated with a low level of Vitamin D [102]. In the last two decades studies show that vitamin D protects against the development of cancer [103, 104]. This fact is strengthened by two studies finding that hypovitaminose D is correlated with a higher incidence of lung cancer [105, 106]. Another evidence for the role of vitamin D in lung cancer is that the TaqI polymorphism of the vitamin D receptor (VDR) gene might be a risk factor for lung cancer [107]. So far there are no evidences that there are a relationship between Vitamin D levels and survival [108].

6.3. BMD and the male skeleton

Sex steroids are important for bone metabolism. Testosterone level decreases only marginally with age in men [109]. Epidemiological studies, investigating the association between testosterone and BMD found no relationship [110]. An epidemiological study found that serum estradiol, but not testosterone, levels was positively associated with BMD in men over age 65 year [111].
It is well established that estrogen deficiency is associated with low BMD/osteoporosis in women. In the last years different studies confirm these findings, that estrogen plays a key role in bone metabolism in men, young and old [112-115].

However, these studies do not exclude an important of testosterone in the male skeleton [116, 117].

7. Chronic inflammation and cancer

The relationship between inflammation and cancer is widely accepted [13]. Inflammation causes tissue injury. This leads to a complex activation of many different reactions, cellular components (neutrophils, macrophages and other inflammatory cells), release of cytokines and growth factors [13].

Carcinogens can induce somatic changes with DNA mutation. These changes can persist in normal tissue until a stimulation or promotor can “activate” these DNA. Those “promotors” can be inflammation, hormones, chronic irritation or chemical irritants [118].

In chronic inflammation inflammatory and phagocytic cells, and their cytokines can induce DNA damage in proliferating cells, by the production of reactive oxygen and nitrogen species [119]. The presence of reactive nitrogen and oxygen released from inflammatory cells, interacts with DNA in proliferative cells which can causes permanent genomic alterations such as point mutations, deletions, or rearrangements.

Cytokines are released in infection and/or inflammation. Normally the cytokines helps in handling infection. However, cytokines play also an important role in cancer pathogenesis. Tumor invasion, promoting tumor growth, apoptosis and promoting metastases are some of the effects of cytokines on tumor cells [120].

8. Risk factors for cardio vascular diseases and the association to lung cancer

Chronic inflammation is documented to play a role in the pathogenesis of cardio vascular disease (CVD), which is frequently seen in patients with lung cancer, with an influence on all stages of the disease [121]. We found no publications on the study of possible association between CVD and the incidence of lung cancer. Regarding other factors of influence on both CVD and LC; tobacco smoking is a common risk factor, whilst, like hypertension, BMI and high cholesterol have been studied in patients with lung cancer. There are evidence that high blood pressure may increase the risk of lung cancer in smoking men [122], that hypertension increases the lung cancer related mortality in patients with lung cancer [123]. High BMI mineral is a known risk factor for CVD [124]. The association between BMI and lung cancer is invers. Smokers with a lower BMI are at higher risk getting lung cancer compared with smokers with high BMI [125]. High cholesterol is associated with a higher risk of CVD. The risk association between cholesterol and lung cancer was investigated in some older studies. No association was found between cholesterol and the risk of lung cancer [126, 127]. Metabolic syndrome, also a known risk factor for CVD, is associated with an over all increased risk of cancer. Most studies investigated this association in patients with colo-rectal cancer and pancreatic cancer [128, 129]. There are some results which suggest that there is no association between the risk of lung cancer and metabolic syndrome [130].

With special interest in co-morbidity in lung cancer as described above, access to large registries, we decided to design studies to look at their possible influence on lung cancer development and survival.

9. Aims of the study

The primary aim of the study was to study the possible influence of comorbidities on the incidence and survival of lung cancer.
The secondary aim was to look for new risk factors that may be used in screening trails for lung cancer.

10. Material and methods

10.1. Material

The study populations for the three papers were recruited from the Nord-Trøndelag Health Study (HUNT study), the Pemetrexed-Gemcitabine Study (PEG) study and the Norwegian Lung Cancer Biobank (NLCB) study.

The HUNT study [131] is a large population-based prospective cohort study in Norway having collected data in three surveys, HUNT 1 (1984-1986), HUNT 2 (1995-1997) and HUNT 3 (2006-2008). Individuals aged 20 years or more were invited each time. In total 77 205 (89% of invited), 65 233 (69% of invited) and 50 806 (54% of invited) people participated in HUNT 1, 2 and 3, respectively. Many people participated two or three times which reduces the total number of subjects to approximately 106 000. Nord-Trøndelag is a county in the middle of Norway having 130 708 inhabitants in January 2009. This population is considered representative of the Norwegian population, but the county lacks larger cities, has a lower educational and income level, and the proportion of smokers is slightly below the mean in Norway.

The PEG study [132] was an open randomized multicenter phase III trial of 436 patients with stage IIIB/IV non-small-cell lung cancer by the Norwegian Lung Cancer Group (NLCG). The aim of the study was to compare pemetrexed plus carboplatin versus gemcitabine plus carboplatin as first line chemotherapy, given as four courses with 3 weeks intervals, with respect to health related quality of life, survival and toxicity. The study was conducted from April 2005 to July 2006.

The NLCB, a registry study started March 15th 2006 aiming to collect tumor tissue, normal tissue and blood samples from patients consecutively admitted to hospital and suspected to have lung cancer. In our study we included only patients with verified lung cancer from the NLCB registry study, which included all clinical stages of lung cancer.

Data from the HUNT study were linked to the Cancer Registry of Norway (CRN) and the Norwegian Cause of Death Registry at Statistics Norway (SSB) [133]. In the PEG-study and in the NLCB registry data were collected from the electronic patient records.

The candidate has contributed with data collection in the PEG study and NLCB registry study.

10.2. The study population

Study 1

The main study population was recruited from the HUNT study 1, 2 and 3 [134]. In this population we found 1206 cases of lung cancer (table 6). In addition we used 436 cases from the Pemetrexed Gemcitabine study (PEG-study) [135]. From the NLCG study 210 patients with lung cancer were included. Only patients with histological verified lung cancer (stage of disease I-IV) until October 15th 2010 were included in the study.

Table 5: Characteristics of lung cancer patients in the HUNT, PEG and NLCB studies by having or not having diabetes mellitus.

	HUNT 1984-2008 N = 1206				PEG 2005-2006 N = 436				NLCB 2006-2010 N = 210				Total N = 1852			
	DM N = 49		Non-DM N = 1157		DM N = 17		Non-DM N = 419		DM N = 18		Non-DM N = 192		DM N = 84		Non-DM N = 1768	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Age (years) [a]	71.0 ± 8.2		70 ± 10.9		73 ± 7.0		64 ± 9.8		68 ± 8.0		68 ± 9.7		70.1 ± 7.7		67.3 ± 10.1	
Gender																
Male	39	80	749	65	12	70	239	57	10	56	112	58	61	73	1100	62
Female	10	20	408	35	5	30	180	43	8	44	80	42	23	27	668	38
Smoking history																
Never	9	18	182	16	0	0	32	8	0	0	5	3	9	11	219	12
Ever	40	82	975	84	17	100	387	92	18	100	187	97	75	89	1549	88
Stage of disease																
Non-metastatic	28	57	507	44	-	-	-	-	8	44	80	42	36	43	587	33
Metastatic	11	22	520	45	17	100	419	100	9	50	110	57	37	44	1049	59
Unknown	10	21	130	11	-	-	-	-	1	6	2	1	11	13	132	8
Histology																
NSCLC	35	71	806	70	17	100	419	100	14	78	157	82	66	78	1382	78
SCLC	6	12	201	17	-	-	-	-	3	17	27	14	9	11	228	13
Unknown	8	17	150	13	-	-	-	-	1	5	8	4	9	11	158	9

N, number; HUNT, Health Study of Nord-Trøndelag; PEG, Pemetrexed Gemcitabine study; NLCB, Norwegian Lung Cancer Bio Bank; LC, lung cancer; DM, diabetes mellitus; NSCLC non small cell lung cancer; SCLC, small cell lung cancer

[a] Values given as median ± SD.

Study 2

In study 2 only participant from the HUNT 2 study were included (table 7). Participants older than 50 years of age, having measured bone density (N=18156), having answered the questions on self reported fracture (N=55052) and osteoporosis (N=52804) and known body mineral index (BMI) (N=18079), were evaluated for inclusion. A total of 6996 persons were included in the study 2. Among these were 132 cases of NSCLC.

Table 6: Baseline characteristics of the study population in study 2

		Women		Men		Total	
		N	%	N	%	N	%
	Low	1622	34	692	31	2314	33
BMD z-score	Medium	1590	33	774	35	2364	33
	High	1601	33	747	34	2348	34
Fractures	Yes	1083	23	268	12	1351	20
	No	3730	77	1945	88	5675	80
Osteoporosis	Yes	515	11	90	4	605	10
	No	4298	89	2123	96	6421	90
Smoking in	0	3139	66	805	37	3944	60
Pack years	1-20	1306	26	687	32	1997	27
	21-40	331	7	509	23	840	10
	>40	37	1	175	8	212	3
BMI	Normal	1458	29	616	28	2074	29
	Underweight	38	1	18	1	56	1
	Overweight	2062	43	1125	51	3187	45
	Obesity	1222	27	437	20	1659	25
Age [a, *]		68 y ± 9 y		65 y ± 9 y			
HRT	Ever	1229	26	-	-	1229	26
	Never	3584	84	-	-	3584	84

Abbreviations: BMD, bone mineral density; N, number; BMI, body mineral index; y, years; a, results reported in mean and standard deviation; *, age at inclusion; HRT, Hormone replacement therapy

Study 3

In study 3 we included data from HUNT1, HUNT2 and HUNT3 representing a total of 97087 people, including 1080 cases of lung cancer (table 8). Only persons with an observation period < 1 year were excluded from the study.

Table 7: Baseline characteristics for the study population with available smoking status. The Hunt study 1864-2008.

		Never smokers N = 38,656		Former smokers N = 20,914		Current smokers N = 26,894	
		CVD		CVD		CVD	
		Yes	No	Yes	No	Yes	No
BMI*	< 18.5	1	1	1	1	2	2
	≥ 18.5 – < 25.0	30	51	29	47	45	58
	≥ 25.0 – < 30.0	45	36	50	40	42	31
	≥ 30.0	23	13	20	12	12	9
Sex	Female	69	59	18	43	25	51
	Male	31	41	82	57	75	49
Chronic cough with phlegm	Yes	5	3	8	4	18	10
Smoking	Light smoker	-	-	60	89	53	84
	Heavy smoker	-	-	40	11	47	16
Person years [a]		14.6 ± 8.3		14.1 ± 9.0		16.5 ± 7.8	
Age at inclusion [a]		44.2 ± 19.1		46.9 ± 17.2		42.4 ± 15.6	

CVD, cardio vascular disease; BMI, Body mineral index; N, numbers; *, kg/m^2; [a], mean and standard deviation; Figures are percentage of participants in each group.

11. Study variables

11.1. Outcome variables

Lung cancer diagnosis and stage of disease

In all three studies lung cancer diagnosis was based on traditional histological classification (World Health Organization. Histological Typing of Lung Tumours, from the 2nd edition in 1981 to date). Lung cancer was classified as NSCLC and SCLC. Based on the TNM classification system for lung cancer (International Association for the Study of Lung Cancer, IASLC) the CRN has categorized lung cancer into non-metastatic and metastatic disease for

the whole period. No tumor invasion of organ or neighboring structures, no lymph node metastasis other than local lymph node metastasis was defined as non-metastatic disease. Patients with all other metastatic features, including organ and lymph node metastasis, were defined as metastatic disease.

We have no information on treatment for patients recruited from the HUNT study and the NLCB registry. However, there are no guidelines recommending different treatment regimens for patients with or without DM.

11.2. Exposure variables:

Diabetes mellitus (study 1)

In the HUNT study diabetes mellitus was defined by the answer "yes" to the question "Do you have or have you had diabetes". Approximately 60 % answered this question either with yes or no. Based on the information about age of the patient, current use of medication and the duration of diabetes mellitus, the majority of the patients were classified as diabetes mellitus type II. In the PEG-study and the NLCB registry diabetes mellitus was diagnosed according to information of diabetes mellitus and/or the use of anti-diabetic medication in the hospital medical record.

Bone mineral density (study 2)

The bone mineral density (BMD) was measured in the non-dominant forearm using Single Energy X-ray Absorptiometry (SXA) (Osteometer DTX 100, Osteometer AS, Copenhagen). Measure point was 24 mm distal from the point where the distance between radius and ulna was 8 mm.

For all analyses we used the z-score. The z-score was calculated in 10 years intervals for male and female separately using following formula: z-score = (observed BMD – mean BMD) divided by with the standard deviation.

The z-score was categorized into tertiles: low z-score, middle z-score and high z-score.

Self reported fracture history

The participants were asked about former fractures in the wrist, hip or vertebra. A total of 55 052 (84%) persons answered this question. An affirmative answer to at least one of these questions was defined as a positive self reported fracture history. To avoid inclusion of high energy fractures, persons with fracture at the age 50 years or younger were excluded.

Self reported osteoporosis

Self reported osteoporosis was defined by an affirmative answer to one of these questions "Has your doctor ever said that you have osteoporosis" or "Do you have or have you had osteoporosis". A total of 52 804 (81%) answered this question.

Cardio vascular disease (study 3)

The definition of CVD was based on baseline questionnaires in all three surveys. CVD was defined by the answer "yes" to one or more of these questions: "Do you have or have you had myocardial infarction?", "Do you have or have you had angina pectoris?" or "Do you have or have you had stroke?". Approximately all participants answered this question (99%).

12. Statistical methods

Different statistical methods were used in the three studies.

Study 1: the Chi-square test, Kaplan Meier method and the Cox regression model.

Study 2: the Chi-square test, the logistic regression model and also the Cox regression model,

Study 3: the Chi square test, the t-test and proportional hazard regression.

In all studies both univariable and the multivariable regressions were performed.

In all three studies two-sided tests were used and statistical significance was defined as $P < 0.05$. The Hazard ratio (HR) and Odds ratio (OR) is reported with 95% confidence interval (CI). The Wald test was used to compare HR's between different groups.

Age, sex, smoking status and pack years were included as confounders in the different statistical models.

Statistical analyses were performed using PASW version 19 (Predictive Analytics Soft Ware, IBM Corporation, New York 10589, USA) and in paper 3 also the statistical program R version 2.15.2 (2012-10-26) for Windows.

12.1. Tests used

Chi square test for independence, This test is used to test the association between to categorical variables.

T-test or Student's t-test, is a hypothesis testing in which the test statistics follows a Student's distribution if the null hypothesis is supported. The test is often used when testing whether two sets of continuous data are significantly different from each other.

The *Kaplan-Meier method* estimates the survival over time even when persons drop out or are studied for different length of time. *The Log rank* used in the Kaplan-Meier model is the hypothesis test to compare the survival distributions of two samples.
Cox regression model is like the Kaplan Meier method an estimator for survival, but the model includes one or more covariates that may be associated with the survival time.
The *logistic regression model* is a type of regression analysis used to assess the relationship between a dichotomous outcome and one or more covariates.
Imputation is one method to handle missing data in data set. In the present study missing data were estimated by use of multiple imputation [136].

13. Approvals

The Regional Committee for Medical and Health Research Ethics have approved the current study (REK# 2010/1081). All participants in HUNT have signed informed consent for use of their data in research also for merging data with other registry.

14. Results

14.1. Study 1

Prolonged survival in lung cancer patients with diabetes mellitus

We found 1677 cases of lung cancer in the HUNT study. From the PEG study we included 436 lung cancer patients and from the NLCB study 210 patients with lung cancer. If the case of death was either unknown (n=77) or other than lung cancer (n=98) patients were excluded.

In total 6 of the 175 excluded lung cancer patients had diabetes mellitus. A total of 1677 lung cancer patient were included in the study, 78 patients with and 1599 without diabetes mellitus. In the HUNT study the Kaplan-Meier survival analysis showed a non-significant trend towards increased survival in lung cancer patients with diabetes mellitus compared to those without diabetes mellitus; median overall survival (OS) was 8.0 months (CI 95%: 5.1-10.9) and 5.0 months (CI 95%: 4.4-5.6) ($p = 0.077$), respectively. The 1-year, 2-year and 3-year survival for patients with and without diabetes mellitus were 33% vs. 28%, 13% vs. 8% and 5% vs. 1%, respectively.

In the PEG study The Kaplan-Meier survival analysis showed an increased 3-years survival in lung cancer patients with compared to patients without diabetes mellitus ($p = 0.048$). Median OS was 16.0 (CI 95%: 5.7-26.3) months and 7.1 (CI 95%: 6.3-7.8) months, respectively. The 1-year, 2-year and 3-years survival for patients with and without diabetes mellitus were 53% vs. 31%, 21% vs. 12% and 0% vs. 0%, respectively. The Kaplan-Meier survival analysis showed equal 3-years survival in patients with and without diabetes mellitus ($p = 0.93$). Median OS was 14.0 (CI 95%: 8.2-19.8) months and 11.0 (CI 95%: 8.0-14.0) months respectively. The 1-year, 2-years and 3-years survival for patients with and without diabetes mellitus were 52% vs. 53%, 31% vs. 38% and 0% vs. 29%, respectively, (table 9).

In a combined survival analysis in the HUNT- and PEG-study and NLCB registry the Kaplan-Meier survival analysis showed an increased 3-years survival in lung cancer patients with diabetes mellitus compared to lung cancer patients without diabetes mellitus ($p = 0.005$) (figure 7). Median OS was 10.0 (CI 95%: 7.7-12.3) months compared to 6.0 (CI 95%: 5.6-6.3) months. The 1-year, 2-year and 3-year survival for patients with and without diabetes mellitus were 43% vs. 28%, 19% vs. 11% and 3% vs. 1%, respectively.

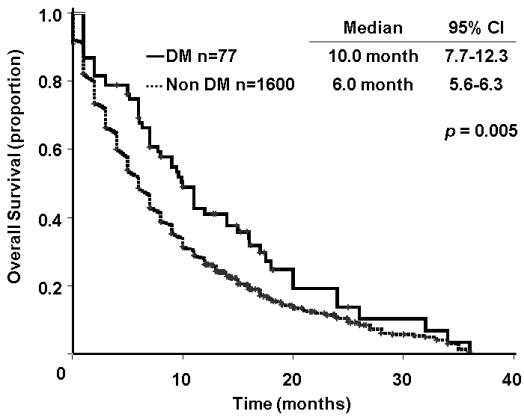
Table 8: Multivariate analysis (Cox regression) for survival in patients with lung cancer.

Table 3: Multivariate analysis (Cox regression) for survival in patients with lung cancer. All available known prognostic factors for survival in lung cancer where entered in the Cox-model.

	HUNT			PEG			NLCB			Total		
	HR	CI 95%	*p*-value	HR	CI 95%	*p*-value	HR	CI 95%	*p*-value	HR	CI 95%	*p*-value
Age (≥70 vs. <70 y)	1.47	1.27-1.69	<0.001	1.05	0.84-1.31	0.652	1.62	1.12-2.36	0.011	1.39	1.24-1.56	<0.001
Gender (male vs. female)	1.15	0.99-1.34	0.071	0.72	0.58-0.89	0.003	1.68	1.13-2.48	0.01	1.32	1.18-1.50	<0.001
Stage of disease (metastatic vs. non-metastatic	1.85	1.59-2.15	<0.001	1.22	0.96-1.55	0.102	2.94	1.92-4.49	<0.001	1.67	1.47-1.89	<0.001
Tumor histology (SCLC vs. NSCLC)	1.05	0.89-1.25	0.54	-	-	-	0.92	0.71-1.18	0.512	1.33	1.14-1.55	<0.001
DM vs. non-DM	0.69	0.46-1.04	0.08	0.51	0.27-0.96	0.037	0.74	0.38-1.44	0.381	0.55	0.41-0.75	<0.001

HUNT, Health Study of Nord-Trøndelag; PEG, Pemetrexed Gemcitabine study; NLCB, Norwegian Lung Cancer Bio Bank; HR, hazard ratio; CI, confidence interval; LC, lung cancer; vs., versus; y, years.

Figure 6: Kaplan-Meier survival curve for patients with and without diabetes mellitus in the HUNT and PEG-study and NLCB registry all combined



14.2. Study 2

Bone mineral density, fracture history, self reported osteoporosis as proxy variables for estrogen and the risk of Non-Small-Cell Lung Cancer – a population based cohort study, the HUNT study: are proxy variables friends or faults?

A total of 9467 persons, 7254 (77 %) women and 2213 (23 %) men, were included in the study. In all 156 (1.6 %) persons developed NSCLC, 83 (1.1%) women and 73 (3.3%) men, which is in accordance with the life-time incidence of lung cancer in Norway. In both sexes those with low BMD z-score were at higher risk of NSCLC compared to those with high BMD z-score, both in unadjusted and adjusted models (men: OR 2.67, 95% CI: 1.39-5.16; women: OR 2.38, 95% CI: 1.09-5.18). In addition women with a medium BMD z-score were at higher risk (OR 2.40, 95% CI: 1.09-5.28) (figure 8 and 9).

Figure 7: The risk of NSCLC in women with low, medium and high BMD

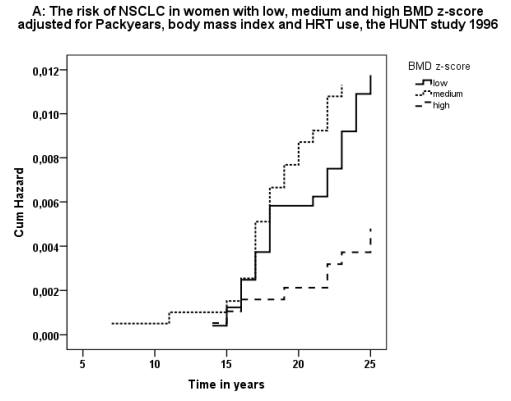
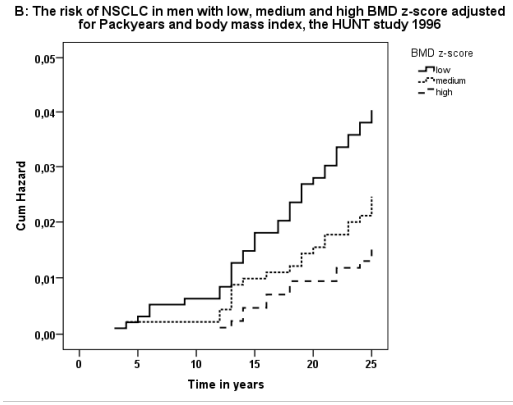


Figure 8: The risk of NSCLC in women with low, medium and high BMD



In both sexes there were no associations between NSCLC diagnosis and self-reported fracture or self-reported osteoporosis (table 10). Sensitivity analysis including lung function and self reported lung symptoms did not change the estimates for BMD.

Table 9: Crude and adjusted odd ratio (OR) of NSCLC according to proxy variable BMD, self reported fracture and self reported osteoporosis, The HUNT study 1995-2008

		Women				Men			
		unadjusted		Adjusted		Unadjusted		Adjusted	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
		BMD z-score							
BMD	High	1	Reference	1	Reference	1	Reference	1	Reference
Medium		1.76	0.98-3.16	2.40	1.09-5.28	1.65	0.83-3.30	1.64	0.81-3.31
Low		1.90	1.07-3.38	2.38	1.09-5.18	3.28	1.73-6.21	2.67	1.39-5.16
PY	0	1	Reference	1	Reference	1	Reference	1	Reference
1-20		4.21	2.49-7.13	4.03	2.12-7.67	1.89	0.85-4.21	1.79	0.81-4.00
21-40		10.28	5.69-18.60	10.77	5.25-22.08	4.98	2.41-10.28	4.31	2.07-8.96
>40		8.34	1.91-36.3	5.70	0.73-44.76	8.55	3.84-19.03	7.74	3.45-17.37
BMI	Underweight	1.02	0.14-7.59	1.23	0.16-9.55	5.16	1.39-19.10	4.02	1.03-15.69
	Normal	1	Reference	1	Reference	1	Reference	1	Reference
	Overweight	0.49	0.29-0.81	0.79	0.44-1.41	0.83	0.48-1.41	1.01	0.58-1.76
	Obesity	0.54	0.31-0.95	0.70	0.33-1.49	0.67	0.32-1.38	0.78	0.37-1.64
HRT		0.99	0.71-1.37	1.07	0.77-1.50	-	-	-	-
		Self reported fracture history							
Fracture yes		0.81	0.47-1.39	0.76	0.39-1.47	0.76	0.35-1.68	0.76	0.34-1.71
PY	0	1	Reference	1	Reference	1	Reference	1	Reference
1-20		4.21	2.49-7.13	4.67	2.41-9.03	1.89	0.85-4.21	1.91	0.86-4.26
21-40		10.28	5.69-18.60	12.70	6.12-26.38	4.98	2.41-10.28	4.75	2.28-9.88
>40		8.34	1.91-36.3	6.63	0.84-52.2	8.55	3.84-19.03	8.56	3.83-19.13
BMI	Underweight	1.02	0.14-7.59	1.25	0.16-9.69	5.16	1.39-19.10	4.03	1.04-15.63
	Normal	1	Reference	1	Reference	1	Reference	1	Reference
	Overweight	0.49	0.29-0.81	0.74	0.42-1.32	0.83	0.48-1.41	0.90	0.52-1.55
	Obesity	0.54	0.31-0.95	0.58	0.27-1.22	0.67	0.32-1.38	0.67	0.32-1.40
Age		0.99	0.96-1.01	1.03	0.99-1.06	1.01	0.98-1.03	1.01	0.98-1.04
HRT		0.99	0.71-1.37	1.03	0.73-1.46	-	-	-	-
		Self reported osteoporosis							
Osteoporosis yes		1.06	0.55-2.06	0.93	0.39-2.22	1.78	0.69-4.52	1.73	0.66-4.51
PY	0	1	Reference	1	Reference	1	Reference	1	Reference
1-20		4.21	2.49-7.13	4.67	2.42-9.03	1.89	0.85-4.21	1.92	0.86-4.28
21-40		10.28	5.69-18.60	12.69	6.11-26.33	4.98	2.41-10.28	4.74	2.28-9.86
>40		8.34	1.91-36.3	6.59	0.84-51.87	8.55	3.84-19.03	8.57	3.83-19.16
BMI	Underweight	1.02	0.14-7.59	1.29	0.17-9.98	5.16	1.39-19.10	3.86	0.99-15.08
	Normal	1	Reference	1	Reference	1	Reference	1	Reference
	Overweight	0.49	0.29-0.81	0.75	0.42-1.33	0.83	0.48-1.41	0.89	0.52-1.53
	Obesity	0.54	0.31-0.95	0.58	0.28-1.23	0.67	0.32-1.38	0.67	0.32-1.40
Age [a]		0.99	0.96-1.01	1.03	0.99-1.06	1.01	0.98-1.03	1.01	0.98-1.04
HRT		0.99	0.71-1.37	1.03	0.73-1.46	-	-	-	-

Abbreviations: OR, Odds ratio; CI, confidence interval; PY, pack years; BMI, Body mineral index; BMD, bone mineral density; a, age at inclusion; HRT, Hormone replacement therapy

14.3. Study 3

Cardio vascular disease and the risk of lung cancer, the HUNT study

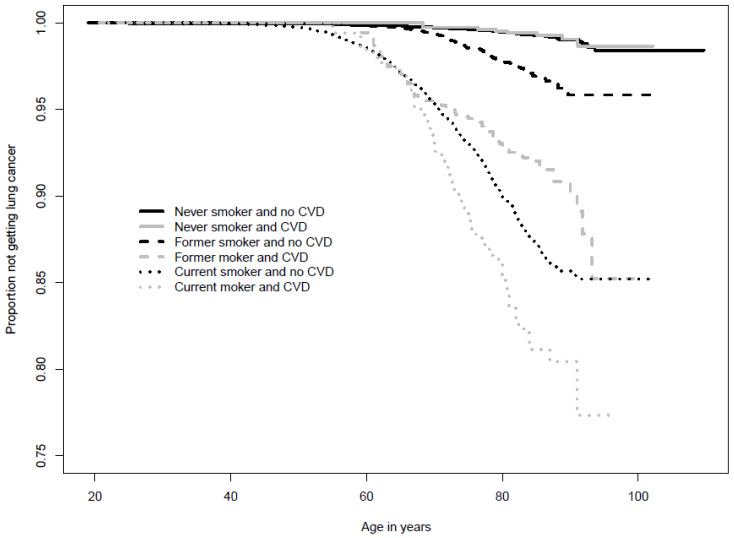
During follow-up from 1984-2008, 1080 cases (1.1%) of lung cancer occurred (20% SCLC, 80% NSCLC), 721 cases (1.5%) among men and 359 cases (0.7%) in women, p<0.001. The mean age at diagnosis of lung cancer was 70.2 ± 10 years for both sexes. The cumulative incidence of lung cancer in never smokers, former smokers and current smokers was 72 (0.2%), 182 (0.9%) and 698 (2.6%) respectively, p<0.001. Missing smoking data was observed in 128 (1.2%).

In never smokers, 37 cases of lung cancer / 100,000 person years were seen in those with CVD vs. 12 cases / 100,000 person years in those without CVD. In former and current smokers 280 cases of lung cancer / 100,000 person years were seen in those with CVD vs. 64 cases of lung cancer / 100,000 person years in those without CVD.

In univariate regression model CVD was not a statistically significant risk factor for lung cancer in never (HR: 1.04, 95% CI: 0.41-2.67), but statistically significant risk factor in former (HR: 2.47, 95% CI: 1.77-3.45) and current smokers (HR: 1.59, 95% CI: 1.26-2.01), (figure 10).

In former and current smokers men were at a higher risk getting lung cancer compared to women (HR: 3.94, 95% CI: 2.59-5.97; HR: 1.86, 95% CI: 1.59-2.16 respectively for former and current smokers). In former and current smokers, chronic chough was a risk factor for lung cancer (HR: 2.19, 95% CI: 1.07-4.44; HR: 2.57, 95% CI: 2.08-3.18, respectively for former and current smokers). In former and current smokers heavy smokers were at a higher risk getting lung cancer compared with light smokers (HR: 8.61, 95% CI: 5.96-12.45; HR: 6.01, 95% CI: 5.17-7.15, respectively for former and current smokers).

Figure 9: Plot of proportion "not getting lung cancer" by age for never smokers (n=38,656), former smokers (n=20,914) and current smokers (n=26,894), separately presented for persons with (n=5,981) and without CVD (n=80,483). The HUNT study 1984-2008



After adjustment for confounders the positive association found in the unadjusted model between CVD and the risk of lung cancer, disappears in never smokers. In former and current smokers CVD was still associated with a 1.4-1.7- fold increased risk getting lung cancer, (table 11 A-C).

Table 10: Cox regression model to analyze the association between CVD and lung cancer (A) never smokers (B) former smokers (C) current smokers

A:

Cox regression model to analyze the association between CVD and lung cancer in never smokers, adjusted for BMI, sex and chronic cough and phlegm, with age used as the time variable. N 33 121, 58 cases of lung cancer.

	HR	95% CI	p-value
CVD	0,87	0,34-2,23	0,778
BMI*			
< 18.5	4,57	0,60-34,76	0,142
≥ 18.5 – < 25.0	1		
≥ 25.0 – < 30.0	2,13	1,16-3,92	0,0154
≥ 30.0	1,03	0,39-2,69	0,958
Sex			
male vs. female	2,34	1,37-3,96	0,0019
Chronic cough with phlegm			
yes vs. no	2,51	0,78-8,09	0,1222

B:

Cox regression model to analyze the association between CVD and lung cancer in former smokers, adjusted for BMI, sex, burden of smoking and chronic cough and phlegm, with age used as the time variable. N 11 776, 102 cases of lung cancer.

	HR	95% CI	p-value
CVD	1,74	1,11-2,73	0,016
BMI*			
< 18.5	2,60	0,36-19,01	0,347
≥ 18.5 – < 25.0	1		
≥ 25.0 – < 30.0	0,81	0,53-1,25	0,339
≥ 30.0	0,80	0,43-1,48	0,473
Sex			
male vs. female	1,43	0,82-2,49	0,206
Chronic cough with phlegm			
yes vs. no	0,88	0,32-2,42	0,808
Heavy vs. light smoker	3,56	2,33-5,45	<0,001

C:

Cox regression model to analyze the association between CVD and lung cancer in current smokers, adjusted for BMI, sex, burden of smoking and chronic cough and phlegm, with age used as the time variable. N 20 931, 521 cases of lung cancer.

	HR	95% CI	p-value
CVD	1,38	1,04-1,83	0,024
BMI*			
< 18.5	1,36	0,67-2,75	0,39
≥ 18.5 – < 25.0	1		
≥ 25.0 – < 30.0	0,98	0,81-1,17	0,788
≥ 30.0	0,89	0,65-1,23	0,494
Sex			
male vs. female	1,09	0,90-1,31	0,390
Chronic cough with phlegm			
yes vs. no	1,55	1,22-1,97	<0,001
Heavy vs. light smoker	2,11	1,75-2,54	<0,001

In former smoker the HR for CVD in cases with missing data for either chronic cough with phlegm or burden of smoking (N=6425) was 2.85 95% CI: 1.68-4.89 and in the analysis including missing cases and complete cases (N=18201) the HR 2.13 CI 95% 1.24-2.45. The estimates for HR for CVD was not significant different in these analyses, Wald test p=0.16.

In current smoker the HR for CVD in cases with missing data for either chronic cough with phlegm or burden of smoking (N=4499) was 1.62 95% CI: 1.02-2.53 and in the analysis including missing cases and complete cases (N=25430) the HR 1.44 CI 95% 1.13-1.82. The estimates for HR for CVD was not significant different in these analyses, Wald test p=0.56.

The results shows that the missing cases did not changed the estimates for CVD and the results were consistent in all groups.

The third study shows that CVD is associated with a higher risk of lung cancer in former smokers and current smokers independent of the smoking burden, but not in never smokers.

15. Discussion

We have studied the possible influence on incidence and survival of comorbidities in patients with lung cancer.

15.1. Diabetes mellitus and lung cancer

The overall survival in lung cancer patients with diabetes mellitus was borderline improved in the HUNT study, compared to patients without diabetes mellitus. This pattern was confirmed in the PEG study whilst corresponding results was not found in the NLCB study. When merging lung cancer patients from all three study populations, increased overall survival was found in lung cancer patients with diabetes mellitus, both in the univariate and multivariate analyses. In addition, the hazard ratio was consistent in all three studies and showed survival benefit in the diabetes population. There was no imbalance in known prognostic factors between the group with DM and without DM.

Results from studies on the impact of diabetes mellitus on lung cancer prognosis are to date conflicting. One study showed an increased survival [76] three referred no change in survival [48, 75, 78] and two studies showed decreased survival [137, 138]. We did not design our study in order to investigate potential mechanisms behind our findings. Different explanations are possible. Patients with diabetes mellitus had less often metastatic LC disease, but this should have been adjusted for by inclusion of stage of disease in our analyses. In addition, increased survival in patients with diabetes mellitus was clearly demonstrated in the PEG study where all patients had advanced lung cancer. It can be argued that the survival benefit seen in patients with diabetes mellitus depends on more frequent and regular consultations that lead to an earlier diagnosis and thereby a survival benefit. However, the fact that the

survival benefit was even more pronounced among the patients in the PEG study, where only patients with advanced lung cancer were included, contradicts the view that frequent consultation leading to earlier diagnosis is the cause of increased survival.

We found only one previous report of increased survival in patients with lung cancer and diabetes mellitus [76]. This report used x^2 analysis and no multivariate analysis and the patient number (lung cancer and diabetes mellitus) was as low as 25. Their main focus was that long-standing diabetes mellitus was suggested to be associated with impeded neoplastic cell spread and metastasis. Three studies have shown decreased survival [48, 137, 138]. The first study was a recently published meta-analysis consisting of 97 studies including hospitalized patients based on either elevated fasting blood glucose (≥ 7 mmol/L), medical records that confirmed the use of anti-diabetics or diabetes mellitus. Patients diagnosed with diabetes mellitus were in a minority. The cause of high fasting blood glucose may have been numerous. The mean age was about 55 years, which is low compared to the median age of 70 years of lung cancer patients that may represent a selection bias. About one third was followed for 2.9 years. The second study was a retrospective registry study of members of a private health system. Patients were black or white people and were included during a 3-years period and followed by two years follow up. The Charlson index of comorbidity was used and the authors did the same analyses as we did. Diabetes with end-organ damage showed an elevated adjusted HR of 1.4, (95% CI 0.2-2.72, p=0.33), which prohibited final conclusion to be drawn. The third study consisted of advanced lung cancer patients. Two research letters showed no association between diabetes mellitus, lung cancer and survival, but it is noteworthy that also the study of Hanbali showed that the frequency of metastasis was lower in patients with lung cancer and diabetes mellitus [75, 78]. This indicates that our observations may not be an incidental finding.

The studies and study reports we found in our literature research were based on a very different design and number of patients, and all have limitations that may have influenced the results.

With the introduction of individualized treatment using targeted therapy, co-morbidity appears to be important to care about. This fact leads to the importance of precise characterization of both the tumor and host. One example on the important interaction between targeted treatment in lung cancer and co-morbidity is the introduction of IGF-1R (Insulin-like growth factor-1 receptor) inhibitors that have serious impact on glucose metabolism [139, 140].

The fact that patients with diabetes mellitus showed a lower frequency of metastatic diseases may partly explain the survival benefit in patients with diabetes mellitus, because the majority of the patients with lung cancer die of metastasis and not of the primary tumor. However, we adjusted for stage of disease in our analyses. Accordingly, this potential advantage can hardly explain the increased survival in patients with diabetes mellitus.

It can be argued that the survival benefit seen in patients with diabetes mellitus as a result of more frequent and regular consultations that lead to an earlier diagnosis and survival benefit. However, the fact that the survival benefit was even more pronounced among the patients in the PEG study, where only patients with advanced lung cancer were included, weakens this argumentation of frequent consultation as the cause.

15.2. Bone mineral density and lung cancer

To our knowledge this is the first study investigating the association between BMD and incidence of lung cancer, having the opportunity to adjust for specific confounders. The association between estrogen and lung cancer is discussed in capture 6.1. According to our

hypothesis we expected a lower risk of lung cancer in the population with low BMD compared to patients with high BMD due to a lower estrogen level and subsequently a lower carcinogenic drive, we presumed. We hypothesis this both in men and women, since also in men BMD is a surrogate for estrogen, capture 6.3.

Unexpectedly, we found that a low BMD z-score was strongly associated with a higher risk of lung cancer in both sexes. The findings remained unchanged after adjusting for smoking, BMI and lung function. After our knowledge there are no other studies investigating BMD and lung cancer. However the association between estrogen and lung cancer was discussed before. Studies investigating estrogen, hormonal replacement therapy (HRT) and the risk of lung cancer have shown conflicting results. Due to different designs, mainly inclusion of women, comparisons are difficult. Taioli et al showed an increased risk of adenocarcinoma in females using HRT [141]. Adami et al did not find any association between the use of HRT and the occurrence of lung cancer in a large cohort study [142]. Kreuzer et al showed that patients who had used oral contraceptives had reduced risk of lung cancer, but it was not the case in those who had used hormonal replacement therapy [143]. In contrast Rodriguez et al showed that hormonal replacement therapy reduced the risk of lung cancer, but this was not the case in former hormonal replacement therapy users [144]. Schabath et al showed a 36% risk reduction of lung cancer in women using hormonal replacement therapy, but this effect decreased with increasing pack years and disappeared in heavy smoker [145]. All these results from former studies include only females.

According to our knowledge there is only one study analyzing the association between osteoporosis and lung cancer. McGlynn et al showed in a large cohort study from Denmark that, men and women younger than 70 years having osteoporosis had an increased risk for lung cancer compared to those without osteoporosis [95]. The authors argued that young patients with osteoporosis were more likely to smoke and suggested that this was the reason

for their results. As far as we know, this study could not answer this question due to lack of data on individual life style factors.

McGlynn had not access on risk factors like smoking and used instead clinical diagnosis as a surrogate for smoking (emphysema) by using the International Classification of Diseases (ICD 10) code system. In addition the authors used the same code system to identify patients with osteoporosis and it is not exactly clear which criteria that were used for the diagnosis.

Inflammation, osteoporosis and lung cancer

Inflammation is found to play a role in osteoporosis. Interleukin promotes osteoclast activation and increases the rate of bone remodeling and bone loss [146]. Inflammation may also play a role in lung cancer development [147]. Both in animal and human studies it has been shown a sex difference in lung development, i.e. lung anatomy and physiology, respiratory function and lung toxicology [148, 149]. Further, smoking women are more susceptible to develop lung cancer compared to smoking men. Apoptosis is an important part of the inflammatory process. Tesfaigzi et al showed a sex difference in the apoptotic mechanism by exposing mice to a lipopolysaccharide and measuring Bcl-2 gene expression (or activation), a regulator of apoptosis. The female mice recovered faster after the exposure compared to male mice. Further, the authors showed that there is a sex difference showing a higher level of interleukin 6 in bronchoalveolar lavage in male mice [150]. These factors may in part explain that there may be sex differences in the occurrence and susceptibility of developing lung cancer.

Vitamin D, osteoporosis and lung cancer

Nutrition plays an important role in bone health, especially calcium and Vitamin D. A prospective study from Finland showed an inversely association between Vitamin D and lung

cancer risk in females and younger participants but not for men [105]. However, Weinstein et al did not find any association between Vitamin D and the risk of lung cancer in smoking males [151]. Unfortunately, we have no access to vitamin D levels in our study. If vitamin D deficiency should explain our findings, we would expect that the result were equal in both study groups, because both low z-score and fracture should be associated with low vitamin D levels. It is known that there are genetic variations of the vitamin D receptor. In other cancer types, like colon cancer, there has been shown that the genetic variation at the VDR locus influences the risk of cancer [152]. It has been not studied yet if this genetic variation may be important in the development of lung cancer and might explain the sex difference.

15.3. CVD and lung cancer

The present study indicates that CVD is an independent risk factor for lung cancer in former and current smokers, with strongest associations in former smokers compared to current smokers. Adjusting for established risk factors for lung cancer like BMI, sex, clinical symptoms indicating chronical inflammation in the lower airways and the burden of smoking, weakened the associations, but CVD was still a risk factor for lung cancer. To our knowledge this is the first study investigating the association between CVD and the incidence of lung cancer.

Chronic inflammation plays a key role in the underlying pathophysiology of both CVD and lung cancer, and could theoretically explain our findings. Chronic inflammation is associated with an increased cell turnover with the potential of generating genetic errors, as well as stimulating angio-neogenesis and apoptosis. Different types of chronic inflammatory diseases are associated with cancer. COPD is known to be a chronic inflammatory disease and is an independent risk factor for lung cancer [153-155]. Also other different types of cancer are

associated with general chronic inflammation. Ankylosing spondylitis is positively associated with kidney cancer [156] and local inflammation like inflammatory bowel disease is associated with a higher risk of colon cancer [157].

CVD has a high prevalence among patients with lung cancer, about 23% [45] and chronic inflammation is associated with the development of CVD [158, 159]. We found a positive association between CVD and lung cancer in both former and current smokers, but not in never smokers in our population. A possible explanation may be that the chronic inflammation responsible for development of CVD is not alone an independent risk factor related to lung cancer development, but smoking and CVD may have an additive or synergistic effect. This inconsistence may also be explained by the fact that heavy former smokers tend to die before they get lung cancer.

Because smoking causes both lung cancer and CVD our results could be confounded by the effect of smoking. Stratification by smoking status and adjustment for smoking burden (light and heavy smokers), did, however, not change the estimates. Nevertheless, residual confounding by tobacco smoking cannot fully be ruled out.

Other confounders like alimentary factors like nutrition, vitamins and less physical activity and indoor air pollution may also contribute to the development of lung cancer [105, 160-162] and are not tested in our study. SCLC was more frequent in former and current smokers compared to never smokers, which is a well known fact. The inflammatory pathway or carcinogenesis may be different in SCLC and NSCLC.

Concerning the interaction between the burden of smoking and CVD in former smokers, this may depend on bias related to self-reported smoking. Those with less education and/or heavy smokers are likely to report accurate number of cigarettes per day [163] and this is especially seen in former smokers [164].

However, our observation in the current study showing that CVD in former and current smokers may provide an additional risk factor for lung cancer is noteworthy but not surprising given the high prevalence of CVD in patients with lung cancer compared with a lower prevalence of CVD in persons without lung cancer (23% vs. 10%). CVD has been shown as a risk factor for colorectal cancer, persons with CVD had nearly twice the prevalence of colorectal cancers [165]. Smokers with CVD may be at high risk developing lung cancer compared to smokers without CVD. If so is the case, it may have an implication for detection of lung cancer since a large proportion of the population is either current smoker or former smoker and CVD has a high prevalence among these.

16. Other considerations

Confounders

A confounder is a variable that correlates (positive or negative) with both the dependent and independent variable.

In all three studies of this PhD thesis potential confounders like medication and physical activity are not included into our models. This might have influence the results.

Different studies have investigated the use of Metformin in patients with lung cancer. Metformin seems to prevent lung cancer carcinogenesis in mice [79]. Another study showed a decreased risk of cancer in general and also of lung cancer [166]. However, other studies showed no association between metformin and the risk of lung cancer [82]. Insulin seems to increase the risk of lung cancer possible via activation of the insulin-like growth factor [73]. Most of the patients in our study with diabetes had diabetes mellitus type II, so it is unclear whether our result can be transferred to patients with diabetes mellitus type I.

Bisphosphonate are a class of drugs that prevent bone loss. They have been used since the beginning of this century in a widely grad.
Literature about the influence of bisphosphonates on the risk of lung cancer is rare, but several studies show that bisphosphonates influence on the progression of the disease. It is shown that bisphosphonates development of skeleton complications [167, 168]. The association between calcium intake and the risk of lung cancer is not well investigated. One study concluded that increased dietary calcium has a preventive effect on the development of lung cancer in non smoking women [169]. The HUNT 2 registry study does not have a design able to solve this question or give possible answers. We have to keep to the descriptive presentation. Use of NSAID as well as statins may reduce the risk of lung cancer [18] [170]. Physical activity is associated with a lower risk of lung cancer [17].

In the HUNT study there are questions about diabetic medication and activity. Unfortunately, the answers response among people developing lung cancer in our study were low for these questions (about 30% response on medication and about 40% on physical activity) and we did not include these data in our analyses. Probably is this low response rate not a random phenomenon, and in case would bias our results.

17. Statistical considerations

17.1. Missing data

Missing data are unavoidable in large epidemiological studies and can undermine the validity of research results. Many different reasons can lead to missing data.

Some missing data can affect the results other does not. Another problem is that some variables may only have small number of missing but in combination the number of patients with some missing data can be large.

Missing data are classified as: missing completely at random, missing at random and missing not at random.

Generally it is not possible to know whether missing data are at random or not at random. Missing data not at random causes problems not those missing data at random. A frequent solution to deal with missing data is the complete case method, in which the analysis uses only observations with all variables present.

The One-way sensitivity analysis is one way to deal with missing data. Results from a complete data set is compared to the same analysis but now with an additional variable included. If the results did not change substantial the “new” variable is not important for the calculation.

Multiple imputation (MI) has been become in the last years a common approach in missing data analysis. Prerequisite for imputation is that data are missing at random. Imputation preserves all cases by replacing missing data with a probable value based on other available information. Once all missing values have been imputed, the data set can then be analyzed using standard techniques for complete data [171]. The results are combined using simple rules to yield estimates, standard errors, and p-values that formally incorporate missing-data uncertainty. Large-scale imputation can introduce bias and can introduce more bias than a complete case analysis if the imputation model does not fit the data well. For this reason, using multiple imputation it is recommend carrying out a complete case analysis in parallel [172].

If the results in the analysis with completely observed data (complete case method) is similar with the results analyzed with missing data, there are reasons to believe that missing data are

at random. Then, it is plausible that the missing observations are unlikely to influence the results.

In study 1 and 2 we used the complete case method without imputation.

In study 3 we used the maximum likelihood estimation to handle cases with missing data. In this study population we had a relatively high number of missing data regarding smoking status (19%), the burden of smoking (22%) and chronic cough with phlegm N=23013 (21.5%). Questions on tobacco smoking were included in a follow-up questionnaire in HUNT1, whilst in HUNT2 and 3 these were included in the main baseline questionnaire. This influences the response rate; 78%, 98% and 97% in HUNT 1, 2 and 3 respectively. In total smoking status was known in 86,674 (81 %) persons. To deal with the high amount of missing data we used imputation for chronic cough with phlegm and burden of smoking. In never smoker the HR for CVD in cases with missing data for either chronic cough with phlegm or burden of smoking (N=2902) was 1.26 95% CI: 0.28-5.68 and in the analysis including missing cases and complete cases (N=36023) the HR 0.96 CI 95% 0.44-2.09. The estimates for HR for CVD was not significant different in these analyses, Wald test p=0.67.

17.2. Combination of data from 3 different studies

Pooling data from different independent studies is common and can be useful. It increases sample size and so statistical power. However pooling data can have some pitfalls, like the study populations can be quite different and therefore not comparable, and not all variables may be comparable, or at last, participants can be counted more than one time [173]. An other way handling different studies is a separately analyses and comparing the results.

In the first study on diabetes mellitus and survival in lung cancer we data from three studies were combined. The main reason for pooling the data was to increase the sample size and thus the power for the analyses. The three studies (HUNT-, PEG- and NLCB-study) recruited patients from the same geographical part in Norway (North- or South-Trøndelag in the middle of Norway) and can therefore subsequently compared. The studies represent different time periods. The HUNT-study included a cohort of persons over an extended time span where the prevalence of lung cancer was 1-3%. The NLCB-study included patients suspected to have lung cancer with a prevalence of lung cancer of 73%. The PEG study included only advanced NSCLC (IIIB/IV). Extracting verified lung cancer patients that were treated after the same national guidelines should not introduce bias related to diagnoses or treatment. All participants in the HUNT, PEG study and NLCB-study are identified with an eleven digits national identification number that all Norwegians are given at birth or immigration. All data are linked by this key and no duplicate data is present in our dataset.

17.3. The HUNT study, sample size, possible bias, variables and other considerations

From 1984 to 2008 a total of 153545 persons have been invited to the HUNT study. This population is considered representative of the Norwegian population, but the county of Nord-Trøndelag lacks larger cities, has a lower educational and income level, and the proportion of smokers is slightly below the mean in Norway. In all 126159 (82%) have participated. A non-responder study showed a possible selection bias [174, 175]. The main reasons for non-attendance for people aged 20–44 years was the lack of time and staying outside the county; from 45–69 the main reasons was being very busy, forgetting the invitation or not being

interested in the study. In the aged 70 and older, the main reasons for non-attendance was having regular follow-up in the health services or being immobilized due to disease [176]. All data have been linked to our national cancer registry [133] with by law regulates nationwide registration of all cancers and to the Cause of death Register [177] what should ensure a high reliability and validity of the cancer diagnosis and other dates.

18. Limitation of the studies

Common limitations for all three studies were the limited information about medication, both daily medications but also lung cancer therapy and the data about smoking habit was not complete.

18.1. Study 1

Prolonged survival in lung cancer patients with diabetes mellitus

A potential shortcoming is that we used a classification system that divided the lung cancer population in limited or advanced disease, however it was based on the TNM system. Further, we had no information about lung cancer- and type of diabetes mellitus treatment in the HUNT study. However, we have no reason to believe that the treatment indication and chosen therapy for lung cancer in the three studies differ between patients with and without diabetes mellitus during the study period. In Norway we practice very similar treatment indications and modalities for the lung cancer population in accordance to the national guidelines, both with and without diabetes mellitus. Performance status (PS) is a known prognostic factor, but was not registered in the HUNT study. Accordingly, PS was not incorporated in our analyses.

18.2. Study 2

Bone mineral density, fracture history, self reported osteoporosis as proxy variables for estrogen and the risk of Non-Small-Cell Lung Cancer – a population based cohort study, the HUNT study: are proxy variables friends or faults?

The limitations of the study are the predominance of women in each of the three study groups. For most of the lung cancer patients we did not have status about estrogen substitution therapy.

BMD is the most reliable surrogate marker for life time estrogen exposure. This is the reason for not discussing fracture history and self reported osteoporosis here, but it is discussed in the paper 2.

18.3. Study 3

Cardio vascular disease and the risk of lung cancer, the HUNT study

One limitation is the number of missing data (about 20%) regarding smoking status, pack years and data about chronic cough with phlegm. To deal with the amount of missing data we used imputation for chronic cough with phlegm and burden of smoking, knowing that this may be bias the results. However, the results showed that the missing cases did not change the estimates for CVD and the results were consistent in all groups.

Further we have adjusted for confounders at baseline and not included changes during observation which might bias the results.

Non-participants in the HUNT study had lower socio-economic status and a higher mortality than participants. In addition the prevalence of CVDs, diabetes mellitus and psychiatric

disorders were higher in non-participants [176]. This fact may bias the results in our three studies.

19. Strengths of the studies

In our study population with approximately 106 000 participants the prevalence of lung cancer (1.9%) is comparable to what is seen in western countries. The median age of the lung cancer patients in the HUNT study was 71 years and indicates good external validity of the present study. In addition to the large and representative study population, it consisted of a cohort from a well-defined geographical area, with a stable number of inhabitants and less migration and emigration of the people. The participation rate in the HUNT study was high, approximately 90, 70 and 50% in the three periods (inclusion waves), respectively. The HUNT-study represents a large database of information about different known risk factors and confounders for NSCLC, like tobacco smoking, BMI, lung function and HRT use. The long observation period, as well as a high mean age further strengthens our study results. At last, the use of our national cancer registry with forced nationwide registration of all cancers ensures a high reliability and validity of the cancer diagnosis.

20. Main conclusions of the studies

- Lung cancer patients with diabetes mellitus seem to have an increased survival compared to lung cancer patients without diabetes.
- Low bone mineral density is associated with a higher risk of lung cancer in men and women.
- Cardio-vascular disease is found to be an independent risk factor for developing lung cancer in former and current smokers.

21. Implications of the results for the future

- The survival benefit in lung cancer patients with diabetes mellitus may justify that lung cancer patients with diabetes mellitus should get the same treatment as the other patients without DM (DM should not be a excluding factor for treatment).
- Patients with low bone mineral density seem to have higher risk for LC and may be a potential candidate for inclusion in future screening trails.
- Patients with a positive smoking history and cardio vascular disease have a higher risk for LC and may be candidates for inclusion in future screening trials.

22. References

23. Paper I-III

24. Poster I-III

25. Appendix I-III

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Paper 1

Prolonged survival in patients with lung cancer with diabetes mellitus.

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Paper 2

Bone mass density, fracture history, self-reported osteoporosis as proxy variables for estrogen and the risk of non-small-cell lung cancer--a population based cohort study, the HUNT study: are proxy variables friends or faults?

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Bone mass density, fracture history, self-reported osteoporosis as proxy variables for estrogen and the risk of non-small-cell lung cancer—A population based cohort study, the HUNT study: Are proxy variables friends or faults?

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ABSTRACT

Lung cancer has the highest mortality of all cancers. Patients with early stage disease have the best cure rates and that emphasizes the importance of early detection. About half of all non-small cell lung cancers (NSCLC) are estrogen receptor positive. The impact of estrogen and its receptors for NSCLC carcinogenesis has been studied but is still unclear. Low estrogen levels are associated with osteoporosis. We hypothesize that low bone mineral density (BMD), a positive history of fracture or self-reported osteoporosis, used as a proxy variable for life time estrogen exposure, are associated with a low incidence of NSCLC. We analyzed data from a cohort study, the Nord-Trøndelag Health Study 2 (1995–1997) linked to the Norwegian Cancer Registry. Using the logistic regression model we calculated the odds ratio (OR) with a 95% confidence interval (CI) for the risk of NSCLC for the three proxy variables, stratified by sex. Participants older than 50 years of age, having measured bone density (N = 18,156), having answered the questions on self-reported fracture (N = 37,883) and osteoporosis (N = 25,701) and known body mass index (BMI) (N = 29,291), were evaluated for inclusion. In 6996 participants all these information was available in addition to tobacco use, and in women also hormonal replacement therapy (HRT). Lung function (FEV1 percent of predicted) was included in a sensitivity analysis. We identified 132 (1.9%) cases of NSCLC, 59 (1.2%) and 73 (3.3%) cases in women and men, respectively. Low BMD was associated with a higher risk of NSCLC, OR: 2.38, 95% CI: 1.09–5.18 and OR: 2.67, 95% CI: 1.39–5.16 in women and men, respectively. No association was found between the two other proxy variables and the risk of NSCLC. Inclusion of lung function in the model did not change the results. Contrary to our hypothesis, women and men with low BMD had a higher risk for NSCLC. In addition the study demonstrates that the risk depends on which proxy variable was chosen, and we may ask: are proxy variables reliable?

1. Introduction

Lung cancer has the second highest incidence rate and the highest mortality rate of all cancers [1]. Early detection followed by surgery provides the best survival rates. This emphasizes the importance of identifying new risk factors, in addition to known factors like age and tobacco smoking [2] that can be included in future screening programs.

About half of all non-small cell lung cancers (NSCLC) are estrogen receptor positive [3,4]. The impact of estrogen and its receptors for NSCLC carcinogenesis has been studied, and contradictory results are published [5–9]. Low estrogen levels are associated with osteoporosis [10–12]. Former studies have indicated that bone mineral density (BMD) in women as well as in men, might reflect life time estrogen exposure [13,14]. BMD should therefore reflect total estrogen exposure better than measured estrogen levels at one or few previous occasions [11,13–18]. In women estrogen therapy prevents bone loss after menopause. The prevalence of osteoporosis increases in both sexes [19]. Accordingly BMD, self-reported

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osteoporosis and self-reported previous fractures might be used as surrogate measures of life time estrogen exposure. BMD has been studied as a possible risk factor for cancer prostate, breast and colon cancer [20–26]. Low BMD is an important risk factor for fracture [27–30]. A positive fracture history was not associated with the risk of ovarian cancer but was associated with a decreased breast and endometrial cancer risk [31,32].

Self-reported osteoporosis has good reproducibility, high specificity, but low sensitivity compared to BMD based osteoporosis diagnosis [33].

Based on the current knowledge we anticipated that a lower cumulative estrogen exposure is associated with a lower risk of NSCLC in both sexes, and hypothesized that corresponding association could be found for surrogate measures of low estrogen exposure as low BMD, self-reported fracture history or self-reported osteoporosis, This hypothesis was studied in the second survey of a large population based cohort study in Norway, the Nord-Trøndelag Health Study (HUNT2).

2. Methods

2.1. Cohort

The HUNT study is a large population-based prospective cohort study in Nord-Trøndelag, Norway, having collected data in three surveys [34]. The county had about 127 000 inhabitants in 1996. In total 65 237 people, age 20 or above, participated in HUNT2 (69% of invited). This population is thoroughly studied and is fairly representative for the whole population in Norway. However, the county, in the middle of Norway, has few larger cities, has a slightly lower educational and income level, and the proportion of smokers is slightly below the Norwegian mean. In the present study we used data from HUNT2 (1995–1997) which were linked to lung cancer data from the Cancer Registry of Norway and the Death Cause Registry of Norway at Statistics Norway [35].

The observation period was from the day of inclusion in the HUNT2 survey until the event of lung cancer, death or the end of the study at December 31st 2008, whichever occurred first.

2.2. Outcome variable

2.2.1. Lung cancer

Lung cancer diagnosis was based on the classification system established by the World Health Organization (WHO) and was histologically verified (biopsy or cytology specimen) [36]. Estrogen receptors have only been found in NSCLC, therefore persons with small-cell lung cancer (SCLC) were excluded from the analyses. Norwegian law dictates that all new cases of cancer must be registered in the Cancer Registry of Norway.

2.3. Exposure variables

2.3.1. Bone mineral density

BMD was measured, in the period from August 1995 to June 1997, in the non-dominant distal forearm using single energy X-ray absorptiometry (SXA) (Osteometer DTX 100, Osteometer AS, Copenhagen). Measure site was 24 mm proximal from the point where the distance between radius and ulna was 8 mm.

BMD was measured as part of two HUNT2 sub studies: the Osteoporosis Study, inviting random samples of women born in the periods 1911–1930, 1936–1945 and 1954–1963, and the Lung Study, inviting a random sample of all participants, and those with self-reported "ever had asthma, use of asthma medication or asthma related symptoms during the last year" [37,38]. Sex specific BMD *z*-scores were calculated as (observed BMD minus mean BMD) divided by standard deviation (SD). Mean BMD and SD were calculated at three years intervals. However two years intervals were used during the ages of 48–62 years, due to increased bone loss within this group. BMD *z*-score were reported in tertiles defining low, medium and high *z*-scores.

2.3.2. Self-reported fracture history

The participants were asked about former fractures in the wrist, hip or vertebra. A total of 55 052 (84%) persons answered this question. An affirmative answer to at least one of these questions was defined as a positive self-reported fracture history. To avoid inclusion of high energy fractures, persons with fracture at the age 50 years or younger were excluded.

2.3.3. Self-reported osteoporosis

Self-reported osteoporosis was defined by an affirmative answer to one of these questions "Has your doctor ever said that you have osteoporosis" or "Do you have or have you had osteoporosis". A total of 52 804 (81%) answered this question.

2.4. Covariates

Potential confounders were evaluated by use of a Directed Acyclic Graph (not shown) and included in logistic regression analyses if they met the criteria for being defined as a confounder. These were tobacco smoking (four categories: 0, 1–20, 21–40 and >40 pack years), BMI (four categories according to the WHO criteria; <18.5 kg/m^2 = underweight, 18.5–24.9 kg/m^2 = normal weight, 25–29.9 kg/m^2 = overweight, ≥30 kg/m^2 = obesity). In women hormone replacement therapy (HRT) use, defined as ever/never users, was also included in the model. Persons invited to the lung study also performed spirometry. In a sensitivity analysis, lung function defined by prebronchodilator forced expiratory volume in one second/forced vital capacity ratio (FEV1/FVC ratio) (≤0.7/>0.7), was included as confounder, data were eligible for 4246 cases (45%). In analyses including BMD we did not adjust for age since BMD *z*-score was already adjusted for age. In analyses including self-reported fracture history or self-reported osteoporosis, the age at inclusion in the HUNT2 was included as a continuous variable in the model.

"Lung symptoms", included in a sub-analysis, was defined by a positive answer to questions on asthma related symptoms.

Only participants older than 50 years, having measured bone mineral density, having answered the questions on self-reported fracture and osteoporosis, with known body mass index (BMI) and tobacco use and in women with known HRT status were included in our study (Fig. 1).

2.5. Statistical analysis

All statistical analyses were stratified by sex. Logistic regression was used to assess odds ratio (OR) with 95% confidence interval for developing NSCLC. Two-sided tests were used and statistical significance was defined as $P < 0.05$. Lung function and self-reported lung symptoms were included in the model as sensitivity analyses.

The Hazard function was calculated by Cox regression models using NSCLC as the defined event, and BMD *z*-score, self-reported fracture history and self-reported osteoporosis as explorative variables, respectively. Included confounders were tobacco use, age at inclusion (not when using BMD *z*-score as the explorative variable), BMI, and in women HRT as well.

Interaction terms between all confounders, used in the model, and the exposure variables were tested, as a product in the logistic regression model.

To test the correlation between the explorative variables (BMD z-score, self-reported fracture and self-reported osteoporosis) the Chi-square test was used.

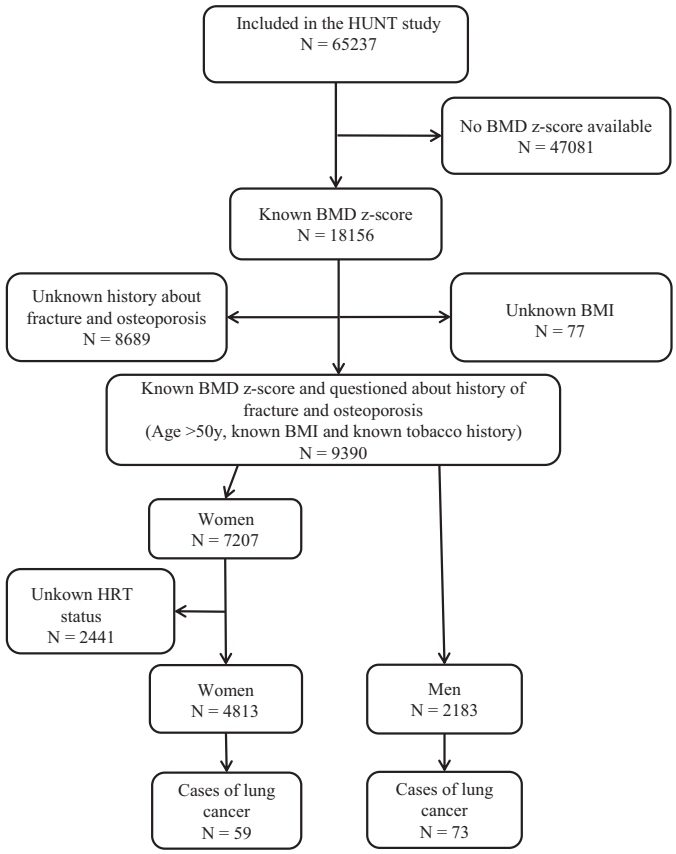

Fig. 1. Selection of the study population. *Abbreviations*: BMD, bone mineral density; *N*, number; BMI, body mass index; HRT, hormone replacement therapy.

Statistical analyses were performed using PASW version 19 (Predictive Analytics Soft Ware, IBM Corporation, New York 10589, USA).

2.6. Ethics

The Regional Committee for Medical and Health Research Ethics have approved the current study (REK# 2010/1081).

3. Results

3.1. Incidence of NSCLC

In our study population 132 (1.9%) persons developed NSCLC, 59 (1.2%) women and 73 (3.3%) men. The mean age at diagnosis of NSCLC was for women 68 ± 9 years and for men 72 ± 9 years and for death of any cause for women 82 ± 7 years and for men 78 ± 8 years. The mean age for hip fracture was 72 ± 7 years, for wrist fracture 63 ± 7 years and for vertebra fracture 69 ± 6 years (Table 1).

3.2. Correlations between BMD z-score, self-reported fracture and self-reported osteoporosis

Of all women reporting a former fracture, 43% had a low BMD *z*-score, and of those reporting osteoporosis 45% had a low BMD *z*-score. Of all men reporting a former fracture, 37% had a low BMD *z*-score, and of those reporting osteoporosis 48% had a low BMD *z*-score.

3.3. The risk of non-small cell lung cancer adjusted for confounders

Men compared to women and ever smokers compared to never smokers were at significant higher risk of developing NSCLC. Further, underweight men and women with a BMI > 25 kg/m^2 were at increased risk of NSCLC in the unadjusted model.

In both sexes those with low BMD *z*-score were at higher risk of NSCLC compared to those with high BMD *z*-score, both in unadjusted and adjusted models. In addition women with a medium BMD *z*-score were at higher risk. In both sexes there were no associations between NSCLC diagnosis and self-reported fracture or self-reported osteoporosis (Fig. 2). Smoking was an independent risk factor for NSCLC (Table 2).

Sensitivity analysis including lung function as a confounder did not change the estimates, but low numbers, 23 cases of NSCLC in women and 67 cases in men, reduced the power, especially among women. Statistical significance was not reached in women but the trend however, that a lower BMD *z*-score is associated with a higher risk of NSCLC, persisted.

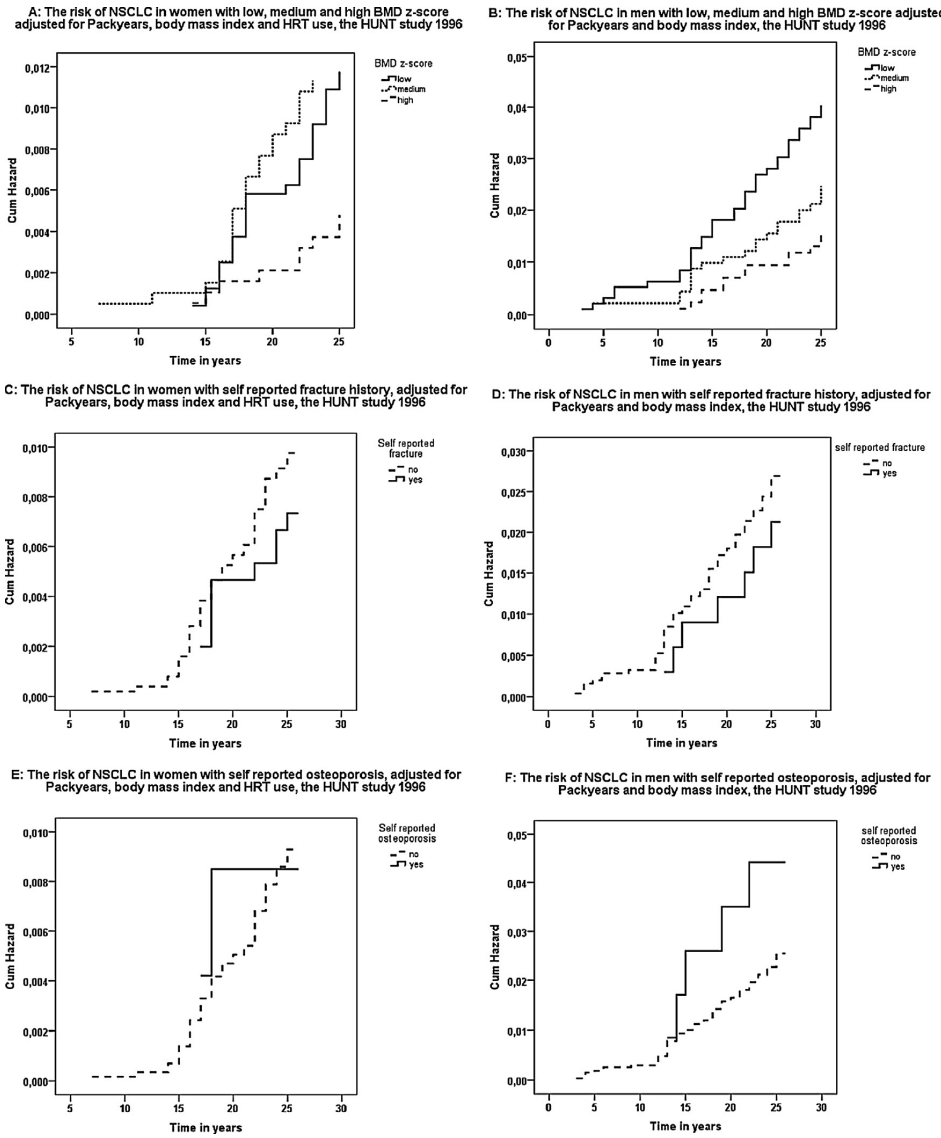

Fig. 2. Hazard function for the risk of NSCLC, adjusted for pack years, BMI, HRT (A–F) and in addition for age at inclusion (C–F). (A) BMD *z*-score in men and (B) in women; (C) self-reported fracture in men and (D) in women; (E) self-reported osteoporosis in men and (F) in women. *Abbreviations*: BMD, bone mineral density; BMI, body mass index; HRT, hormone replacement therapy; NSCLC, non-small cell lung cancer.

We did not find any interaction between the confounders and the exposure variables (data not shown).

3.4. Inclusion of lung symptoms in the logistic regression model

A total number of 725 (34%) women and 1568 (76%) men specified self-reported lung symptoms in the survey. We included self-reported lung symptoms in the logistic regression models in addition to tobacco use, BMI and HRT (only in women). As shown in Table 3 the inclusion of self-reported lung symptoms did not change the results.

4. Discussion

4.1. Main findings

Low BMD *z*-score was strongly associated with increased risk of NSCLC in both sexes. These findings remained unchanged after adjusting for tobacco use, BMI and in women, HRT. Sensitivity analysis including lung function as a confounder did not change the estimates in men, in women the estimates were no longer significant but the pattern, that lower BMD is associated with a higher risk of NSCLC, was still present.

Table 1
Characteristics of the study populations, The HUNT study, 1995–2008.

	Women		Men		Total	
	N	%	*N*	%	*N*	%
BMD z-score						
High	1601	33	747	34	2348	34
Medium	1590	33	774	35	2364	33
Low	1622	34	692	31	2314	33
Fractures						
Yes	1083	23	268	12	1351	20
No	3730	77	1945	88	5675	80
Osteoporosis						
Yes	515	11	90	4	605	10
No	4298	89	2123	96	6421	90
Tobacco use in						
0	3139	66	805	37	3944	60
Pack years						
1–20	1306	26	687	32	1997	27
21–40	331	7	509	23	840	10
>40	37	1	175	8	212	3
BMI						
Underweight	38	1	18	1	56	1
Normal	1458	29	616	28	2074	29
Overweight	2062	43	1125	51	3187	45
Obesity	1222	27	437	20	1659	25
Age[a,b]	68 y ± 9 y		65 y ± 9 y			
HRT						
Ever	1229	26	–	–	1229	26
Never	3584	84	–	–	3584	84

Abbreviations: BMD, bone mineral density; *N*, number; BMI, body mass index; y, years; HRT, hormone replacement therapy.
[a] Results reported in mean and standard deviation.
[b] Age at inclusion.

No association between the risk of NSCLC and other measures of bone strength as self-reported osteoporosis or self-reported fractures was found, neither in men nor in women.

Our hypothesis, that the surrogate variables for low estrogen exposure like low BMD, self-reported fracture history or self-reported osteoporosis, were associated with a lower occurrence of NSCLC, was rejected.

Noteworthy, our results demonstrate that the choice of proxy variable may have important effects on the results. To our knowledge this is the first study to investigate three proxy variables of life time estrogen exposure and its association to the incident of NSCLC, and in the same study having registered specific confounders and adjusted for them, like tobacco use, BMI and HRT.

4.2. Estrogen and non-small cell lung cancer

An increasing numbers of studies show that estrogen is essential in the bone metabolism also in men [13,16,39–41]. It is demonstrated in different studies that BMD reflects the life-time estrogen exposure over years in men [13,42,43]. In studies analyzing the risk of cancer BMD was used as a marker for life-time estrogen exposure [20,24]. Accordingly available literature provides good evidence for the appropriateness of using BMD as a proxy variable for long-time estrogen exposure in men.

Earlier studies investigating estrogen, HRT and the risk of NSCLC have shown conflicting results [5–8]. Studies on HRT included only women. The fact that women and men with low BMD *z*-score and women with medium BMD *z*-score, show a higher risk of NSCLC contradicts the assumption that estrogen influences the development of NSCLC. This is in accordance with the results reported by Adami et al. [5].

4.3. Osteoporosis and NSCLC

According to our knowledge there is only one study analyzing the association between the diagnosis of osteoporosis and NSCLC. In a large Danish registry study McGlynn et al. showed that men and women younger than 70 years with osteoporosis had an increased risk of NSCLC compared to those without osteoporosis [44]. This is in agreement with our results, but our results does not support that tobacco use explains the entire association as indicated by McGlynn et al.

In contrast to our population based study with available data on life style habits like tobacco smoking, McGlynn included patients admitted to the hospital, used the diagnosis of emphysema as a surrogate measure of tobacco use and registry diagnosis of osteoporosis based on unknown criteria, opposed to the objective measure of BMD used in our study.

4.4. Proxy variables

Proxy variables are increasingly being used on a wide basis in epidemiological studies. BMD, history of fracture and self-reported osteoporosis has been used in different studies as proxy variables for long time estrogen exposure. Obviously, the three proxy variables reflect the estrogen exposure over time better than a single estrogen measurement at an earlier random point in time. Like other researchers we have no access to former estrogen levels and we are therefore dependent on the variables mentioned above.

There is an association between BMD and endogenous estrogen level in both sexes [10,12–14]. We have identified only one previous study investigating the association between self-reported fracture and endogenous estrogen level. The EPIC-Oxford prospective cohort study found an inverse association between estradiol and fracture risk in both sexes [45]. Cauley et al. showed that low BMD is associated with a higher risk of fracture in men and women [46]. We found no study investigating endogenous estrogen level, BMD and self-reported osteoporosis. However, in our study population we confirmed a highly significant correlation between low BMD *z*-score and the reporting of osteoporosis in men and women and a positive correlation between BMD *z*-score and the answer to the question on self-reported fracture. The fact that BMD is a predictor for future fractures was the reason why we used fracture as the second proxy variable [27–30]. At last, there was a positive correlation between fracture history and reported osteoporosis. Based on available literature and the good correlation between the three proxy variable observed in our population, we assume that all three proxy variables should be suitable as proxy variables for life-time estrogen exposure. However, the associations to the occurrence of lung cancer came out differently when comparing the three proxy variables. Correlation and outcome differs from cause and outcome, where possible interaction and unknown confounders must be thought of. Therefore, the three proxy variables for estrogen exposure used in our study may also be proxy variables for other factors related to bone formation or degradation. Such factors may include vitamin D, vitamin A and physical activity, and these are not studied in the present study. BMD appears to be a good objective measurement and proxy variable for life-time estrogen exposure, but it is difficult to rank the three proxy variables, and the two last variables are less studied in the literature as well.

The mean age for debut of NSCLC and the mean age for debut of fracture were approximately the same, and fractures may therefore not be recognized as a risk factor for NSCLC.

Self-reported lung symptoms did not influence the results for any of the three proxy variables.

People with previous fracture history, low BMD *z*-score and self-reported osteoporosis may be more multi morbid than others, and could therefore die before the development of lung cancer.

Table 2
Crude and adjusted odd ratio (OR) of NSCLC according to proxy variable BMD (3 categories), self-reported fracture and self-reported osteoporosis: the HUNT study, 1995–2008.

	Women				Men			
	unadjusted		adjusted		unadjusted		adjusted	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
BMD *z*-score								
BMD								
High	1	Reference	1	Reference	1	Reference	1	Reference
Medium	1.76	0.98–3.16	2.40	1.09–5.28	1.65	0.83–3.30	1.64	0.81–3.31
Low	1.90	1.07–3.38	2.38	1.09–5.18	3.28	1.73–6.21	2.67	1.39–5.16
PY								
0	1	Reference	1	Reference	1	Reference	1	Reference
1–20	4.21	2.49–7.13	4.03	2.12–7.67	1.89	0.85–4.21	1.79	0.81–4.00
21–40	10.28	5.69–18.60	10.77	5.25–22.08	4.98	2.41–10.28	4.31	2.07–8.96
>40	8.34	1.91–36.3	5.70	0.73–44.76	8.55	3.84–19.03	7.74	3.45–17.37
BMI								
Underweight	1.02	0.14–7.59	1.23	0.16–9.55	5.16	1.39–19.10	4.02	1.03–15.69
Normal	1	Reference	1	Reference	1	Reference	1	Reference
Overweight	0.49	0.29–0.81	0.79	0.44–1.41	0.83	0.48–1.41	1.01	0.58–1.76
Obesity	0.54	0.31–0.95	0.70	0.33–1.49	0.67	0.32–1.38	0.78	0.37–1.64
HRT	0.99	0.71–1.37	1.07	0.77–1.50	–	–	–	–
Self-reported fracture history								
Fracture								
Yes	0.81	0.47–1.39	0.76	0.39–1.47	0.76	0.35–1.68	0.76	0.34–1.71
PY								
0	1	Reference	1	Reference	1	Reference	1	Reference
1–20	4.21	2.49–7.13	4.67	2.41–9.03	1.89	0.85–4.21	1.91	0.86–4.26
21–40	10.28	5.69–18.60	12.70	6.12–26.38	4.98	2.41–10.28	4.75	2.28–9.88
>40	8.34	1.91–36.3	6.63	0.84–52.2	8.55	3.84–19.03	8.56	3.83–19.13
BMI								
Underweight	1.02	0.14–7.59	1.25	0.16–9.69	5.16	1.39–19.10	4.03	1.04–15.63
Normal	1	Reference	1	Reference	1	Reference	1	Reference
Overweight	0.49	0.29–0.81	0.74	0.42–1.32	0.83	0.48–1.41	0.90	0.52–1.55
Obesity	0.54	0.31–0.95	0.58	0.27–1.22	0.67	0.32–1.38	0.67	0.32–1.40
Age	0.99	0.96–1.01	1.03	0.99–1.06	1.01	0.98–1.03	1.01	0.98–1.04
HRT	0.99	0.71–1.37	1.03	0.73–1.46	–	–	–	–
Self-reported osteoporosis								
Osteoporosis								
Yes	1.06	0.55–2.06	0.93	0.39–2.22	1.78	0.69–4.52	1.73	0.66–4.51
PY								
0	1	Reference	1	Reference	1	Reference	1	Reference
1–20	4.21	2.49–7.13	4.67	2.42–9.03	1.89	0.85–4.21	1.92	0.86–4.28
21–40	10.28	5.69–18.60	12.69	6.11–26.33	4.98	2.41–10.28	4.74	2.28–9.86
>40	8.34	1.91–36.3	6.59	0.84–51.87	8.55	3.84–19.03	8.57	3.83–19.16
BMI								
Underweight	1.02	0.14–7.59	1.29	0.17–9.98	5.16	1.39–19.10	3.86	0.99–15.08
Normal	1	Reference	1	Reference	1	Reference	1	Reference
Overweight	0.49	0.29–0.81	0.75	0.42–1.33	0.83	0.48–1.41	0.89	0.52–1.53
Obesity	0.54	0.31–0.95	0.58	0.28–1.23	0.67	0.32–1.38	0.67	0.32–1.40
Age[a]	0.99	0.96–1.01	1.03	0.99–1.06	1.01	0.98–1.03	1.01	0.98–1.04
HRT	0.99	0.71–1.37	1.03	0.73–1.46	–	–	–	–

Abbreviations: OR, odds ratio; CI, confidence interval; PY, pack years; BMI, body mass index; BMD, bone mineral density; HRT, hormone replacement therapy.
[a] Age at inclusion.

Table 3
Risk of NSCLC in the three study groups (BMD *z*-score, self-reported fracture and self-reports osteoporosis): results from the unadjusted and the adjusted regression model included self-reported lung symptoms in the model. Stratified by sex. The HUNT study, 1995–2008.

	Women				Men			
	Not adjusted for lung symptoms		Adjusted for lung symptoms		Not adjusted for lung symptoms		Adjusted for lung symptoms	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
BMD z-score								
High	1	Ref	1	Ref	1	Ref	1	Ref
Medium	2.40	1.09–5.28	2.77	1.22–6.33	1.64	0.81–3.31	1.95	0.89–4.23
Low	2.38	1.09–5.18	2.75	1.22–6.20	2.67	1.39–5.16	3.39	1.64–7.01
Fracture history	0.76	0.39–1.47	0.81	0.42–1.58	0.76	0.34–1.71	0.90	0.40–2.02
Osteoporosis history	0.93	0.39–2.22	1.12	0.47–2.65	1.73	0.66–4.51	1.68	0.64–4.41

Abbreviations: OR, odds ratio; CI, confidence interval; Ref, reference; BMD, bone mineral density.

However, in our population the mean age of death was nearly the same, about 82 years, which is about 12 year after the mean age of debut of NSCLC.

4.5. Strengths and limitations

First, the registry variables in the present prospective cohort show a high level of representativity and reliability. It profits from the high participation rate (69%). A non-responder study did not find support for a selection bias [47]. Second, HUNT2 represents a large database of information about different known risk factors and confounders for NSCLC, like tobacco smoking, BMI, lung function and HRT use. Third, the long observation period of approximately 13 years, as well as a high mean age further strengthens our study results. Fourth, the use of our national cancer registry with forced nationwide registration of all cancers ensures a high reliability and validity of the cancer diagnosis.

The limitations of the study are primarily that we have no information about the estrogen receptor status of the NSCLC cases and about the estrogen serum levels.

5. Conclusion

Our hypothesis that women and men with high BMD *z*-score show an increased risk of NSCLC was rejected. Most likely estrogen does not promote NSCLC development. Despite that proxy variables are often used in epidemiologically studies, this study demonstrates that the evaluation of the potential role of estrogen in developing NSCLC depends on which proxy variable for long term estrogen exposure was chosen. This reminds us of the limitations of using proxy variables in epidemiological studies, and one may question if they are friends or faults.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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None.

Role of fundings

None.

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Manuscript 3

Cardio vascular disease and the risk of lung cancer, the HUNT study

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Plan to submit the manuscript in October/November 2013

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Poster 1

ERS 2011 Amsterdam,

Prolonged survival in lung cancer patients with diabetes mellitus: a large cohort study

NTNU – Trondheim
Norwegian University of Science and Technology

ST. OLAVS HOSPITAL
UNIVERSITETSSYKEHUSET I TRONDHEIM

Prolonged survival in lung cancer patients with diabetes mellitus

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Aim

Lung cancer patients have a high frequency of co-morbidity. Data on the impact of diabetes mellitus, the most frequent endocrine disorder, on the prognosis of lung cancer is conflicting. The aim was to investigate the impact of diabetes mellitus (DM) on survival in lung cancer.

Methods

We analyzed data from a cohort, the Nord-Trøndelag Health Study (HUNT study) linked to the Norwegian Cancer Registry, the Pemetrexed-Gemcitabine study (PEG study), an open randomized multicenter phase III trial of 436 patients with stage IIIB/IV non-small-cell lung cancer by the Norwegian Lung Cancer Group (NLCG), and the Norwegian Lung Cancer Biobank (NLCB). Kaplan-Meier method was used to compare survival and the Cox-regression model to adjust for confounders. All p-values are two-sided.

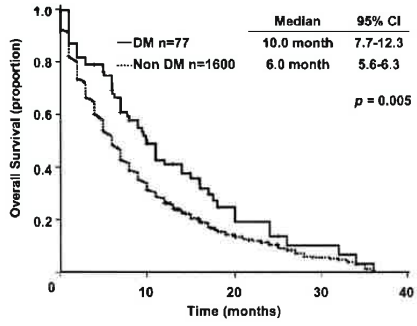
Flow sheet of the number of eligible patients included in the study

Results

The HUNT study showed a trend towards increase survival in patients with DM (p = 0.077). In the PEG study there was a significant increase (p = 0.048), but not in the NLCB study (p = 0.93).

In the combined analysis lung cancer patients with DM had increased survival compared to those without (p = 0.005). The 1-year, 2-year and 3-year survival were 43% vs. 28%, 19% vs. 11% and 3% vs. 1%, (DM vs. non DM) respectively.

Kaplan-Meier survival curve for patients with and without DM in the combined analysis



Multivariate analysis (Cox regression) for survival in patients with lung cancer

	HUNT		PEG		NLCB		Total	
	HR	CI 95%	HR	CI 95%	HR	CI 95%	HR	CI 95%
Age (≥70 vs. <70 y)	1.47	1.27-1.69	1.05	0.84-1.31	1.62	1.12-2.36	1.39	1.24-1.56
Male vs. female	1.15	0.99-1.34	0.72	0.58-0.89	1.68	1.13-2.48	1.32	1.18-1.50
Stage of disease *	1.85	1.59-2.15	1.22	0.96-1.55	2.94	1.92-4.49	1.67	1.47-1.89
SCLC vs. NSCLC	1.05	0.89-1.25	-	-	0.92	0.71-1.18	1.33	1.14-1.55
DM vs. non-DM	0.69	0.46-1.04	0.51	0.27-0.96	0.74	0.38-1.44	0.55	0.41-0.75

* Metastatic vs. non metastatic disease

Conclusions

Lung cancer patients with diabetes mellitus showed increased survival

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Poster 2

ERS 2012 Vienna,

Osteoporosis and the risk of NSCLC, the HUNT study

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Bone mineral density and risk of Non-small Cell Lung Cancer, the HUNT study

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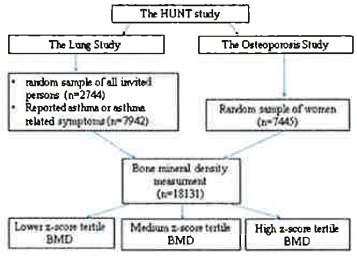
Background and aim

- Estrogen receptors α and β have been found in non-small cell lung cancer (NSCLC) tissue.
- About 50 % of all NSCLC are estrogen receptor positive, but contradictory associations between lung cancer risk and estrogens have been reported
- Bone mineral density (BMD) could be a proxy variable for estrogen

•Our aim was to investigate whether low BMD is associated with lower risk for lung cancer.

Methods

- We analyzed data from a cohort study, the Nord-Trøndelag Health Study 2 (HUNT-study 2)
- A total of 65 337 persons participated in HUNT – study 2 and of these underwent 18131 subjects bone densitometry of the forearm.
- All analyses were stratified by sex.
- Body mass index (BMI), lung function and smoking were tested as confounders in logistic regression models.



Results

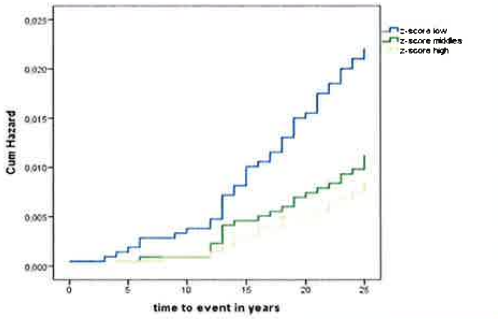
- 72% of the 18156 participants were females.
- In the low z-score group we found more ever smokers ($P < 0.001$), but no difference in age and sex distribution between the three z-score groups.
- 194 cases with NSCL were identified. Among these 56 % were females, 87% were ever smokers and the mean age at lung cancer diagnosis was 72 ±11 y.
- In men, low z-score was associated with a higher risk of lung cancer, OR 3.3 (95% CI: 1.85-5.99) and adjusted for smoking OR 2.93 (95% CI: 1.62-5.31).
- In women no association with BMD was seen.

Risk of NSCLC by tertiles of forearm BMD Z-scores

	Female			Male		
	OR	CI 95%	p	OR	CI 95%	p
z-score						
High	1	reference		1	reference	
Medium	1.32	0.803, 2.163	*	1.58	0.83, 3.02	*
Low	1.33	0.825, 2.161	*	2.93	1.62, 5.31	***
Pack years	2.99	2.38, 3.74	***	2.38	1.89, 2.98	***

Abbreviations OR, odds ratio; CI, confidence interval
p-value: *, not significant; **, <0.05; ***, <0.001

Occurrence of lung cancer (NSCLC) in males as a function of z-score (Hazard function), ajusted for smoking



Conclusions

Low bone mineral density is associated with a higher risk of NSCLC, only in men.

Oral presentation (study 3)

Oral presentation, ERS 2013 Barcelona,

Cardio vascular disease and the risk of lung cancer the HUNT study

ERS Barcelona September 6th – 11th 2013

Room 3.9 Session 441 14:45-16:45 14:45
OP Oral Presentation : Biology and molecular pathology of lung cancer

Abstract 4636 (Late breaking abstract)

Cardio vascular disease and the risk of lung cancer, the HUNT study

T. Amundsen, P. Hatlen, S. Carlsen, O. Salvesen, A. Langhammer (Trondheim, Norway)

Background: Inflammation is involved in both lung cancer (LC) and cardio-vascular disease (CVD).

Aims: We hypothesize that CVD is an independent risk factor for LC.

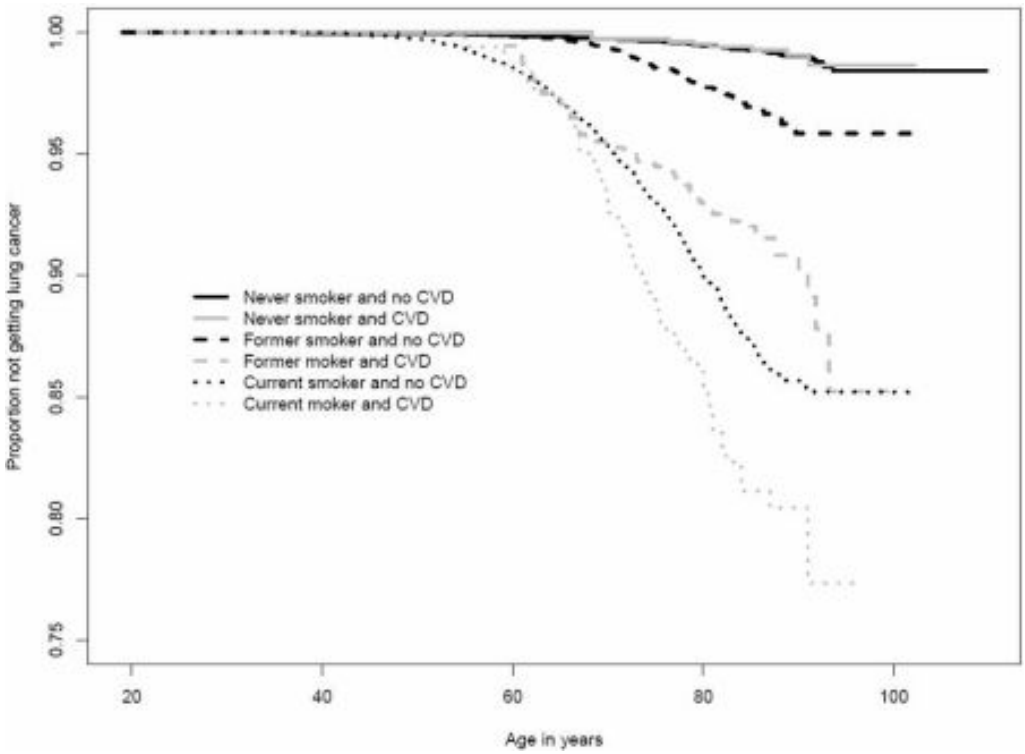
Methods: Data from the Nord-Trøndelag Health Study linked to the Norwegian Cancer and Death Cause Registry, analyses stratified by smoking. 97,087 persons (1,634,967 person years), never smokers n=38,656, former smokers n=20,914, current smokers n=26,894, follow-up 15 years. The proportional hazard model (HR CI 95%) for CVD on LC incidence, adjusted for age, sex, BMI, burden of smoking and chronic cough.

Results: LC = 1,080, cases (1.1%), CVD = 5,981 (6.9%). CVD vs LC in former smokers = HR 1.74 (CI95% 1.11-273), current smokers = HR 1.38 (CI95% 1.04-1.83), never smokers = HR 0.87 (CI95% 03.4-2.23),

Table 1

Former smokers	HR	CI 95%	Current smokers	HR	CI 95%
CVD	1,74	1,11-2,73		1,38	1,04-1,83
BMI Below 18.5	2,60	0,36-19,01		1,36	0,67-2,75
18.5-25	1			1	
25-30	0,81	0,53-1,25		0,98	0,81-1,17
Above 30	0,80	0,43-1,48		0,89	0,65-1,23
Sex (M vs F)	1,43	0,82-2,49		1,09	0,90-1,31
Chronic cough with phlegm (Y vs No)	0,88	0,32-2,42		1,55	1,22-1,97
Heavy vs light smoker	3,56	2,33-5,45	Heavy vs light smoker	2,11	1,75-2,54

Association CVD and lung cancer, former and current smokers. HR, hazard ratio,



Conclusion: CVD was an independent risk factor for lung cancer in former and current smokers. CVD may be a novel risk factor for lung cancer screening.

Appendix I

Questionnaire HUNT1

Questionnaire 1
For people 20 years old and over, both sexes
HUNT 1

Page 1

INVITATION TO CHEST X-RAY EXAMINATION AND EXAMINATION OF BLOOD PRESSURE AND BLOOD SUGAR

The chest x-ray examination is now coming to your district. This time the results are part of a larger health study. Please see the enclosed brochure for information about the study.

You will find the time and location of the examination below.

Please fill in the questionnaire on the other side of this paper and bring it to the examination. Bring an X-ray certificate, tuberculosis vaccination card or your employee medical card if you have one.

It is important that you attend even if you have recently had your blood pressure and blood sugar measured and even if you are receiving treatment for high blood pressure or diabetes.

Sincerely,
National Mass Radiography Service
Box 8155 Dep., Oslo 1

County Medical Officer
Health Council
National Institute of Public Health

Page 2

A. How is your health at the moment?
(Put an X in only one box)
Poor
Not so good
Good
Very good

B. During the past 12 months, have you visited any of the following:
<yes, no>
A general practitioner (district medical officer, doctor in private practice, house physician)
A company physician
A military doctor
A doctor at hospital (without being hospitalized)
Another doctor

C. Have you been hospitalized during the last 5 years? <yes, no>

D. Are you taking or have you taken medicine for high blood pressure?
<yes, no>

E. Do you have or have you had any of the following illnesses? <yes, no>
Diabetes
Myocardial infarction (heart attack)
Angina pectoris (chest pain)
Stroke or cerebral haemorrhage

F. Do you suffer from any long-term illness or injury of a physical or psychological nature that impairs your functioning in your everyday life? (Long term means that it has lasted or will last for at least one year.) <yes, no>

If YES, would you describe your impairment as slight, moderate or severe? < slight, moderate or severe >
Motor impairment
Vision impairment
Hearing impairment
Impairment due to physical illness
Impairment due to mental health problems

G. Do you have any siblings? (living or deceased) <yes, no>

If YES, has one or more of them ever had any of the following illnesses? <yes, no, don't know>
Diabetes
Heart attack/angina pectoris
High blood pressure

H. Thinking about your life at the moment, would you say that you by and large are satisfied with life, or are you mostly dissatisfied?
(Put an X in only one box)
Very satisfied
Satisfied
Somewhat satisfied
Neither satisfied nor dissatisfied
Somewhat dissatisfied
Dissatisfied
Very dissatisfied

SEE THE PICTURE OF THE BLOOD PRESSURE MEASUREMENT IN THE ENCLOSED BROCHURE

I. Have you ever had your blood pressure measured? <yes, no, don't know>
If you answered NO, proceed to question M.

J. In what year was your blood pressure <u>last</u> measured? <year, don't know>
19___ Give year here (about)

K. Where did you last have your blood pressure measured?
(Put an X in only one box)
At a general practitioner (district medical officer, doctor in private practice, house physician)
At a company physician
At a military doctor
At a hospital
At another doctor
Don't know

L. What was the result of the blood pressure measurement?
(Put an X in only one box)
Start or continue taking medicine for high blood pressure
Go in for a follow-up examination, but not take medicine
No follow-up examination and no medication necessary

M. Which general practitioner would you prefer to be referred to if this health survey indicates that you should undergo a more thorough examination?
Write the name of the doctor here _____
No particular doctor <cross>

ABOUT YOUR JOB

N. Are you currently employed?
(Put an X in only one box)
Yes, full-time employment (not including housework)
Yes, part-time employment (not including housework)
Yes, full-time housework
No, not employed

O. If you are not in full-time employment, is the reason:
(Put an X in only one box)
Unemployment/redundancy
Retirement or disability pension
Education or military service
Other reason

IF YOU ARE EMPLOYED, PLEASE ANSWER THE NEXT TWO QUESTIONS

P. Does your work involve a lot of stress and hassles?
(Put an X in only one box)
No, not at all
Rarely
Yes, a certain amount
Yes, almost all the time

Q. Do you decide how your work is planned?
(Put an X in only one box)
No, not at all
A little
Yes, for the most part
Yes, I decide

Appendix II

Questionnaire HUNT2

HUNT 2 Questionnaire 1

For people 20 years old and over, both sexes

Page 1

Page one is a personal invitation to the screening with information on where and when to attend. The participants were asked to fill in the questionnaire at home and bring it with them to their examination. The screening nurse at the examination location was to ensure that all questions on page two were filled in, explain misunderstandings if necessary and help participants complete and correct the questionnaire.

Page 2

This questionnaire is an important part of the Health Study. Here you will find questions about previous illnesses and other important conditions regarding your health. Please complete the form and take it with you to the health examination.

If any questions are not clear, leave them unanswered until you come to the examination where you can discuss them with the person on staff who examines you. All information you give will be treated in the strictest confidence.

Several places on this questionnaire we ask you to give your age when an illness occurred. If you do not know exactly how old you were, give the age that is closest to what you think may be correct.

When the results of the examination are available, there will be some people who need to be re-examined by their own doctor. If this is the case for you, you will be informed of this in a letter that we will send with your results. At the same time, your doctor will be sent your results. This is why in the section at the end of the questionnaire you are asked to give the name of your general practitioner, community doctor or health care centre where results are to be sent and possible follow-up examination are to be carried out.

Sincerely,
The Nord-Trøndelag Health Service - The State Health Examiners - The State Institute for Public Health

THIS IS ABOUT YOUR HEALTH

How is your health at the moment? (Put an X in only one box)
Poor
Not so good
Good
Very good

RESPIRATORY DISORDERS

Do you cough daily during periods of the year? <yes, no>

If YES, answer the next two questions.
Do you usually bring up phlegm when coughing? <yes, no>
Have you had a cough with phlegm for periods of at least 3 months during each of the last two years? <yes, no>

Have you had attacks of wheezing or breathlessness during the last 12 months? <yes, no>

Do you have or have you had asthma? <yes, no> Age first time ____

Do you use or have you used asthma medication? <yes, no>

CARDIOVASCULAR DISEASES, DIABETES

Have you had or do you have:
Myocardial infarction (heart attack) <yes, no> Age first time ____
Angina pectoris (chest pain) <yes, no> Age first time ____
Stroke/brain haemorrhage <yes, no> Age first time ____
Diabetes <yes, no> Age first time ____

What was the result the last time your blood pressure was measured? (Put an X in only one box)
Start or continue taking medicine for high blood pressure
Go in for a follow-up examination, but not take medicine
No follow-up examination and no medication necessary
Have never had blood pressure measured

Are you taking medication for high blood pressure? (Put an X in only one box)
Currently taking medication
Previously, but not now
Have never taken it

Has one or more of your parents or siblings had a myocardial infarction (heart attack) or angina pectoris (chest pains)? <yes, no, don't know>

METABOLISM

Have you ever had:
Hyperthyroidism (too high metabolism) <yes, no> Age first time ____
Hypothyroidism (too low metabolism) <yes, no> Age first time ____
Goitre <yes, no> Age first time ____
Other disease of the thyroid gland <yes, no> Age first time ____

Do you take or have you ever taken either of these medicines:

Thyroxin <yes, no> Age first time ____
NeoMercazole <yes, no> Age first time ____

Have you had a thyroid gland operation? <yes, no> Age first time ____

Have you had radioiodine treatment? <yes, no> Age first time ____

MUSCULOSKELETAL DISORDERS

During the last year, have you had pain and/or stiffness in your muscles and limbs that has lasted for at least 3 consecutive months? <yes, no>
If NO, go on to the next section.
If YES, answer the following questions:

Where did you have pain and/or stiffness? <yes, no>
Neck
Shoulders
Elbows
Wrists, hands
Chest/stomach
Upper part of back
Lumbar region
Hips
Knees
Ankles, feet

(If you had complaints in several areas for at least 3 months in the last year, put a circle around the yes-X for the complaint that lasted longest.)

How long did the pain and/or stiffness last? (Answer for the area where it lasted the longest)
If less than 1 year, give the number of months. ____ Number of months
If 1 year or more, give the number of years. ____ Number of years

Have these complaints reduced your ability to work during the last year? (Also applies to those working at home. Put an X in only one box.)
No, not significantly
To some degree
Significantly
Don't know

Have you been on sick leave due to these complaints during the last year? <yes, no, not working>

Have the complaints caused you to reduce your leisure activities? <yes, no>

Page 3

Has a doctor ever said that you have/have had any of the following diseases? <yes, no>
Osteoporosis
Fibromyalgia (fibrositis/chronic pain syndrome)
Arthritis (rheumatoid arthritis)
Degenerative joint disease (osteoarthritis)
Bechterew's disease (AS)
Other long-term skeletal or muscular diseases

Have you ever had: <yes, no> Age last time ____

A fractured femur
A fractured wrist or forearm
Neck injury (whiplash)
Injury that led to hospitalisation

OTHER COMPLAINTS

To what degree have you had the following complaints in the last 12 months? <not at all, slightly, very much>
Nausea
Heartburn/ acid regurgitation
Diarrhoea
Constipation
Palpitations
Breathlessness

OTHER DISEASES

Do you have or have you ever had: <yes, no> Age first time ____
Epilepsy
Mental health problems for which you sought help
Cancer
Other long-term disease

EVERYDAY TASKS

Do you suffer from any long-term illness or injury of a physical or psychological nature that impairs your functioning in your everyday life? <yes, no>
(Long-term means at least one year.)
If YES, would you describe your impairment as slight, moderate or severe? <slight, moderate, severe>
Motor ability impairment
Vision impairment
Hearing impairment
Impairment due to physical illness
Impairment due to mental health problems

MEN continue after this section

TO BE ANSWERED BY WOMEN ONLY

How many children have you had? <_____ Number of children>
(Put 0 if you have had no children)

If you have had children, answer these questions:
How old were you when you had your first child? <Age _____>
How old were you when you had your last child? <Age _____>
(Do not answer if you have only had one child)

How old were you when you started menstruating? <Age ____>
(Put 0 if you have never menstruated)

Continue to the next section

SMOKING

Did any of the adults where you grew up smoke indoors? <yes, no>

After you were 20 years old, do you live or have you lived with a daily smoker(s)? <yes, no>

How long are you usually in a smoky room each day? <Number of hours ____>
(Put 0 if you are not usually in a smoky room)

Do you smoke? <yes, no>
Daily cigarette smoker?
Daily cigar/cigarillo smoker?
Daily pipe smoker?
Have never smoked daily (Put an X) □

If you previously smoked, how long has it been since you stopped? <Number of years ____>

If you, now or previously, smoke(d) daily, answer these questions:

How many cigarettes do you or did you usually smoke daily? <Number of cigarettes - ____>
How old were you when you started smoking? <Age ____>
How many years in total have you smoked daily? <Number of years ____>

COFFEE/TEA/ALCOHOL

How many cups of coffee/tea do you drink daily? <Number of cups ____>
(Put 0 if you do not drink coffee/tea daily)
Brewed coffee
Other coffee
Tea

Concerning alcohol, are you a non-drinker? <yes, no>

How many times a month do you normally drink alcohol? <Number of times ____> (Do not include low-alcohol beer. Put 0 if less than once a month.)

How many glasses of beer, wine or spirits do you usually drink in the course of two weeks? (Do not include low-alcohol beer. Put 0 if less than once a month.)

Beer <Number of glasses ____>
Wine <Number of glasses ____>
Spirits <Number of glasses ____>

PHYSICAL ACTIVITY

DURING LEISURE TIME

How much of your leisure time have you been physically active during the last year? (Think of a weekly average for the year. Your commute to work counts as leisure time.)
<Hours per week: None, Less than 1, 1-2, 3 or more>
Low physical activity (no sweating/not out of breath)
Vigorous physical activity (sweating/out of breath)

AT WORK

(For both paid or unpaid work)
How would you describe your work? (Put an X in only one box)
Mostly sedentary work (e.g. at a desk, on an assembly line)
Much walking at work (e.g. delivery work, light industrial work, teaching)
Much walking or lifting at work (e.g. postman, nurse, construction work)
Heavy physical work (e.g. forestry work, heavy agricultural work, heavy construction work)

HOW DO YOU FEEL?

In the last two weeks, have you felt: <no, a little, a good amount, very much>
Confident and calm?
Happy and optimistic?
Have you felt:
Nervous and restless?
Troubled by anxiety?
Irritable?
Down/depressed?
Lonely?

Read each item below and place an X next to the reply that comes closest to how you have been feeling **in the past week** (only one X per item). Do not take too long over your replies; your immediate reaction to each item will probably be more accurate than a long, thought-out response.

I still enjoy the things I used to enjoy
Definitely as much
Not quite so much
Only a little
Hardly at all

I get a sort of frightened feeling as if something awful is about to happen
Very definitely and quite badly
Yes, but not too badly
A little, but it doesn't worry me
Not at all

I can laugh and see the funny side of things
As much as I always could
Not quite so much now
Definitely not so much now
Not at all

Worrying thoughts go through my mind
A great deal of the time
A lot of the time
Not too often
Very little

I feel cheerful
Never
Not often
Sometimes
Most of the time

I can sit at ease and feel relaxed
Definitely
Usually
Not often
Not at all

I feel as if I'm slowed down
Nearly all the time
Very often
Sometimes
Not at all

I get a sort of frightened feeling like 'butterflies' in the stomach
Not at all
Occasionally
Quite often
Very often

I have lost interest in my appearance
Definitely
I don't take as much care as I should
I may not take quite as much care
I take just as much care as ever

I feel restless as if I have to be on the move
Very much indeed
Quite a lot
Not very much
Not at all

I look forward with enjoyment to things
As much as I ever did
Rather less than I used to
Definitely less than I used to
Hardly at all

I get sudden feelings of panic
Very often indeed
Quite often
Not very often
Not at all

I can enjoy a good book or radio or television programme
Often
Sometimes
Not often
Very seldom

EDUCATION

What is your highest level of education?

Primary school 7-10 years, continuation school, folk high school
High school, intermediate school, vocational school, 1-2 years high school
University qualifying examination, junior college, A levels
University or other post-secondary education, less than 4 years
University/college, 4 years or more

WORK

What kind of work do you currently do? (One or more Xs)
Paid work
Self-employed
Full-time housework
Student, military service
Unemployed, laid off
Retired/on Social Security

How many hours of paid work do you have a week? <Number of hours ____ >

Do you work shifts, at night, or on call? <yes, no>

IN GENERAL

Thinking about your life at the moment, would you say that you by and large are satisfied with life, or are you mostly dissatisfied?

(Put an X in only one box)
Very satisfied
Satisfied
Somewhat satisfied
Neither satisfied nor dissatisfied
Somewhat dissatisfied
Dissatisfied
Very dissatisfied

Which general practitioner would you prefer to be referred to if this health survey indicates that you should undergo a more thorough examination?
Write the doctor's name here_____

Thank you for completing this questionnaire!
And once again, Welcome to the examination!

Appendix III

Questionnaire HUNT3

HUNT 3 Questionnaire 1

Health and daily life

1. How is your health at the moment?

Poor ☐ Not so good ☐ Good ☐ Very good ☐

	Yes	No
2. Do you suffer from long-term (at least 1 year) illness or injury of a physical or psychological nature that impairs your functioning in your daily life?	☐	☐

If Yes,
Would you describe your impairment as slight, moderate or severe?

	Slight	Moderate	Severe
Motor ability impairment	☐	☐	☐
Vision impairment	☐	☐	☐
Hearing impairment	☐	☐	☐
Impairment due to physical illness	☐	☐	☐
Impairment due to mental health problems	☐	☐	☐

3. Do you have physical pain now that has lasted more than 6 months?

Yes ☐ No ☐

4. How strong has your physical pain been during the last 4 weeks?

No pain	Very mild	Mild	Moderate	Strong	Very strong
☐	☐	☐	☐	☐	☐

5. To what extent has your physical health or emotional problems limited you in your usual socializing with family or friends during the last 4 weeks?

Not at all	Very little	Somewhat	Much	Was not able to socialize
☐	☐	☐	☐	☐

Health services

6. During the last 12 months, have you visited any of the following:	Yes	No
General practitioner	☐	☐
Another specialist outside the hospital	☐	☐
Consultation w/ a doctor without being admitted		
to the psychiatric out-patient dept.	☐	☐
to another hospital out-patient dept.	☐	☐
Chiropractor	☐	☐
Homeopath, acupuncturist, reflexologist, laying on of hands or other alternative treatment practitioner	☐	☐
7. Have you been admitted to hospital in the last 12 months?	☐	☐

Illness and Injury

	Yes	No
8. Have you had any kind of attack of wheezing or breathlessness during the last 12 months?	☐	☐
9. Have you at any time during the last 5 years taken medicine for asthma, chronic bronchitis, emphysema or COPD?	☐	☐
10. Do you take or have you taken medication for high blood pressure?	☐	☐

11. Have you had or do you have any of the following:
(Put an X on each line)

	Yes	No	If Yes, how old were you the first time *Ex:* (34 years old)
Myocardial infarction (heart attack)	☐	☐	years old
Angina pectoris (chest pain)	☐	☐	years old
Heart failure	☐	☐	years old
Other heart disease	☐	☐	years old
Stroke/brain haemorrhage	☐	☐	years old
Kidney disease	☐	☐	years old
Asthma	☐	☐	years old
Chronic bronchitis, emphysema or COPD	☐	☐	years old
Diabetes	☐	☐	years old
Psoriasis	☐	☐	years old
Eczema on hands	☐	☐	years old
Cancer	☐	☐	years old
Epilepsy	☐	☐	years old
Arthritis (rheumatoid arthritis)	☐	☐	years old
Bechterew's disease	☐	☐	years old
Sarcoidosis	☐	☐	years old
Osteoporosis	☐	☐	years old
Fibromyalgia	☐	☐	years old
Degenerative joint disease (osteoarthritis)	☐	☐	years old
Mental health problems you sought help for	☐	☐	years old

12. Has it ever been verified that you had high blood sugar (hyperglycaemia)?

Yes ☐ No ☐

If Yes, **in what situation was this discovered the first time?**

At a health examination ☐ While sick ☐

While pregnant ☐ Other ☐

Injuries

13. Have you ever had:

	Yes	No	If Yes, how old were you the **first** time *Ex: (34 years old)*
Hip fracture	☐	☐	☐ years old
Fractured wrist/forearm	☐	☐	☐ years old
Fracture/compressed dorsal vertebrae?	☐	☐	☐ years old
Whiplash	☐	☐	☐ years old

Illness in immediate family

14. Do your parents, siblings or children have, or have they had, the following illnesses? *(one X per line)*

	Yes	No	Don't know
Stroke or brain haemorrhage before the age of 60	☐	☐	☐
Myocardial infarction (heart attack) before the age of 60	☐	☐	☐
Asthma	☐	☐	☐
Allergies/hay-fever/nasal allergies	☐	☐	☐
Chronic bronchitis, emphysema or COPD	☐	☐	☐
Cancer	☐	☐	☐
Mental health problems	☐	☐	☐
Osteoporosis	☐	☐	☐
Kidney disease (not kidney stone, urinary tract infection, urinary incontinence)	☐	☐	☐
Diabetes	☐	☐	☐

15. Have your parents' siblings, your cousins or either of your grandparents been diagnosed with diabetes (type 1 or type 2)?

Yes ☐ No ☐

How do you feel?

16. In the last two weeks, have you felt: *(one X per line)*

	No	A little	A good amount	Very much
Confident and calm	☐	☐	☐	☐
Happy and optimistic	☐	☐	☐	☐
Nervous and restless	☐	☐	☐	☐
Troubled by anxiety	☐	☐	☐	☐
Irritable	☐	☐	☐	☐
Down/depressed	☐	☐	☐	☐
Lonely	☐	☐	☐	☐

17. Has anyone at any time in your life tried to oppress, degrade or humiliate you over an extended period of time?

Yes ☐ No ☐

Lifestyle

Smoking

	Yes	No
18. Did any of the adults where you grew up smoke indoors?	☐	☐
19. Did your mother smoke when you were growing up?	☐	☐

20. Do you smoke? *(Put an X in only one box)*

- **No**, I have never smoked ☐
  - *If you never smoked, skip to question **22***
- **No**, I quit smoking ☐
- Yes, cigarettes occasionally (parties/vacation, not daily) ☐
- Yes, cigars/cigarillos/pipe occasionally ☐
- Yes, cigarettes daily ☐
- Yes, cigars/cigarillos/pipe daily ☐

21A. Answer this if you smoke daily now or previously smoked daily:

1. How many cigarettes do/did you usually smoke daily?	☐ Cigarettes pr day
2. How old were you when you started smoking daily?	☐ years old
3. If you previously smoked daily, how old were you when you quit smoking?	☐ years old

21B. Answer this if you smoke/previously smoked occasionally, but not daily:

1. How many cigarettes do/did you usually smoke in a month?	☐ Cigarettes pr mo.
2. How old were you when you started smoking occasionally?	☐ years old
3. If you previously smoked occasionally, how old were you when you quit?	☐ years old

22. Do you use, or have you used snuff?

No, never	☐	Yes, occasionally	☐
Yes, but I quit	☐	Yes, daily	☐

*If you answered No, never, skip to question **23***

If Yes,

How old were you when you began using snuff?

☐ years old

How many portions snuff do/did you use a month?

☐ Portions snuff a month

If you use(d)/smoke(d) both cigarettes and snuff, which did you begin with first?

Snuff	☐	About the same time (within 3 months)	☐
Cigarettes	☐	Don't remember	☐

Did you begin using snuff to try to quit or cut down on smoking?

No ☐

Yes, to quit smoking ☐ Yes, to cut down on smoking ☐

Diet

23. How often do you normally eat these foods?
(one X on each line)

	0-3 times a month	1-3 times a week	4-6 times a week	Once a day	Twice or more a day
Fruits, berries	☐	☐	☐	☐	☐
Vegetables	☐	☐	☐	☐	☐
Chocolate/candy	☐	☐	☐	☐	☐
Boiled potatoes	☐	☐	☐	☐	☐
Pasta/rice	☐	☐	☐	☐	☐
Sausages/hamburgers	☐	☐	☐	☐	☐
High-fat fish on bread or for dinner (salmon, trout, herring, mackerel, haddock)	☐	☐	☐	☐	☐

24. Do you take the following dietary supplements?
(One X for each supplement)

	Yes, daily	Occasionally	No
Cod-liver oil	☐	☐	☐
Omega-3 capsules	☐	☐	☐
Vitamins and/or minerals	☐	☐	☐

25. How <u>many glasses</u> do you usually drink of the following? ½ litre = 3 glasses (one X on each line)

	Seldom/ never	1-6 gl. a week	1 gl. a day	2-3 gl. a day	4 gl or more a day
Water, Farris, etc.	☐	☐	☐	☐	☐
Whole milk (sweet/sour)	☐	☐	☐	☐	☐
Other milk (sweet/sour)	☐	☐	☐	☐	☐
Soda/juice w/sugar	☐	☐	☐	☐	☐
Soda/juice w/out sugar	☐	☐	☐	☐	☐
Juice or nectar	☐	☐	☐	☐	☐

26. How many cups of coffee do you drink a day?
(write 0 if you do not drink coffee/tea daily)

	Boiled coffee	Other coffee	Tea
Number of cups			

27. How many cups of coffee do you drink in the evening (after 6pm)?

Number of cups ☐

Alcohol

28. About how often in the last 12 months did you drink alcohol? *(do not include low-alcohol beer)*

4-7 times a <u>week</u> ☐ About once a <u>month</u> ☐

2-3 times a <u>week</u> ☐ A few times a <u>year</u> ☐

About once a <u>week</u> ☐ Not at all the last <u>year</u> ☐

2-3 times a <u>month</u> ☐ <u>Never</u> drink alcohol ☐

29. Did you drink alcohol during the last 4 weeks?

Yes ☐ No ☐

If Yes,
Did you drink so much that you felt very intoxicated (drunk)?

No ☐ Yes, 1–2 times ☐ Yes, 3 times or more ☐

30. How many glasses of beer, wine or spirits do you usually drink <u>in the course of two weeks</u>: *(do not include low-alcohol beer, write 0 if you do not drink alcohol)*

	Beer	Wine	Spirits
Number of glasses			

31. How often do you drink <u>5 glasses or more</u> of beer, wine or spirits in one sitting?

Never ☐ Monthly ☐ Weekly ☐ Daily ☐

Exercise

By exercise we mean going for walks, skiing, swimming and working out/sports.

32. How often do you exercise? *(on the average)*

Never ☐

Less than once a week ☐

Once a week ☐

2-3 times a week ☐

Nearly every day ☐

33. If you exercise as often as once or several times a week: How hard do you exercise? (average)

I take it easy, I don't get out of breath or break a sweat ☐

I push myself until I'm out of breath and break into a sweat ☐

I practically exhaust myself ☐

34. For how long do you exercise each time?(average)

Less than 15 minutes ☐ 30 min.-1 hour ☐

15-29 minutes ☐ More than 1 hour ☐

35. Do you have at <u>least 30 minutes</u> of physical activity daily at work or in your leisure time?

Yes ☐ No ☐

36. About how many hours do you sit during a normal day? *(include work hours and leisure time)*

☐ hours

Employment

37. If you have had paid or unpaid employment, how would you describe your job? *(One X only)*

- Work that mostly involves sitting *(ex: desk work, assembly worker)* ☐
- Work that requires much walking (*ex: clerk, light industry worker, teacher*) ☐
- Work that requires much walking and lifting (*ex: mail carrier, nurse, construction worker*) ☐
- Heavy physical labour (ex: *forester, farmer, heavy construction worker*) ☐

Height/Weight

38. About how tall were you <u>at age 18</u>?

☐☐☐ cm Don't remember ☐

39. About how much did you weigh <u>at age 18</u>?

☐☐☐ kg Don't remember ☐

40. Are you satisfied with your weight now?

Yes ☐ No, don't weigh enough ☐ No, weigh too much ☐

41. Have you tried to diet <u>in the last 10 years</u>?

No ☐ Yes, a few times ☐ Yes, many times ☐

42. Do you weigh at least 2 kg less than you did 1 year ago?

Yes ☐ No ☐

If Yes, **what is the reason for this?**

Dieting ☐ Illness/stress ☐ Don't know ☐

Serious events in the last 12 months

43. Has a member of your immediate family died?
(Child, spouse/partner, sibling or parent)

Yes ☐ No ☐

44. Have you been in imminent mortal danger because of a serious accident, catastrophe, violent situation or war?

Yes ☐ No ☐

45. Has your relationship with your spouse or long-term partner ended?

Yes ☐ No ☐

46. If you answered Yes to one or more of the above questions (43, 44 or 45), how much have you reacted to this in the last <u>7 days</u>?

Not at all ☐ Moderate amount ☐
A little ☐ Very much ☐

Childhood – When you were <u>0-18 years old</u>

47. Who did you grow up with?

Mother ☐ Other relatives ☐
Father ☐ Adoptive parents ☐
Stepmother/ stepfather ☐ Foster parents ☐

48. Did your parents leave each other, or get a divorce, when you were a child?

No ☐
Yes, before I was 7 years old ☐ Yes, when I was 7-18 years old ☐

49. Did either of your parents die when you were a child?

No ☐
Yes, before I was 7 years old ☐ Yes, when I was 7-18 years old ☐

50. Did you grow up with pets?

No ☐
Yes, cat ☐ Yes, dog ☐ Yes, horse ☐ Yes, other animal ☐

51. How much milk or yoghurt did you usually drink?

Seldom/ never	1-6 glasses pr. week	1 glass pr. day	2-3 glasses pr. day	More than 3 glasses pr. day
☐	☐	☐	☐	☐

52. Did you grow up on a farm with farm animals? Yes ☐ No ☐

53. When you think about your childhood, would you describe it as:

Very good ☐ Average ☐ Very difficult ☐
Good ☐ Difficult ☐

In General

54. Thinking about your life at the moment, would you say that you by and large are satisfied with life, or are you mostly dissatisfied? *(One X only)*

- Very satisfied ☐
- Satisfied ☐
- Somewhat satisfied ☐
- A bit of both ☐
- Somewhat dissatisfied ☐
- Dissatisfied ☐
- Very dissatisfied ☐