Clinical outcomes for men diagnosed with prostate cancer

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By

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A. Acknowledgement

When I decided to study medicine in Argentina I did not know how affected I was going to be by patients' lives and experiences. I soon realised that I preferred the biology and theory. Thus, after finishing my medical degree and meeting my Belgian husband I moved to London where I completed an MSc degree in Medical Molecular Biology with the objective of becoming a lab-based researcher. I then met Dr Mieke Van Hemelrijck, who with her passion for teaching, introduced me to the world of epidemiology. She encouraged me to pursue a PhD in prostate cancer epidemiology and she showed me that with perseverance and my background in medicine and molecular biology I would be able to complete this incredible journey. I therefore would like to thank her for her unconditional support through every step and basically every day of the last three years.

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B. Abstract

Background and Aims

Early diagnosis of prostate cancer and improvement in treatment and palliative care has translated in more men living with prostate cancer for prolonged periods of time. The current thesis therefore assessed clinical outcomes for men diagnosed with prostate cancer by specifically focusing on those outcomes related to the disease itself and those related to prostate cancer-specific treatments.

Methods

1) Disease-related Outcomes

a. Serum biomarkers and second primary tumours:

The Swedish AMORIS cohort was used to investigate how a variety of serum biomarkers of different metabolisms (i.e. glucose, lipids, gamma-glutamyl transferase (GGT) and fructosamine) measured before prostate cancer diagnosis as a first primary tumour, are associated with patterns of secondly diagnosed primary tumours (SDPTs). This database contains information on >350,000 men who provided measurements for these biomarkers. Cox proportional hazard models and multiple imputations were used to quantify these associations.

2) Treatment-related Outcomes

For the next three projects, I used the PCBaSe Sweden database which covers >96% of prostate cancer patients in Sweden between 1998 and 2006.

b. The association between radiotherapy and risk of thromboembolic disease:

Using Cox proportional hazard models, I investigated the risk of thromboembolic disease (TED) after receiving radiotherapy for prostate cancer.

c. *Drugs for metabolic conditions and prostate cancer death in men on gonadotropin releasing hormone receptor agonists:*

I investigated how having a treatment for metabolic disease-related components ('metabolic drugs') at the time of androgen deprivation therapy initiation was associated with prostate cancer mortality and overall mortality. Cox proportional hazard models, cumulative incidence and competing risk analyses were applied.

d. Anti-androgens versus on gonadotropin releasing hormone receptor (GnRH) agonists in relation to prostate cancer death:

Using propensity score matching, Cox proportional hazard models and cumulative incidence analyses, I investigated whether there is any difference in terms of prostate cancer survival and overall survival amongst men treated either with anti-androgens or GnRH agonists.

Results

My findings in the AMORIS study support the hypothesis that alterations in metabolic factors like cholesterol, triglycerides and GGT present several years before prostate cancer diagnosis may indicate a common biological background between prostate cancer and SDPTs. In more detail, my results showed higher risk of SDPTs for those with high serum levels of triglycerides (HR: 1.37, 95%CI: 1.17-1.60), total cholesterol (HR: 1.22, 95%CI: 1.04-1.42) and GGT (HR: 1.32, 95%CI: 1.02-1.71), as compared to the normal levels.

My findings in the PCBaSe studies show that:

a. After adjusting for all available confounding covariates, no association was found between radiotherapy (in the forms or external beam radiotherapy and brachytherapy) and TED (HR: 1.05, 95% C.I.: 0.61-1.79 and HR: 0.97, 95%C.I.: 0.29-1.44 respectively). Radiotherapy was not associated with an increased risk of thromboembolic events within 5 years of receiving this treatment.
b. After competing risk analysis, I observed that 'metabolic drugs' did not improve or worsen prostate cancer mortality amongst men being treated with GnRH agonists. However, men on 'metabolic drugs' were more likely to die of cardiovascular disease than men not on these drugs (i.e. HR 1.87; 95%CI: 1.56-2.24 for anti-hypertensive drug use and HR 2.46; 95%CI: 2.03-2.98 for anti-hypertensive + lipid lowering drug use).
c. Following propensity score matching, men on GnRH agonists had a similar risk of death from prostate cancer as men on anti-androgens, HR 1.09 (95% CI: 0.94-1.27), but a higher risk of death from all causes, HR 1.25 (95% CI: 1.14-

1.37). Anti-androgens showed similar overall and prostate cancer mortality rates to GnRH agonists.

Conclusion

Overall, my results showed that metabolic alterations in terms of high levels of lipids and GGT might have an impact on men after prostate cancer diagnosis by an association with an increased risk of SDPTs. However, treatment for metabolic syndrome related conditions did not increase the risk of prostate cancer death amongst those treated with GnRH agonists, but did increase the risk of CVD-related deaths. Also, my results help elucidate potential treatment side-effects and outcomes by showing that: a. radiotherapy did not increase the risk of TED, allowing patients and physicians to focus on other well-established RT side effects (i.e. erectile dysfunction, urinary incontinence or bowel incontinence); and b. that anti-androgens may be an alternative to GnRH agonists for men with advanced non-metastatic prostate cancer, given similar prostate cancer death and overall mortality risks.

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F. List of abbreviations

AA	Anti-androgens
ADT	Androgen Deprivation Therapy
AMORIS	Apolipoprotein-related MORtality RISk
AR	Androgen receptor
AS	Active Surveillance
BT	Brachytherapy
BHP	Benign Prostatic Hyperplasia
BMI	Body Mass Index
BRCA	Breast Cancer Gene
CALAB	Central Automation Laboratory
CCI	Charlson Comorbidity Index
CRPC	Castrate Resistant Prostate Cancer
CVD	Cardiovascular Disease
DHT	Dihydrotestosterone
DNA	Deoxyribonucleic acid
DRE	Digital rectal examination
DVT	Deep Venous Thrombosis
EBRT	External Beam Radiotherapy
EPIC	European Prospective Investigation into Cancer and Nutrition
ERSPC	European randomized study of screening for prostate cancer
EUA	European Urology Association
FDA	Food and drug administration
fPSA	Fee circulating PSA
FSH	Follicle stimulant hormone
GnRH	Gonadotropin-releasing Hormone
GGT	Gamma-glutamyl transferase
GWAS	Genome-wide association studies
HCA	Heterocyclic amines

HDL	High-density lipoprotein
HR	Hazard Ratio
ICD	International Classification of Disease
IGF-1	insulin-like growth factor-1
IGRT	Image guided radiation therapy
IMRT	Intensity modulated radiation therapy
INCA	IT platform Information Network for CAncer
LH	Luteinizing hormone
MetS	Metabolic syndrome
MICE	multivariate imputation using chained equations
NPCR	National Prostate Cancer Register
NSAA	Non-steroidal antiandrogens
NSAIDs	Non-steroidal anti-inflammatory drugs
PCBaSe	Prostate cancer database Sweden
PE	Pulmonary embolism
PLCO trial	Prostate, lung, colorectal and ovarian cancer screening
PRIAS Surveillance	Prostate Cancer Research International Active
PCPT	Prostate Cancer Prevention Trial
PRECISION Sampling Using Image Guidance or Not?	Prostate Evaluation for Clinically Important Disease:
ProtecT	Prostate Testing for Cancer and Treatment
PSA	Prostate-specific Antigen
PSAD	PSA density
PSADT	PSA doubling time
PSAV	PSA velocity
RALP	Robot-assisted laparoscopic radical prostatectomy
REDUCE	REduction by DUtasteride of prostate Cancer Events
ROS	Reactive oxygen species
RT	Radiotherapy
RANKL	Receptor activator of nuclear factor kappa B ligand

SAS	Statistical Analysis Software
SBRT	Stereotactic body radiation therapy
SD	Standard Deviation
SDPT	Secondly diagnosed primary tumours
SEER	United States of America Surveillance, Epidemiology and End Results program
SNPs	single nucleotide polymorphisms
SPT	Second primary tumours
STAMPEDE	Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy
TED	Thromboembolic disease
UK	United Kingdom
UK NSC	UK National Screening Committee
US	United States
WCRF	World Cancer Research Fund
WW	Watchful waiting
95%CI	95% Confidence Interval
3D-CRT	Three-dimensional conformal radiation therapy

1. Chapter I: Introduction and research objectives

Prostate cancer is the second most common cancer in men, with almost 70% of the cases occurring in more developed regions. Prostate cancer incidence varies greatly worldwide with the highest rates being reported in Oceania, North America, western and northern Europe, followed by less developed regions, such as the Caribbean, southern Africa and South America. The lowest rates are found in Asian populations (1).

Established risk factors include black race, older age and family history. However, an increasing body of evidence suggests that Westernized lifestyle can also increase the risk of having prostate cancer. For instance, migration studies have shown that after migration to the United States of America, Chinese and Japanese show substantial increase in prostate cancer incidence (2).

Even though prostate cancer represents the fifth leading cause of death from cancer in men, overall mortality rates are decreasing. These trends translate into men living longer with prostate cancer diagnosis – which is thought to be mainly attributed to the introduction of prostatic specific antigen (PSA) testing (see below: PSA-screening) (3). Five-year survival rates can be more than 90% in some countries (e.g. 99% in the United States of America). However, as encouraging as these numbers are, living with prostate cancer entails several health consequences related to the disease itself as well as its treatments (4).

This thesis therefore aims to provide more insight into the impact of a prostate cancer diagnosis and its treatments on a man's quality and quantity of life by investigating different disease and treatment-related clinical outcomes. More specifically, this thesis comprises of the following four projects:

(1) Using the Swedish AMORIS study, I investigated how a variety of serum biomarkers of different metabolisms measured prior to prostate cancer

diagnosis were associated with patterns of secondly diagnosed primary tumours (SDPTs).

(2) Using data from PCBaSe Sweden, I evaluated how radiotherapy for prostate cancer is associated with risk of thromboembolic disease (TED).

(3) In the same database, I assessed the association between drugs used to treat symptoms of the metabolic syndrome and prostate cancer death among men on primary Gonadotropin-releasing Hormone (GnRH) agonists.

(4) Finally, I compared prostate cancer-specific and overall survival between men treated with primary anti-androgen monotherapy (AA) and GnRH agonists using data from PCBaSe Sweden.

The next chapter provides an overview of the current state of the art with respect to prostate cancer epidemiology and outcomes – with specific focus on the four projects described above. The methods and results of the first project are described in Chapter III, whereas the other three projects are all described in Chapter IV. Finally, chapter V is the concluding chapter, interpreting the results and providing guidance for future research.

2. Chapter II: Background

2.1. The normal prostate: Anatomy, histology, physiology

A normal prostate gland is approximately 20 to 30 g in volume and has the shape of a chestnut. It is located posterior to the pubic symphysis, under to the bladder, and anterior to the rectum. The prostate surrounds part of the urethra (prostatic urethra) which explains some of the common symptoms reported for prostate pathologies such as urine retention, decreased force of stream or urinary frequency.(5)

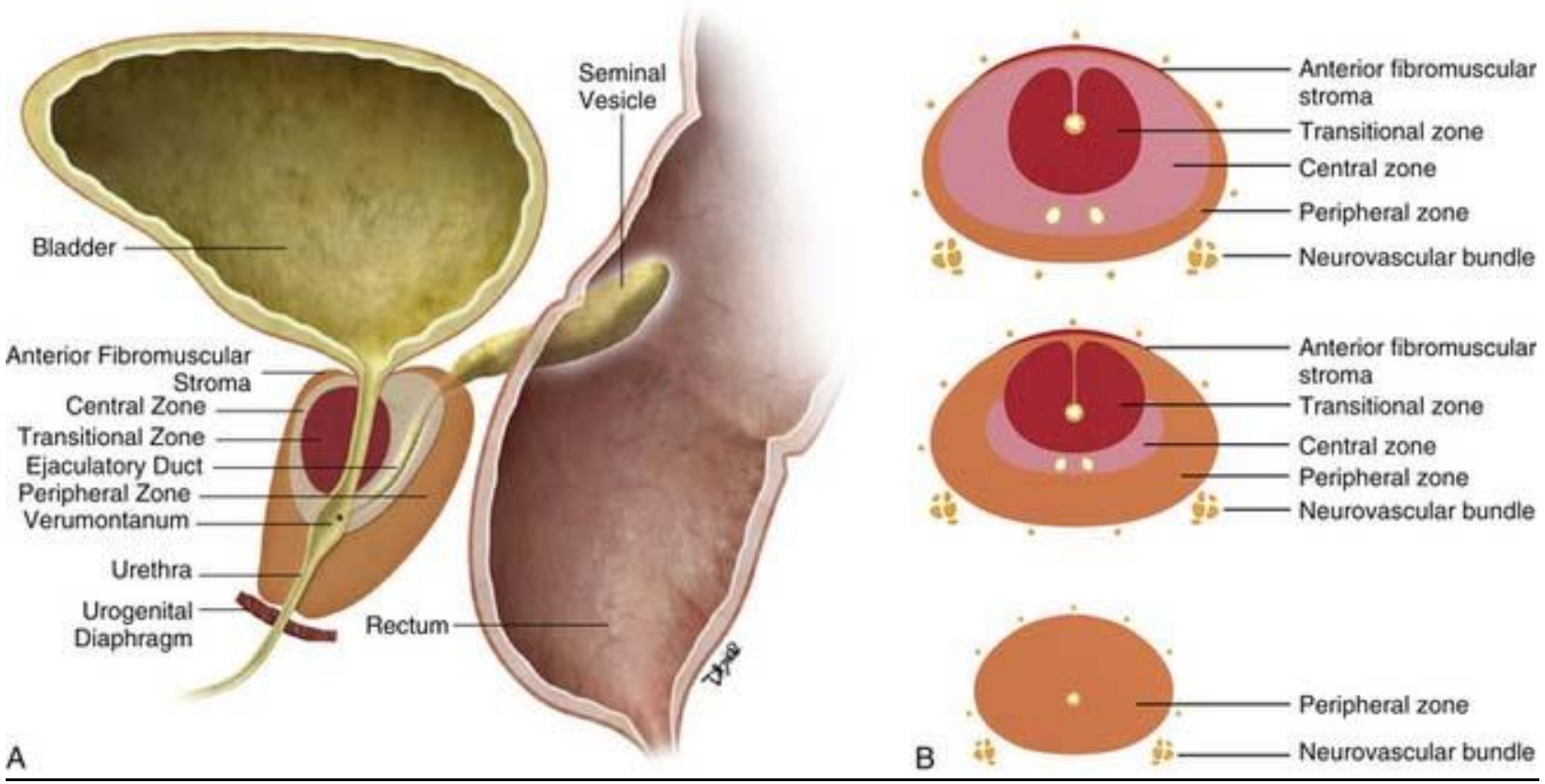
Prostate tissue consists of 70% glandular tissue and 30% fibromuscular-stroma and can be divided into three zones (Figure 1):

The transition zone: represents 10% of the prostatic glandular tissue and 20% of the adenocarcinomas can be found in it.

The central zone: surrounds the ejaculatory ducts and 25% of the glandular tissue. 1-5% of adenocarcinomas can be found in this zone.

The peripheral zone: represents 70% of the glandular tissue. It is located in the posterior and lateral segments of the prostate. About 70% of the adenocarcinomas are found in this zone (5).

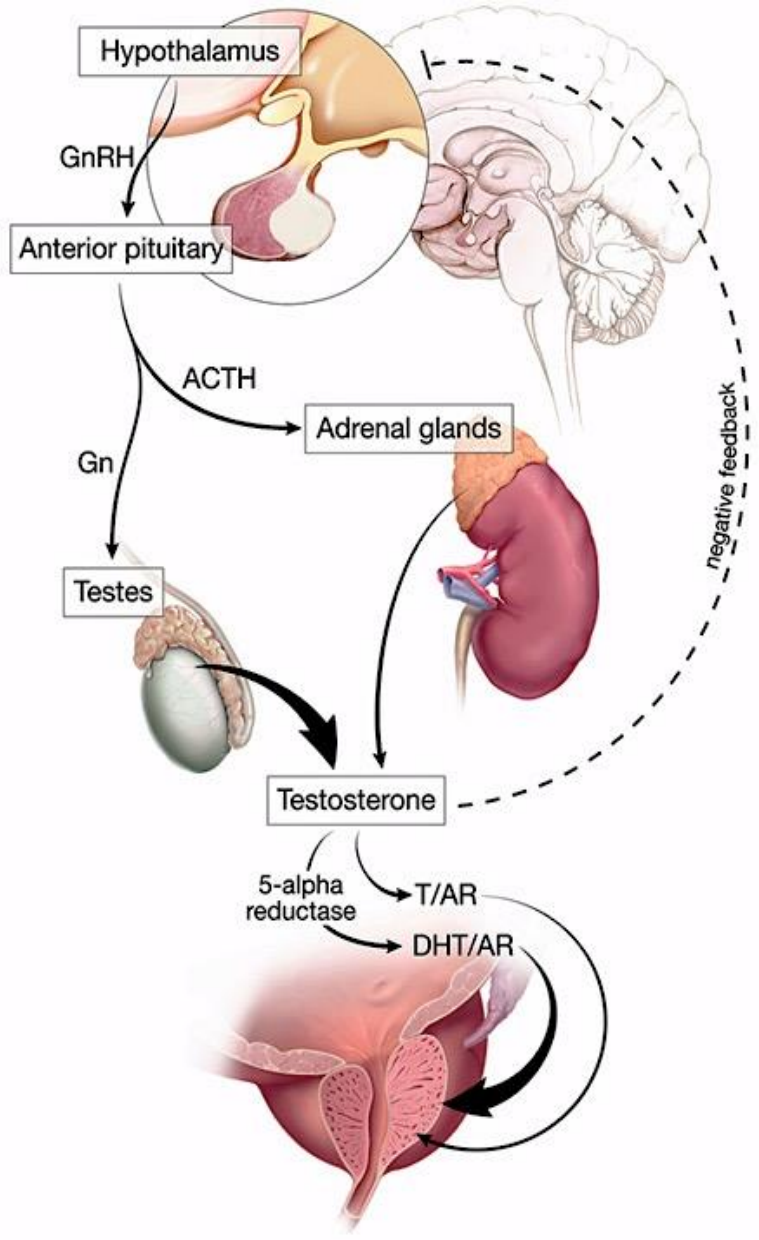
Figure 1 Anatomy of the normal prostate gland (taken from (6)



The prostate's main function is the production of a fluid that gives a liquid consistency to semen with the necessary properties that will provide spermatozoids good motility, protection and prolonged survival.

In order to develop and function, prostate cells need androgens: testosterone and its more active metabolite dihydrotestosterone (DHT) converted to by the enzyme 5a-reductase in the prostate (7). These hormones are part of a well-described endocrine feedback loop. Simplified, the hypothalamus produces Gonadotropin Releasing Hormone (GnRH), which acts on the pituitary gland cells. These cells release luteinizing hormone (LH) and follicle stimulant hormone (FSH). LH and FSH act on the testis, where they promote the synthesis and liberation of androgens and inhibin. These two molecules have effects on different tissues and a negative feedback effect on the hypothalamus and the pituitary gland, which as a result downregulates the synthesis of androgens (8). Once in the prostate cells, testosterone and DHT bind to the androgen receptor (AR) located on nuclear membrane, which acts as a transcription factor involved in prostate development and function (Figure 2).

Figure 2 The Hypothalamic-Pituitary-Gonadal Axis (9)



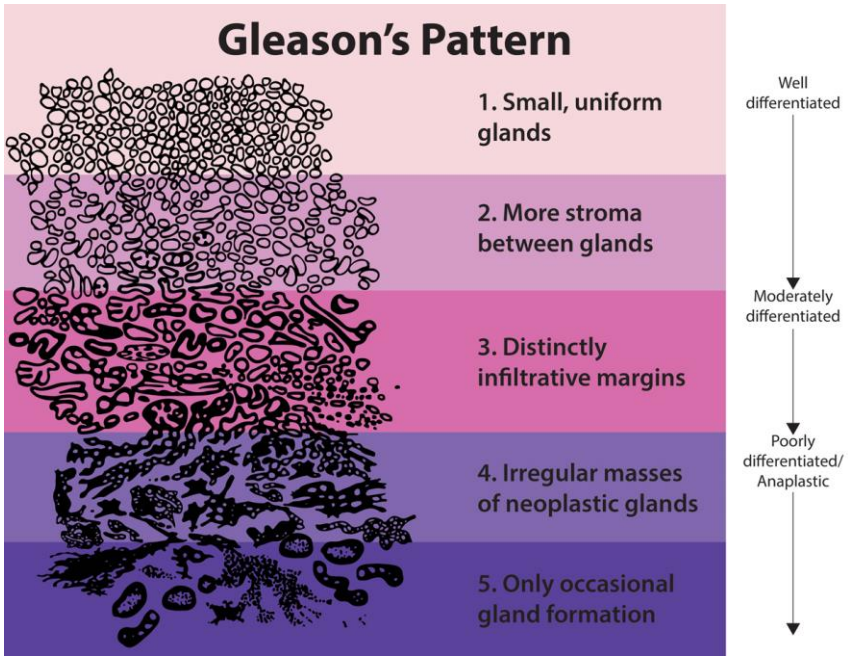
2.2. Prostate Cancer

2.2.1. Clinical presentation

Currently, most prostate cancers are diagnosed in men who are asymptomatic. This is due to screening programs where a molecule found in prostate cells, the prostatic surface antigen (PSA- see below), is measured in blood. Usually these patients present local disease. However, for those with more advanced disease common symptoms go from urinary complaints to weight loss, bone pain, neurologic alterations due to spinal cord compression and other symptoms of metastasis. During physical examination, physicians perform a digital rectal examination (DRE) in order to determine size, consistency and detect any abnormalities on the posterior surface of the prostate. This procedure complements further studies for clinical staging of the disease.

Once prostate cancer is suspected, either by elevated blood levels of PSA, physical examination or by symptoms, a prostate biopsy is usually the confirmatory test where different histological characteristics are scored according to the grade of cell differentiation, meaning how much tumour cells resemble the normal prostatic tissue. The less alike the tumour cells look compared to the normal prostatic cells the less differentiated the cancer is considered. The standardized scoring system based on these microscopic characteristics is the Gleason score. The score is based on the sum of the grades of the two most common tumour cells patterns. Gleason grade ranges from 1 to 5 and the Gleason score ranges from 2 to 10. Higher grades and scores indicate worse prognosis (Figure 3).

Figure 3 Gleason's pattern (10)



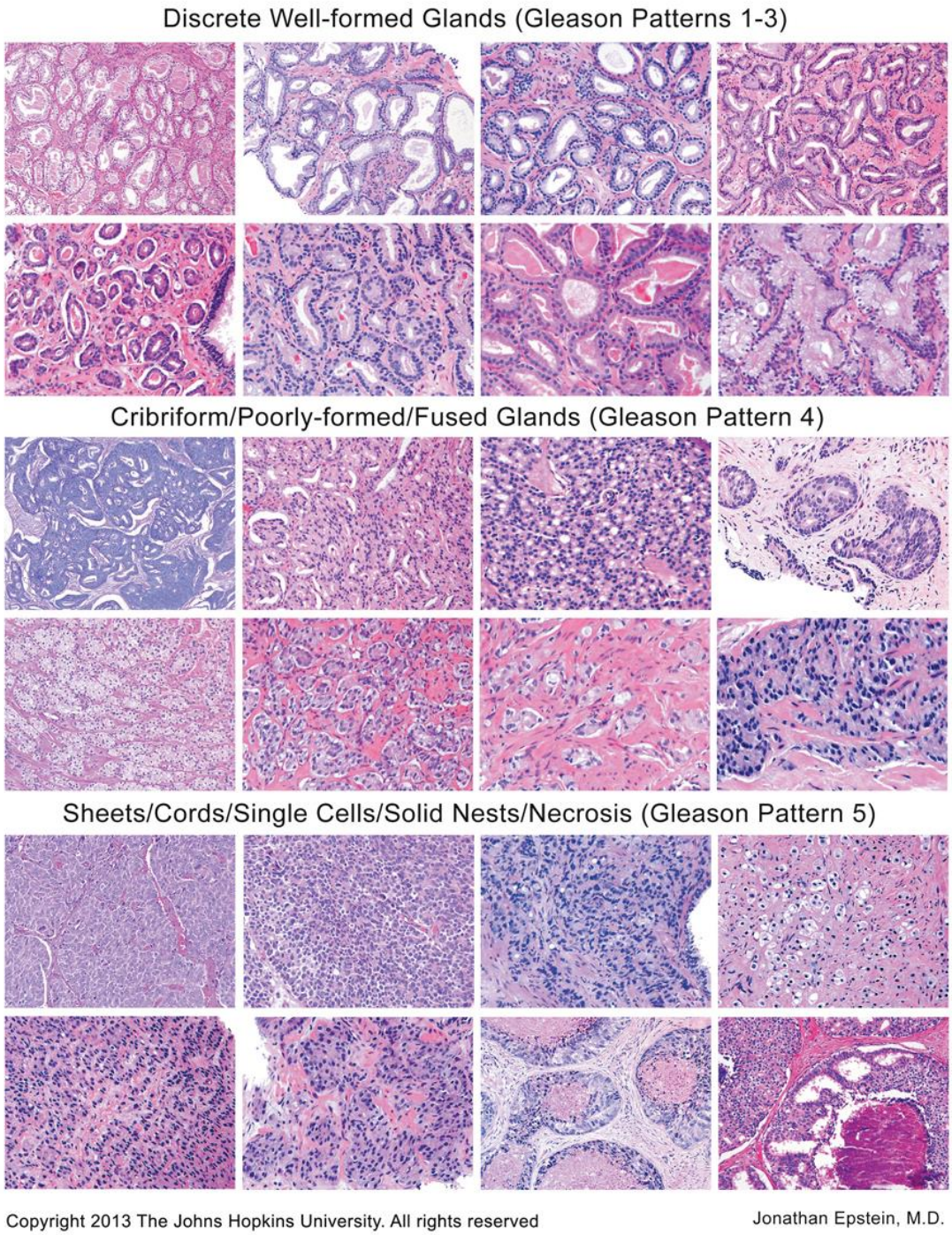
Recently Eppstein et al. introduced the following grading system (Figure 4):

- Grade group 1 (Gleason score 3 + 3 = 6): Only individual discrete well-formed glands.
- Grade group 2 (Gleason score 3 + 4 = 7): Predominantly well-formed glands with lesser component of poorly formed/fused/cribriform glands
- Grade group 3 (Gleason score 4 + 3 = 7): Predominantly poorly formed/ fused/cribriform glands with lesser component of well-formed glands*.
- Grade group 4 (Gleason score 8): only poorly formed/fused/cribriform glands or - Predominantly well-formed glands and lesser component lacking glands ** - Predominantly lacking glands and lesser component of well-formed glands **
- Grade group 5 (Gleason scores 9–10): Lack of gland formation (or with necrosis) with or without poorly formed/fused/cribriform glands*(11)

**For cases with >95% poorly formed/fused/cribriform glands or lack of glands on a core or at radical prostatectomy, the component of <5% well- formed glands is not factored into the grade.*

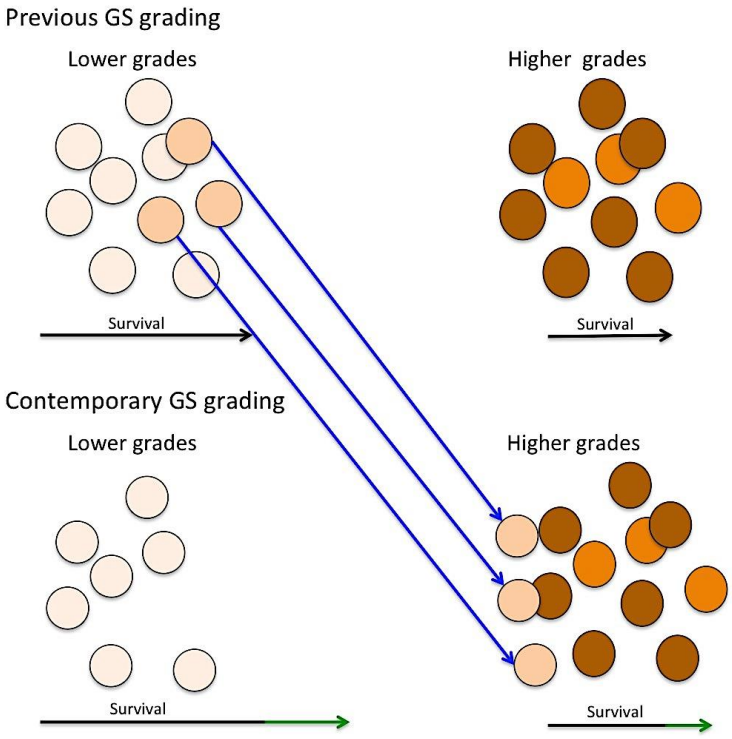
*** Poorly formed/fused/cribriform glands can be a more minor component.*

Figure 4 Eppstein Gleason Grading (11)



Due to this change in GS grading, many prostate cancers have been reclassified to a higher grade in what is known as stage migration or the "Will Rogers phenomenon" (Figure 5) (12). Studies assessing this effect in prostate cancer have shown an artificial improved survival for prostate cancers both previously classified as low grade and high grade. This occurs because among those prostate cancers that were historically classified as low grade, there were some of a higher grade according to the new grading system which probably had a lower survival than those who were classified as a low grade in both systems (13). Thus, by including those who had a higher grade and lower survival, the lower grade group's survival improves. The same occurs by adding those previously classified as low grade to the newly higher grade, assuming that they had a better survival than those who had been classified from the beginning as a high grade. Consequently, survival seems to improve across the new grading system, which in many instances has been attributed to improvement in treatment modalities (14). Although important to acknowledge, this phenomenon does not affect the results in this thesis, as survival or other clinical outcomes were not assessed between men diagnosed in these different time periods.

Figure 5 Will Rogers phenomenon



Based on the biopsy results and the PSA values, imaging studies may be performed to assess macroscopic tumour characteristics. The TNM Classification of Malignant Tumours is then used, where T stands for tumour size and infiltration of nearby structures, N for lymph node invasion and M for distant metastasis (Table 2).
Using several parameters such as age at diagnosis, PSA, Gleason score, TNM classification and percentage of positive biopsy cores, different prediction models and risk categories have been created to aid clinicians and patients with treatment decisions (15) (Table 1). Tissue based molecular assays are available for prognostication in prostate cancer; these can distinguish between low and intermediate risk disease (i.e. Oncotype DX, Prolaris). Currently they are only used in the US (16).

Table 1. European Urology Association prostate cancer risk assessment (17)

Definition			
Low-risk	Intermediate-risk	High-risk	
PSA < 10 ng/mL and GS < 7 (ISUP grade 1) and cT1-2a	PSA 10-20 ng/mL or GS 7 (ISUP grade 2/3) or cT2b	PSA > 20 ng/mL or GS > 7 (ISUP grade 4/5) or cT2c	any PSA any GS cT3-4 or cN+ Any ISUP grade
Localised			Locally advanced

GS=Gleason score; ISUP=International Society for Urological Pathology;

PSA=prostate-specific antigen.

Recently an increasing body of evidence indicates that multiparametric MRI before biopsy and MRI guided biopsy may improve prostate cancer detection in particular those considered clinically significant (18-20). Although some studies have not shown the superiority of an MRI protocols over ultrasonography–guided biopsies (21, 22) a recent RCT, the PRECISION (Prostate Evaluation for Clinically Important Disease: Sampling Using Image Guidance or Not?) trial, has shown that MRI, with or without targeted biopsy, leads to fewer men undergoing biopsy, more clinically significant cancers being identified, less overdetection of clinically insignificant cancer, and fewer biopsy cores being obtained than standard transrectal ultrasonography–guided biopsy. However, an important limitation of the trial was the moderate agreement (78%) between the site and the central radiologist reading, indicating the need for further research to improve the standardization, reproducibility, and reporting of multiparametric MRIs (23).

Table 2. TNM classification of prostate cancer (24)

T - Primary Tumour		
TX	Primary tumour cannot be assessed	
T0	No evidence of primary tumour	
T1	Clinically inapparent tumour that is not palpable	
	T1a	Tumour incidental histological finding in 5% or less of tissue resected
	T1b	Tumour incidental histological finding in more than 5% of tissue resected
	T1c	Tumour identified by needle biopsy (e.g. because of elevated prostate-specific antigen (PSA) level)
T2	Tumour that is palpable and confined within the prostate	
	T2a	Tumour involves one half of one lobe or less
	T2b	Tumour involves more than half of one lobe, but not both lobes
	T2c	Tumour involves both lobes
T3	Tumour extends through the prostatic capsule[1]	
	T3a	Extracapsular extension (unilateral or bilateral) including microscopic bladder neck involvement
	T3b	Tumour invades seminal vesicle(s)
T4	Tumour is fixed or invades adjacent structures other than seminal vesicles: external sphincter, rectum, levator muscles, and/or pelvic wall	
N - Regional Lymph Nodes[2]		
NX	Regional lymph nodes cannot be assessed	
N0	No regional lymph node metastasis	
N1	Regional lymph node metastasis	
M - Distant Metastasis[3]		
M0	No distant metastasis	
M1	Distant metastasis	
	M1a	Non-regional lymph node(s)
	M1b	Bone(s)
	M1c	Other site(s)

1Invasion into the prostate apex or into (but not beyond) the prostate capsule is not classified as T3, but as T2.

2Metastasis no larger than 0.2 cm can be designated pNmi.

2T2a to c only exist for clinical T2 (cT2). For pathological T2 they are no longer present in the 2017 TNM. Only pT2 exists.

3When more than one site of metastasis is present, the most advanced category is used. (p)M1c is the most advanced category.

2.2.2. Prostate cancer biology and natural history

About 95% of all prostate cancers are adenocarcinomas; followed by 4% that present transitional cell characteristics and are believed to develop from urothelial cells form the prostatic urethra. The remaining few cases are either neuroendocrine or squamous cell carcinomas (15).

Prostate cancer, in most cases, is a slow growing tumour detected in adults and elderly adults. Prostate cancer is likely to be, as many other cancers, the result of various genetic hits that occur over many years. Therefore, lifestyle and environmental risk factors are thought to also impact prostate cancer development and progression throughout most of men's life.

Different studies have looked at associations between the exposure to some factors in utero and prostate carcinogenesis. For instance, birth weight has been used in some studies as a proxy for nutrients and other exposure factors during pregnancy. However, results are inconsistent (7). Nonetheless, lack of consistency between studies does not rule out a possible association. In Rothman's sufficient cause model different component causes may add up to different sufficient causes for the same outcome. Meaning that depending on the context and the population, some of these factors can be associated or not with the outcome. Furthermore, temporality and other exposures to potential preventive factors need to be taken into account (25). Extreme hormonal changes that occur during adolescence have also been suggested as possible elements that predispose to genetic alterations that together with other factors that occur later in life, even aging itself, lead to prostate cancer carcinogenesis. It is well established that androgens are necessary for prostate cancer development. In laboratory studies prostate cancer growth is inhibited in the absence/ blockage of androgens and stimulated in their presence. Furthermore, one of the current treatment protocols for prostate cancer, androgen deprivation therapy (ADT), consists on lowering levels of androgens by chemical or surgical castration. However, many prostate cancers become androgen resistant or refractory tumours, and although they still show activity in response to androgens, lack of these hormones does not stop growth and proliferation of the cancer cells. This may be a consequence of structural

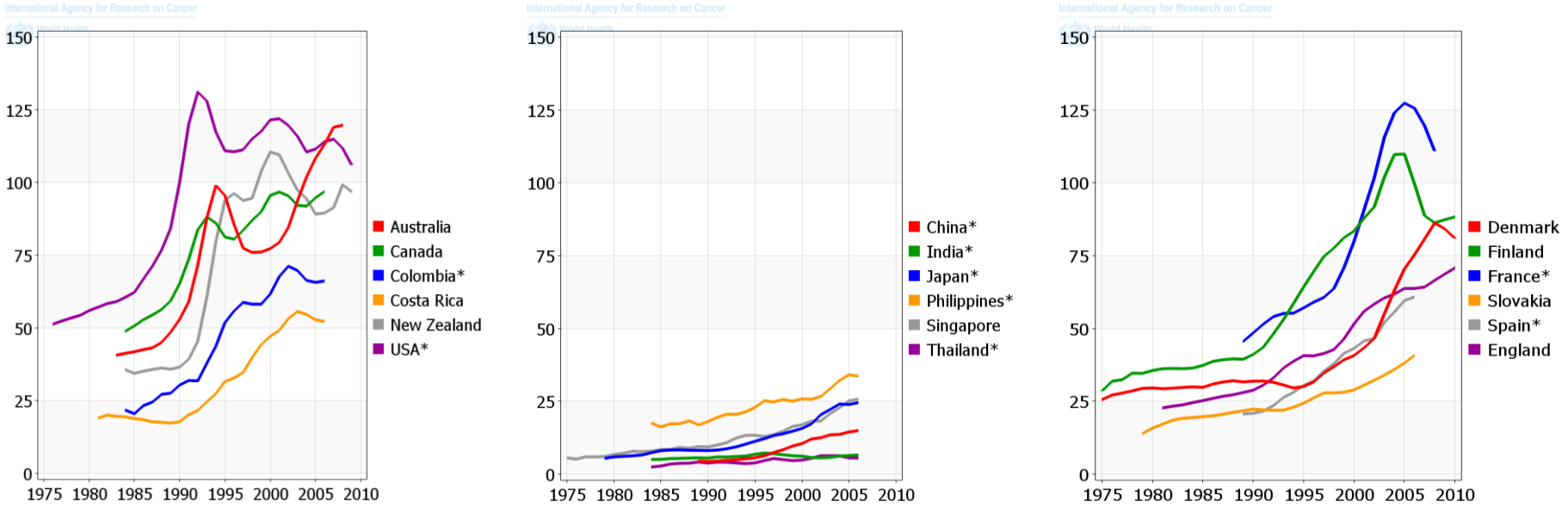
changes in the androgen receptor that lead to its downstream activation in the absence of a ligand (26).

2.2.3. Descriptive epidemiology

2.2.3.1. Incidence

Prostate cancer is the second most commonly diagnosed male cancer worldwide. However, these worldwide incidence rates are strongly influenced by large Asian populations where the most common cancer is of the lung. When looking at Europe, North and South America, Oceania and most of Africa, the most commonly diagnosed cancer is prostate cancer. Part of these variations in incidence rates across populations can be attributed to differences in PSA screening (see below) access and/or recommended protocols and a smaller part due to lifestyle factors (27). For instance, globally age standardized incidence rates have increased over time mainly in countries where PSA screening has been routinely applied like the US or Europe. Nonetheless, incidence rates have also increased in countries from East Asia, where PSA testing has not been commonly used (Figure 6). Some researchers have suggested that this could be due to a nutrition transition characterized by an increased intake of energy, animal fat and red meat (28).

Figure 6 Worldwide trends in incidence of prostate cancer. From: GLOBOCAN 2012 (IARC)(1)

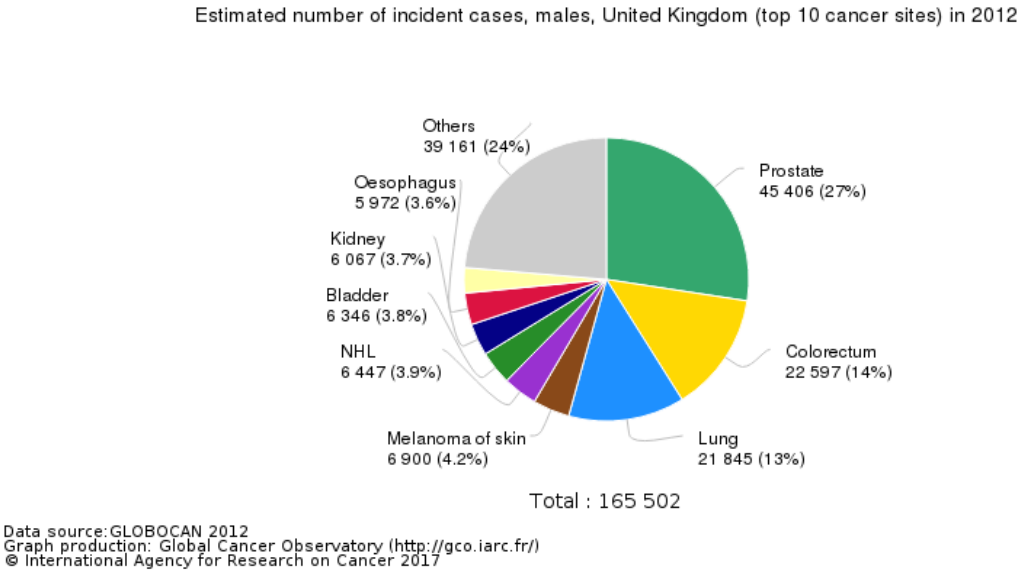


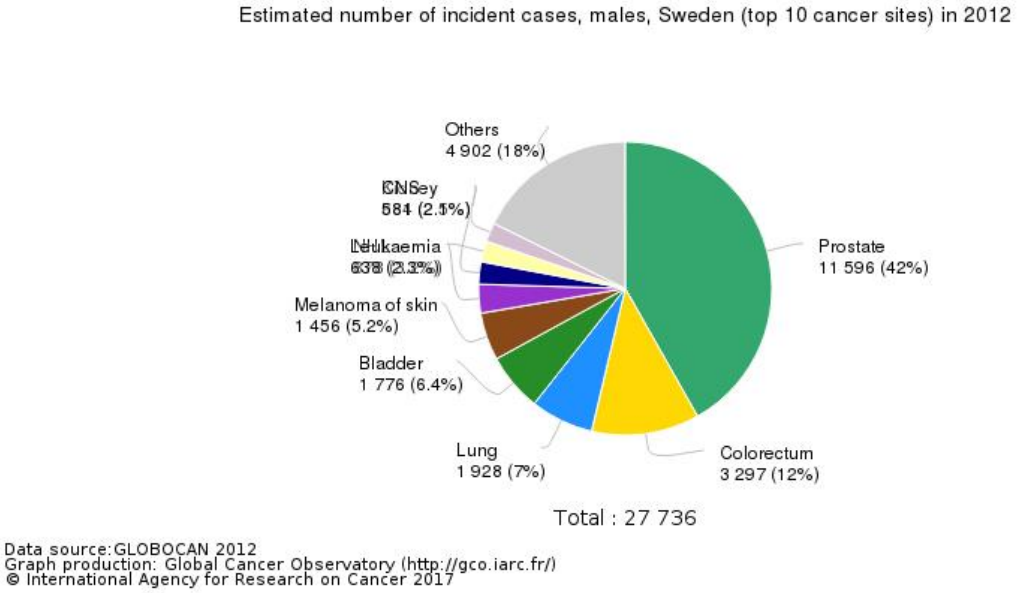
In the UK, prostate cancer is the most common cancer among men, accounting for about 45,406 cases annually, which represent 27% of all new cancer diagnoses in 2012 (Figure 7).

Prostate cancer incidence rates have increased by 44% in the UK since the 1990s due to the boost in detection with PSA screening. In the last years more than 54% of all prostate cancer cases have been diagnosed in men aged over 70 years. Age-standardized rates for black males are 1.2 to 2.5 times higher than rates for white males. Furthermore, lifetime risk of being diagnosed with prostate cancer is 13.2-15.0% for white males and 23.5-37.2% in black males (29).

In Sweden, prostate cancer is also the most common cancer among men. About 11,600 new cases were diagnosed in 2012, representing 42% of all newly diagnosed cancers (age-standardized incidence rate of 101.9 per 100,000) (Figure 7). PSA screening, implemented in 1997, is predominantly responsible for this rapid increase in prostate cancer incidence (30).

Figure 7 Overview of incidence male cancers in the UK and Sweden in 2012.





2.2.3.2. Mortality

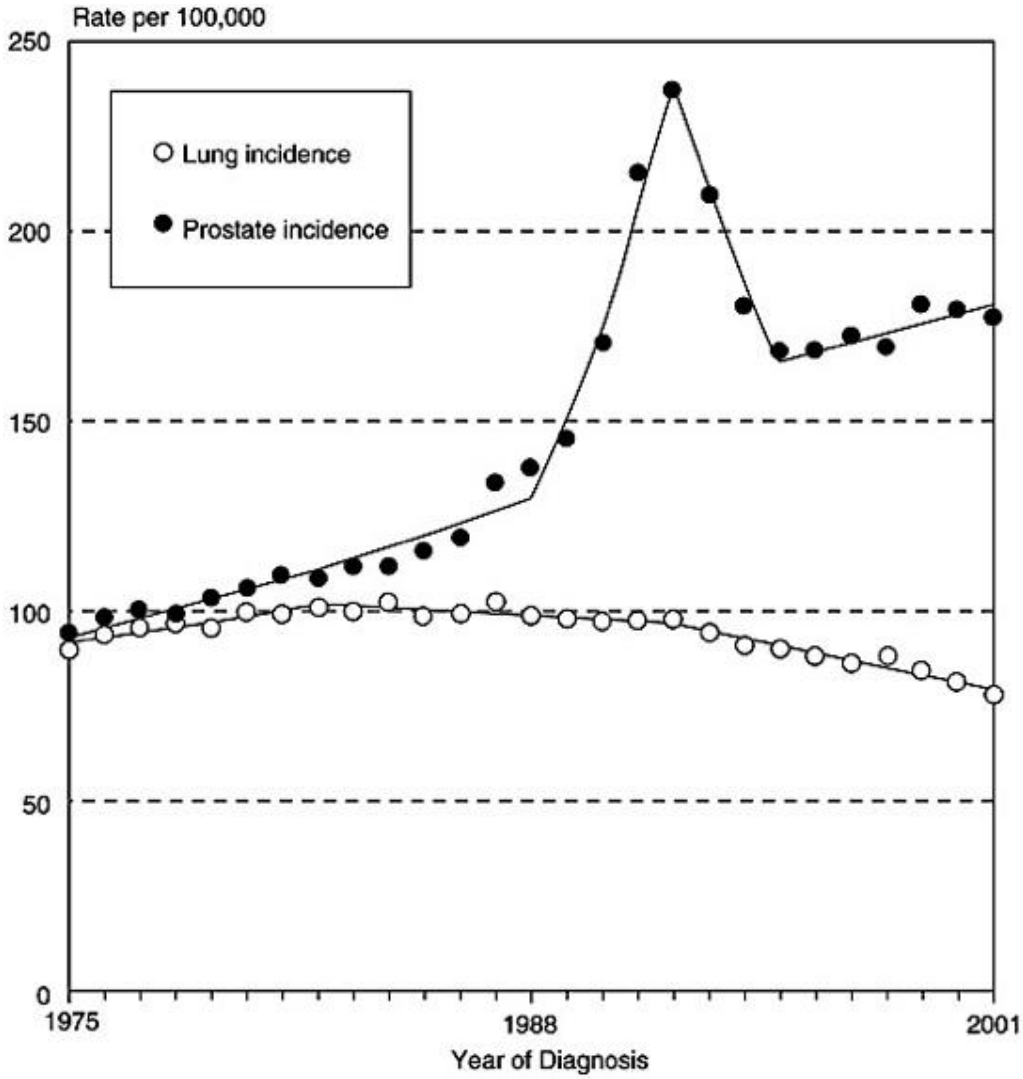
Prostate cancer is the fifth leading cause of death from cancer in men worldwide, the second in the UK and the first in Sweden (31). Overall, mortality rates in more financially developed regions are decreasing. This is likely due to advances in treatment as well as increased detection of early stage disease through PSA testing. However, prostate cancer mortality is increasing, as well as incidence, in less resourced areas, due to a combination of the more recent introduction of PSA testing,

westernized lifestyle changes and less knowledge and access to curative treatment (32). For instance, a recent study using the world's health organization (WHO) Ranking of the World's Health Systems and the expenditure on health/gross domestic product (GDP; e/GDP) and life expectancy showed that prostate cancer mortality to incidence ratios (MIR) are higher for less developed countries. This means that besides the below described risk factors for prostate cancer, good management of health care (i.e. early screenings, advanced surgical and personalized therapy) has a direct impact on improving clinical outcomes resulting in low MIRs (33). Prostate cancer survival rates vary according to disease stage at diagnosis. For those diagnosed with localised disease and low GS prostate cancer, 5-year survival rates are above 90% in most countries. However, for those with stage IV (metastatic prostate cancer or GS 8/9) disease, survival rates are rather low (i.e. 30% in the UK), compared to earlier stages (29).

2.2.3.3. Prostate cancer screening: PSA and other markers

PSA is a glycoprotein produced by the prostate gland epithelial cells. Its discovery, purification and clinical use are the result of collective contribution by many scientists since the late 1960s until the beginning of the 1980s (34). By the mid 1980s, the PSA test was first approved by the Food and Drug Administration (FDA) for monitoring prostate cancer progression. Later, in 1994, PSA testing was approved for screening (35). After the introduction of the PSA test, in most western countries, prostate cancer incidence showed a sharp rise and subsequent fall. For instance, in the US this occurred between 1989 and 1995 with a peak at 1992, which represents undiagnosed preclinical cases detected by PSA (25) (Figure 8).

Figure 8 Prostate versus lung cancer incidence in the US between 1975 and 2001. (25)



Normally, very low levels of PSA can be found in blood. PSA blood levels can increase not only due to malignant processes but also due to any inflammatory condition such as prostatitis, benign prostatic hyperplasia (BPH) and even after intense exercise or recent ejaculation. Increased levels of PSA may indicate the presence of prostate cancer. Nonetheless, false negative results are not rare, as prostate cancer can be present in the absence of elevated PSA levels (36). Once in the blood stream, most of PSA binds to blood proteins and a smaller fraction remains free, also known as fPSA. PSA tests using a cut-off of 4.0 ng/mL have a sensitivity of 67.5-80%. The specificity of PSA at levels higher than 4.0 ng/mL is about 60-70% (37). In a prospective blinded study by Catalona et al. it was concluded that percentage of fPSA might reduce unnecessary biopsies in men being screened for prostate cancer, with a minimal loss in

sensitivity in detecting cancer (38). Tests to analyse PSA kinetics are also available. PSA velocity (PSAV) is the absolute annual increase in serum PSA (ng/ml/year), PSA doubling time (PSADT) measures the exponential increase in serum PSA over time and PSA density (PSAD) is calculated dividing serum PSA by prostate volume. Currently these measurements have a prognostic application but due to conflicting results they are not used in diagnosis (16).

The main aim of screening is to reduce the diagnosis of advanced disease and mortality from prostate cancer, while also improving quality of life. Screening should be ethical and cost-effective. The PSA test is relatively easy and inexpensive. However, screening of asymptomatic men could lead to detection of large numbers of men with latent, clinically indolent disease (39). In fact, overdiagnosis rates are estimated to be between 27% and 56% due to the lack of specificity of PSA screening. Potential benefits and risks or limitations of PSA testing are listed in Table 3. In the last decade new PSA, genetic tests and prediction algorithms based on these tests and other parameters have been developed. The 4Kscore is a prediction model, which combines several variables such as: DRE results, biopsies, age, PSA, fPSA and human kallikrein 2 in order to predict the patient's risk of GS ≥ 7 in biopsies (40). Another model is the prostate health index (PHI), which combines total PSA, fPSA and serum isoform (-2) pro-PSA to predict Gleason Score 7 on biopsy, upgrading/upstaging on prostatectomy and recurrence (41). Other blood, urine and tissue based tests are the 'Stockholm-3' test, the PCA3, the TMPRSS2: ERG and the Mi-Prostate Score (MiPS). All of these tests have shown promising results in the prediction of prostate cancer diagnosis and progression however they need to be validated in large-scale studies in order to be consistently recommended in prostate cancer guidelines (16).

Table 3. Benefits and risks of prostate cancer PSA based screening (modified from (42))

Benefits	*Risks or limitations*
1. It may lead to detection of cancer before symptoms develop. 2. It may lead to detection of cancer at an early stage when the cancer could be cured or treatment could extend life. 3. Repeat PSA tests may provide valuable information, aiding a prostate cancer diagnosis.	1. The PSA test is not diagnostic. 2. PSA is not tumour specific in the prostate. 3. The PSA test may give false-positive results. A man may have an elevated PSA level, but no cancer. About 75% of men who have an elevated PSA level have a false-positive result. 4. The PSA levels may not be elevated and provide false reassurance. 5. The PSA test may lead to the identification of prostate cancers, which might not become clinically significant.

Thus, the only test that can be found in most prostate cancer guidelines is blood levels of PSA. To evaluate the effectiveness of the PSA test in reducing mortality, two randomized trials have been conducted and updated: the European randomized study of screening for prostate cancer (ERSPC) and the prostate, lung, colorectal, and ovarian (PLCO) cancer screening trial.

The ERSPC study randomized 162,388 men for prostate cancer PSA-based screening or no screening. Men were between 55 to 69 years of age. Recruitment initiated in 1991 in the Netherlands and in Belgium, and then between 1994 and 1998 five more countries joined: Sweden, Finland, Italy, Spain, and Switzerland. After a median follow-up time of nine years, the study showed that prostate cancer deaths decreased by 20% in men assigned to screening, but there was a high risk of overdiagnosis (43). For instance, the relative risk for prostate cancer between the screening and control groups at 9-year follow-up was 1.91, at 11-year follow-up was 1.66, and at a median of 13 years of follow-up this was 1.57. The most recent update shows that the numbers needed to invite for screening to prevent 1 prostate cancer death decreased from 979 at 9 years to 781 at 13 years and the numbers needed to diagnose to prevent 1 death decreased from 35 at 9 years to 27 at 13 years. No significant differences for overall

mortality have been reported (44). This study has been criticized for not publishing de-identified individual data, in order to confirm the findings. Another point of criticism that has been raised is the fact that five of the seven countries included found no differences – which questions the validity of data pooling (45).

The PLCO recruited from 10 study centres across the United States, men and women between the ages of 55 and 74 years from 1993 through 2001. For prostate cancer PSA-based screening, 74,000 men were randomized to either screening or no screening. The authors found, after seven to ten years of follow-up, a non-statistically significant increase in prostate cancer mortality in the annual screening group (46). However, nearly 50% (some claim 90%) of the men in the control group had undergone at least one PSA test, and thus the PLCO study does not have the statistical power to reach any valid conclusion (47). Yet, in a recent study authors combined data from the two trials and performed an analysis in which they took into account differences in screening implementation and the settings of both trials. It was concluded that both the ERSPC and PLCO trials provide compatible evidence that screening reduces prostate cancer mortality (48).

In the UK, the UK National Screening Committee concluded that the evidence to support PSA testing as the basis for a screening program is not conclusive, thus their recommendation is not to offer screening for prostate cancer. Nonetheless, the test is free for men aged 50 or more who request it, on the condition that they receive clear and balanced information about the advantages and disadvantages of the PSA test, biopsy and treatments for prostate cancer (42).

In Sweden, testing for PSA is not part of a prostate cancer-screening programme. Many men have PSA tests on their own initiative. However, a study by Godtman et al. found that organized screening reduces prostate cancer mortality and that opportunistic PSA testing could result in more overdiagnosis, with almost twice the number of men needed to be diagnosed to save one man from dying from prostate cancer compared to men included in an organized screening program (49).

Hence, the screening debate for prostate cancer has not been solved. Following the results of the above described trials it is difficult to disentangle whether the overdiagnosis following screening is due to detection of cancers with malignant potential, but which have a long lead time, or the diagnosis of truly biologically indolent cancers.

However, lack of consistency amongst reference guidelines led a group of prostate cancer experts to elaborate a series of consensus statements at the 2013 Prostate Cancer World Congress in Melbourne, Australia based on all available evidence. The 5 statements and brief justifications can be found in table 4 (50).

Table 4. Prostate cancer early detection consensus statements 2013 Prostate Cancer World Congress in Melbourne, Australia

Statement	Justification
1. Men Aged 50–69 Years, PSA testing reduces the incidence of metastatic prostate cancer and prostate cancer-specific mortality rates.	Based on evidence from the PLCO, ERSPC and the Götenborg studies healthy, well-informed men in this age group should be fully counselled about the positive and negative aspects of PSA testing to reduce their risk of metastases and death.
2. Prostate cancer diagnosis must be uncoupled from prostate cancer intervention.	Many men with low-risk prostate cancer do not need immediate aggressive treatment thus, active surveillance strategies need standardization and validation to ensure patients and clinicians that this is a safe strategy.
3. PSA testing should not be considered on its own, but rather as part of a multivariable approach to early prostate cancer detection.	Ethnicity, family history, medical history, DRE findings, prostate volume, risk prediction models and new tools, such as the Prostate Health Index (phi) test and prostate cancer antigen 3 (PCA3) test, can help to better risk stratify men.
4. Baseline PSA testing for men in their 40s is useful for predicting the future risk of prostate cancer and Its aggressive forms.	For men whose baseline PSA level is in the highest centiles above the median the risk of developing life-threatening disease later in life is greater. Thus, this option should be discussed with men in this age group.
5.Older men in good health with a >10-year life expectancy should not be denied PSA testing based on their age.	As life expectancy improves in many countries around the world a small proportion of older men may benefit from an early diagnosis of more aggressive forms of localised prostate cancer. Therefore, men should be assessed on an individual basis rather than applying an arbitrary chronological age beyond which testing should not occur.

PSA concentrations can also be predictive of the future risk of prostate cancer metastasis and cancer specific death. In a recent case control study by Vickers et al., frozen blood samples were used to select a group of men aged between 27 and 56 years old. PSA levels were measured and the association between PSA concentrations and prostate cancer metastases was analysed. It was found that for men in the highest 10^{th} of PSA concentration (≥1.3 µg/L) at age 40, the risk of prostate cancer metastases after 15 years of follow-up was low (0.6%). However, the risk of metastases within 15 years was three times higher (1.6%) for men in the highest 10th at age 45-49, and close to tenfold higher (5.2%) at age 51-55. They concluded that their results suggest that not starting PSA based screening until age 51-55 may translate into an important number of men at increased risk of later being diagnosed with an incurable cancer (51).

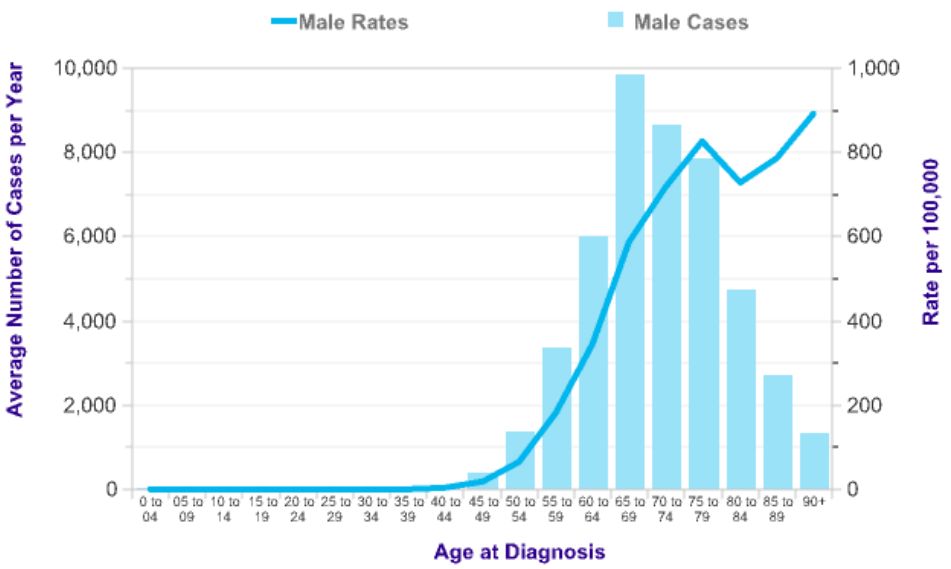
For the current thesis, PSA screening is not affecting the work conducted in the Swedish Apolipoprotein-related Mortality Risk (AMORIS) cohort as most cancers were diagnosed prior to the introduction of PSA testing (52). The work done using data from PCBaSe Sweden (53, 54) focuses on outcomes for men already diagnosed with prostate cancer – so that detailed information on tumour characteristics was available to account for the potential effects of screening. More details of this work are described in Chapters III and IV.

2.2.4. Risk factors

2.2.4.1. Ageing

The most important risk factor for developing prostate cancer is ageing; the chance of having prostate cancer rises rapidly after age 50. Prostate cancer is often found in autopsy studies and most of these men were never diagnosed with the disease (Figure 9) (55) (56).

Figure 9 Average number of new cases per year and age-specific incidence rates, males, UK, 2012-2014 (56)



During aging, there is an increased frequency of DNA damage and of DNA strand-break in most of the body tissues. These age-related changes are partly the consequence of oxidative stress, characterized by the accumulation of cellular oxidants, such as free radicals and reactive oxygen species (ROS). ROS can cause direct damage to DNA as well as oxidative modification of proteins, including enzymes involved in DNA repair leading to tumourigenesis. Most cells in young, healthy individuals have ROS detoxification enzymes. With aging a decline in ROS detoxification enzyme activities occurs (57).

2.2.4.2. Race

Although several other factors need to be taken into account when making conclusions about prostate cancer incidence and mortality in different ethnicities (i.e. socio-economic status, access to health services and screening programs, health seeking behaviour), overall it is considered that black men are at higher risk of presenting and dying from prostate cancer when compared to other races (58). Several studies from the US and the UK, have shown that men with African ascendance (either American, European or Caribbean) are 2.5 to 3 times more likely to develop prostate cancer than the Caucasian or Hispanic counterparts. Regarding mortality, black men present 20 to 30% higher rates than white men (59).

It has been shown that the androgen receptor expression is down-regulated in the stromal cells of the prostate in black men (60), but has a high expression in malignant epithelium (61). Furthermore, it has been suggested that high testosterone levels in black men may play a role in the pathogenesis of prostate cancer (62). Another possible explanation is the higher prevalence of polymorphisms associated with the 5-alpha reductase and with the androgen receptor genes which regulate testosterone metabolism and function in prostate cancer cells (63).

Diet may also play a role in the difference in incidence of prostate cancer amongst different ethnicities. For instance, an epidemiological study showed a positive statistically significant association between prostate cancer risk and saturated fat intake among all ethnic groups combined. However, fat intake differed among different ethnic groups: African-Americans consumed more than white individuals, followed by the Asian-Americans (64).

2.2.4.3. Family history and prostate cancer genetics

First degree relatives of men with prostate cancer are two times more likely to develop prostate cancer than the general population. For first degree relatives of men with prostate cancer diagnosed at ≤60 years of age this risk is three times or more than the risk for men without a family history (Table 5). Furthermore, it has been shown that the risk is greater for brothers than for sons of men with prostate cancer, suggesting that environmental factors and detection bias may also play a role (65).

Table 5. Effect of family history of prostate cancer on lifetime risk of clinical prostate cancer(65)

Family History	Relative Risk	% Absolute Risk
Negative	1	8
Father affected at 60 yrs. or older	1.5	12
1 Brother affected at age 60 yrs. or older	2	15
Father affected before age 60 yrs.	2.5	20
One brother affected before age 60 yrs.	3	25
Two affected male relatives	4	30
Three or more affected male relatives	5	35–45

With the advance in molecular biology techniques, several chromosomes and genes have been linked to prostate cancer risk. Moreover, evidence suggests that less common genetic variants entail higher risk. However, these variants only account for a small proportion of the overall familial risk (66). Of particular interest are the single nucleotide polymorphisms (SNPs). These are a variation in a single DNA building block, called a nucleotide (67). Genome-wide association studies (GWAS) allow the simultaneous assay of millions of common (≥5%) SNPs. So far, 100 prostate cancer risk related SNPs have been identified which account for approximately 33% of the familial risk of prostate cancer in populations of European ancestry (68).

Furthermore, in a recent study using genotype data from men with European and African American ancestries, authors found a high degree of similarity between these groups suggesting a similar genetic background underlying prostate cancer risk (69). Some of the identified regions where SNPs can be found are in chromosomes 8, 10 and 19. In chromosome 19 SNPs in the genes encoding PSA have been found to affect the antigens levels, suggesting a potential role for SNPs mapping in prostate cancer screening (66).

Less common variants have also been associated with higher risk of prostate cancer. One mutation known to increase the risk of prostate cancer approximately five to sevenfold is the one present in the tumour suppressor gene BRCA2. Less evidence has been reported regarding BRCA1, although some studies have found that carriers of a mutation on this gene have doubled the risk of prostate cancer for males aged <65 years. Furthermore, a study analysing the role of prostate cancer screening in men that had a mutation in either gene, showed that PSA screening had a high positive predictive value and that screening amongst these men detected clinical significant cancer (70). Germline mutations in the homebox gene HOXB13 are also rare, however they have been found to be strongly associated with increased risk of prostate cancer (71). Moreover, in a recent study it was found in a cohort of patients with Lynch syndrome that their risk of prostate cancer was five times higher than the rest of the population, but it did not appear to have earlier onset or a more aggressive phenotype. Lynch syndrome, which is characterised by a series of germline mutations

in one of the mismatch repair genes, increases the risk of colorectal, endometrial, ovarian, gastric, small intestinal, pancreatic, ureteral, brain, and sebaceous gland adenocarcinomas (72).

Regarding treatment, some researchers used GWAS to look into SNPs and radiotherapy response and found that men carrying SNPs in the FSHR (follicle stimulating hormone receptor) gene and in chromosome 9 had an increased incidence of erectile dysfunction and urinary toxicity, respectively. Furthermore, men who underwent radical prostatectomy and had a SNP in the EGFR (epidermal growth factor receptor) gene present had less risk of biochemical relapse conferring a protective effect (66, 68, 70).

2.2.4.4. Lifestyle factors: Nutrition

Even though genetic variants explain, in part, the aetiology of prostate cancer and some treatment discrepancies between patients, their complex interactions with environmental factors are not fully understood yet. There is a growing body of evidence supporting the hypothesis that obesity is associated with increased risk of prostate cancer in particular advanced stage, although this association may be biased as obese men are less likely to be health conscious and to present a health seeking behaviour (i.e. less likely to undergo PSA screening). Furthermore, it has been reported that obese men have lower PSA levels due to hemodilution and DRE can be difficult to perform and interpret for the practitioners (73). Nonetheless, epidemiological and laboratory-based studies have suggested potential biological links between obesity and prostate cancer. In a review collecting all available evidence regarding the association between obesity and prostate cancer, it was found that three meta-analyses were in agreement. They all reported weak, but statistically significant positive associations. Furthermore, they also reported the results from studies looking at obesity and prostate cancer treatment and disease outcomes, and showed that obesity was associated with higher risk of biochemical recurrence and prostate cancer specific death after radical treatment. Strong and consistent evidence across several studies showed a positive dose–response relationship between increasing BMI and fatal prostate cancer (74).

Regarding specific dietary components, the evidence is less conclusive. Another large review reported that results from most studies are either inconclusive or contradictive. According to some studies, simple refined carbohydrates might increase the risk of prostate cancer by stimulating insulin peaks, insulin resistance and insulin-like growth factor-1 (IGF-1)(75). Moreover, this review highlighted that one of the main sources of protein for humans, animal meat, may be linked to an increased risk of prostate cancer. Some of the mechanisms suggested include the presence of saturated fats and cholesterol as well as the formation of heterocyclic amines (HCAs) during high-temperature cooking. HCAs are compounds that can cause genomic instability through DNA damage. Furthermore, consumption of well-cooked meat has been associated with increased prostate cancer incidence (76).
Experimental studies have shown that reducing fat intake slows down tumour growth, however human case-control and cohort studies have found no association between total fat consumption and prostate cancer risk (77). Yet, in a questionnaire-based prospective study, Giovanucci *et al*. found that total fat consumption, primarily of animal origin, was associated with risk of advanced prostate cancer (RR: 1.79; 95%CI: 1.04–3.07) for high versus low quintiles of intake (78).

Dairy products and calcium intake have also been associated with risk of prostate cancer, although the evidence is not conclusive. Results from a study using data from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort showed that prostate cancer is associated dairy proteins and dairy calcium consumption (79). A possible mechanism would be through the downregulation of vitamin D by calcium given the high calcium content of dairy. High vitamin D levels may regulate gene expression, inhibit cellular proliferation, and induce the differentiation of normal and neoplastic cells (80). However, in a nested case-control study within EPIC cohorts, no associations were found between serum concentrations of vitamin D and prostate cancer suggesting that other mechanisms and factors may also be involved in the dairy calcium-prostate cancer previously motioned association (81).

Studies looking at lycopene, vitamin E, cruciferous vegetables, zinc and isoflavones have found positive or no associations between these nutrients and prostate cancer (15, 82). Lycopene is a carotene found in many fruits and vegetables and its consumption has been linked to lower risk of prostate cancer incidence and progression. However, in the latest World Cancer Research Fund (WCRF) International's report on diet, weight, physical activity and prostate cancer, the level of evidence for lycopene-rich foods was changed from "probable" to "limited, no conclusion". It was clarified that although lycopene has been shown to have strong anti-cancer effects in experimental models, currently the data are too mixed to reach a conclusive decision (83).

Table 6 WCRF levels of evidence regarding diet, weight, physical activity and prostate cancer (83)

DIET, NUTRITION, PHYSICAL ACTIVITY AND PROSTATE CANCER			
		DECREASES RISK	INCREASES RISK
STRONG EVIDENCE	Convincing		
	Probable		Body fatness (advanced prostate cancer)[1,2] Adult attained height[3]
LIMITED EVIDENCE	Limited-suggestive		Dairy products Diets high in calcium Low plasma alpha-tocopherol concentrations Low plasma selenium concentrations
	Limited-no conclusion	Cereals (grains) and their products, dietary fibre, potatoes, non-starchy vegetables, fruits, pulses (legumes), processed meat, red meat, poultry, fish, eggs, total fat, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, plant oils, sugar (sucrose), sugary foods and drinks, coffee, tea, alcoholic drinks, carbohydrate, protein, vitamin A, retinol, alpha carotene, lycopene, folate, thiamin, riboflavin, niacin, vitamin C, vitamin D, vitamin E supplements, gamma-tocopherol, multivitamins, selenium supplements, iron, phosphorus, calcium supplements, zinc, physical activity, energy expenditure, vegetarian diets, Seventh-day Adventist diets, individual dietary patterns, body fatness (non-advanced prostate cancer), birth weight, energy intake	
STRONG EVIDENCE	Substantial effect on risk unlikely	Beta-carotene[4,5]	

1. Body fatness is marked by body mass index (BMI), waist circumference and waist-hip ratio. The effect was observed in advanced prostate cancer only.
2. Advanced in this report includes advanced, high grade, and fatal prostate cancers (see section 5.2).
3. Adult attained height is unlikely to directly influence the risk of cancer. It is a marker for genetic, environmental, hormonal, and also nutritional factors affecting growth during the period from preconception to completion of linear growth.
4. Includes both foods naturally containing the constituent and foods which have the constituent added.
5. The evidence includes studies using supplements at doses of 20, 30, and 50 mg/day.

Yet, as highlighted by Prof Holmberg in his comment on reviews looking into diet and prostate cancer, lifestyle markers such as body weight, nutrition, physical exercise and socioeconomic status are very closely related and interdependent. Therefore, considering all these factors and their effects together in one setting and completely deconfounding the analyses in observational studies is rather difficult (84). Furthermore, food intake questionnaires can be subject to recall bias, as participants may not always give accurate data due to the time intervals, or due to differences in

answers based on people's dietary routines, more than on the real consumption. Answers are usually influenced by sex, age, and concerns about weight or body image. Therefore, for some studies analyses based on physical or chemical measurement (i.e. Lipids blood levels) of lifestyle reflectors are likely to be more reliable in order to determine the associations between cancer and micro and macronutrients (85-87).

In chapter III, I describe in more detail the current evidence for the associations between blood levels of the glucose and lipid metabolism and prostate cancer.

2.2.4.5. Physical activity

Physical activity is associated with lower body fat and testosterone levels, and therefore in theory active men should present lower risk of having prostate cancer. However, studies results are not consistent and physical activity has been associated with decreased and similar prostate cancer risk compared to a sedentary lifestyle (64, 88). In a more recent systematic review and meta-analysis, 19 cohort studies and 24 case-control studies were compared to find a significant prostate cancer risk reduction for individuals between 20 and 45 years of age (RR: 0.93; 95% CI, 0.89-0.97) and between 45 and 65 years of age (RR: 0.91; 95% CI, 0.86-0.97) who performed activities as compared to those with limited physical activity, but not for individuals <20 years of age or >65 years of age. It was concluded that even though the risk reduction for prostate cancer may be small, the benefits of physical activity on other health related aspects are well known and thus should be encouraged (89).

2.2.4.6. Cigarette smoking and alcohol consumption

Cigarette smoking and alcohol consumption have been linked to several different types of cancers. Although evidence used to be less conclusive (90) in the last 10 years several studies and a meta-analysis have linked cigarette smoking to prostate cancer risk (91, 92). Additionally, cigarette smoking has been found to play a further role in prostate cancer progression. Men who continue to smoke after prostate cancer initial

treatment are more likely to present metastasis, biochemical recurrence or progression to castrate resistant prostate cancer than those that cease smoking (93).

Regarding alcohol consumption, previous studies used to show no association between this habit and prostate cancer (94, 95). However, more recently an increasing body of evidence, which has dealt with type of drinker misclassification bias (i.e., former drinkers, occasional drinkers), supports the hypothesis that alcohol may increase prostate cancer risk in a dose response manner (96).

2.2.4.7. Chemoprevention

2.2.4.7.1. 5-Alpha-reductase inhibitors (5-αRIs).

5-αRIs are used for the treatment of BPH and inhibit the conversion of testosterone to DHT. Currently there are two 5-αRIs: finasteride and dutasteride. Both drugs reduce the prostate size and levels of PSA (97) and have been assessed in clinical trials for their use as prostate cancer chemopreventive agents. The Prostate Cancer Prevention Trial (PCPT) evaluated finasteride; after 7 years of follow up those in the finasteride arm had a 25 % reduction in prostate cancer incidence (98).
Dutasteride was studied in the REduction by DUtasteride of prostate Cancer Events (REDUCE) trial. Those who received dutasteride had a 23 % reduction in prostate cancer incidence after four years of follow up (99).
However, 5-αRIs are more likely to prevent low-grade cancers with no risk reduction in GS ≥7. As previously mentioned, it is unlikely that low-grade cancers lead to prostate cancer mortality. It is unknown if this is a result of study design or a real consequence of 5-αRIs use. Nonetheless, in 2010, the US FDA Oncology Drugs Advisory Committee reviewed the data for finasteride and dutasteride and concluded that the risks of high-grade cancer were likely to be real and that the trials provided no evidence of a reduction in prostate cancer mortality (100).

2.2.4.7.2. Non-steroidal anti-inflammatory drugs

Experimental studies have shown that prostate cancer is linked to inflammation; regression of precancerous lesions occurred in animal models treated with non-

steroidal anti-inflammatory drugs (NSAIDs)(101). Furthermore, a meta-analysis of epidemiological studies showed that aspirin use was associated with a 17% reduction in prostate cancer risk. However, authors concluded that data was not conclusive and further well-designed observational studies with adequate exposure measurements, accurate case definition, attention to latency effects and careful adjustment for screening and other biases are needed (102) .

2.2.4.7.3. Statins

In a recent Nature review on the use of statins and prostate cancer prevention, it was concluded that although preclinical research shows that statins can inhibit prostate cancer growth and that results from more than thirty observational support the hypothesis that statin use reduces the risk of advanced prostate cancer and prostate cancer progression, so far no primary prevention trials have been performed to obtain the highest possible level of evidence for statins as prostate cancer chemopreventive agents. Furthermore, it was highlighted that the results of the observational studies may be biased by health-conscious behaviour. Therefore, considering that once advanced disease is diagnosed (either by higher GS or metastases), progression occurs in a short period of time, implementing secondary and tertiary prevention trials may be more feasible and could provide a better insight into the biological actions of statins in prostate cancer development (103).

2.2.5. Prostate cancer treatments

2.2.5.1. Deferred treatment: Active surveillance and watchful waiting

Active surveillance is the periodic monitoring of tumour parameters through repeated biopsies, DRE, PSA testing and image studies in men with confirmed low risk localised prostate cancer. It is intended to reduce overtreatment in those men whose tumour show no or very slow progression, allowing curative treatment when the parameters show increased tumour activity or when the patients require it for other reasons (i.e. anxiety). Inclusion criteria for active surveillance vary according to institutions or

regional guidelines, however the following characteristics are found in most protocols (104):

- *Clinically confined prostate cancer (T1–T2)*
- *Gleason score ≤6*
- *Three or fewer biopsies involved with cancer*
- *50% of each biopsy involved with cancer*
- *PSA <10 ng/ml*

Interestingly, men who fulfil the inclusion criteria for active surveillance after an initial biopsy are recommended to undergo a confirmatory biopsy, given that ~30% of Gleason scores at initial biopsies are upgraded at confirmatory biopsies (105). Indicators to switch to active treatment have also been debated. In the latest report of the Prostate Cancer Research International Active Surveillance (PRIAS) study, it was concluded that Gleason upgrading and cT3 should be the only indicators for an immediate change to curative treatment and that other indicators (e.g., fast-rising PSA) should indicate further investigation to confirm the suspicion of higher risk disease (106).

In 2016, results from the UK based Prostate Testing for Cancer and Treatment (ProtecT) trial were published. In this trial investigators looked at the effects of active monitoring, radical prostatectomy, and radical radiotherapy on prostate-cancer mortality at a median of 10 years of follow-up. Secondary outcomes included the rates of disease progression, metastases, and all-cause deaths. A total of 1,643 men were randomly assigned to active monitoring (n=545), radical prostatectomy (n=553), and radiotherapy (n=545). Regarding prostate cancer mortality, no significant difference was found among the three randomised groups: there were 17 prostate cancer specific deaths overall: 8 in the active monitoring group, 5 in the surgery group and 4 in the radiotherapy group. Disease progression occurred more in men in the active surveillance group, and no significant difference was observed for overall mortality. This study results, although not considered practice changing, have strongly

contributed to reassuring practitioners and patients that active surveillance as a valid form of treatment for men with low-risk prostate cancer (107).

Watchful waiting is another type of deferred treatment and consist on the observation of symptoms and palliative treatment in men diagnosed with prostate cancer who have a limited life expectancy due to other comorbidities or in older men with localized prostate cancer with less aggressive tumours (104).

2.2.5.2. Curative treatment: radical prostatectomy and radiotherapy

For men diagnosed with low risk prostate cancer, radical prostatectomy shows similar overall survival to conservative treatments (108). However, a study showed that for men with intermediate and high-risk disease, radical prostatectomy improved overall survival and reduced the risk of bone metastases (104). Furthermore, for intermediate and high-risk disease adjuvant hormonal and/or radiation treatment may be used. Depending on the T status from the TNM classification, surgeons may also perform a pelvic lymph node dissection. In most reference centres of developed countries, prostatectomies are being performed with the use of robots a modality known as robot-assisted laparoscopic radical prostatectomy (RALP) (104).

Common surgical side effects include:

- Urinary incontinence, erectile dysfunction
- Peripheral Neurological Injuries
- Bowel Complications
- Rectal, ureteral, bladder Injury
- Mortality
- Thromboembolic complications (109).

There are two main types of radiotherapy (RT) for prostate cancer: external beam radiation therapy (EBRT) and Brachytherapy. EBRT can be used in earlier stage cancers or to help relieve symptoms in more advanced stages. Subtypes of EBRT include:

- Three-dimensional conformal radiation therapy (3D-CRT)
- Intensity modulated radiation therapy (IMRT)

- Image guided radiation therapy (IGRT)
- Stereotactic body radiation therapy (SBRT) used for delivering large doses of radiation to a certain precise area, such as the prostate.
- Proton beam radiation therapy

Brachytherapy is usually applied in early stages of disease and consists of implanting small radioactive seeds or pellets in the prostate that will release radiation locally with little impact on surrounding tissues (110).

Common side effects for both types of RT include erectile dysfunction, urinary incontinence, radiation cystitis, bowel problems/discomfort, tiredness and lymphedema (111).

2.2.5.3. Hormonal treatment

Hormonal treatment blocks the endocrine loop that provides the prostate with androgens and is therefore also called ADT. It is considered a palliative therapy or adjuvant in combination with RP or RT in men with intermediate or high-risk prostate cancer. In addition to surgical castration (i.e. orchiectomy), there is also chemical castration, which can be achieved through several medications (15):

- Gonadotropin releasing hormone agonists (GnRH): These aim to reach medical castration. They are used early and late in the course of the disease. GnRH agonists bind to the GnRH receptors on pituitary gonadotropin-producing cells, which cause a temporary release of LH and FSH. However, after a few weeks desensitation occurs, meaning that the pituitary cells internalize the GnRH receptor reducing the synthesis of LH and FSH and eventually testosterone to castrate levels or below the castrate threshold (50 ng/dL) (112). Formulations include: leuprolide, triptorelin, goserelin, histrelin. Common side effects of GnRH agonists are hot flashes, decreased libido, anemia, gynecomastia, fatigue, headache, depression, changes in skin texture, and bone mineral depletion(113). Side effects more relevant to this thesis are obesity, impact on

cardiovascular diseases incidence and mortality, diabetes, and lipid profile alterations which will be described in chapter IV (109).

- GnRH antagonists: These drugs bind competitively to GnRH receptors, leading to an immediate inhibition of LH, FSH and testosterone synthesis. As a consequence no flare effect is observed. Furthermore, an extended follow-up study has been published suggesting better progression-free survival compared with monthly leuprolide (114). Moreover, in a study looking at cardiovascular events in men receiving either GnRH agonists or antagonists, it was found that antagonists halve the number of cardiac events experienced by men with preexisting cardiovascular disease during the first year of ADT (115).
- Antiandrogens: These competitively bind to the androgen receptor and inhibit its interaction with testosterone and dihydrotestosterone. There are two types of antiandrogens: the steroid antiandrogens and the non-steroidal antiandrogens (NSAA). The most used steroid antiandrogen is cyproterone acetate. These types of antiandrogens are weak partial agonists and competitive inhibitors of the androgen receptor. They also have progestational agonist actions that, by a negative feedback effect, lower LH secretion. Subsequently LH stimulated testosterone production decreases leading to the loss of libido, decreased sexual potency and low testosterone levels.
  The NSAA also block androgen receptor testosterone binding in the central nervous system interrupting the negative feedback of testosterone on gonadotropin secretion. Therefore, testosterone levels increase presenting less sexual side effects than the steroid antiandrogens. However, testosterone excess is converted into estrogens by aromatases and as a consequence gynecomastia and breast tenderness may occur. Common formulations are flutamide, nilutamide and bicalutamide.
  Antiandrogens are generally used in combination with a GnRH agonist, however in some countries they are used as monotherapy (116).
- Other hormones: Currently there is one medication that combines an alkylating agent to estradiol: Estramustine. Alkylating agents are compounds that introduce DNA changes (alkyl groups) that halt cell division and are used as treatment for different types of cancers (117).

Estrogens were the first drugs used to block the synthesis of LH and thus androgen concentrations in blood inducing tumour regression. However, due to adverse effects and the discovery of new drugs, their application as single therapy agents was discontinued (118). Nonetheless, in the last decades it has been observed that the main side effects of estrogens were due to liver metabolism of the drug and it can be avoided by parenteral administration (i.e. transdermal patches). As a result, estrogens are currently being studied in comparison to GnRH agonists in a clinical trial with primary outcomes overall survival and progression free survival (119).

2.2.5.4. Other prostate cancer drugs

For metastatic and castrate resistant prostate cancer (CRPC) several other drug combinations are used. Antimicrotubule drugs such as docetaxel and cabazitaxel have demonstrated improvements in overall survival in patients with very advanced disease. Another drug used for CRPC is Abiraterone acetate: a first-in-class inhibitor of cytochrome P-450c17, a critical enzyme in extragonadal and testicular androgen synthesis (120). Furthemore, Abiraterone has been found to reduce the risk of death when combined with ADT compared to ADT alone in metastatic hormone sensitive prostate cancer (121).

Enzalutamide is an androgen-receptor inhibitor that inhibits androgen-receptor translocation to the cell nucleus, recruitment of androgen-receptor cofactors, and androgen-receptor binding to DNA (122).These drugs can be used in combination with corticosteroids (prednisone, hydrocortisone, dexamethasone), which are used as palliative medication due to their anti-inflammatory and immune suppressive effects. Currently, in the Phase II/II Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy (STAMPEDE): A Multi-Stage Multi-Arm Randomised Controlled Trial, several combinations of these drugs are being evaluated in the treatment of advanced or metastatic prostate cancer with overall survival as the primary outcome. Some of the treatment arms are still recruiting and the reference group is treated with ADT (plus radiotherapy for newly-diagnosed non-metastatic disease) (123).

Monoclonal antibodies (denosumab) are being used to prevent skeletal related injuries due to bone metastasis. This antibody binds to the receptor activator of nuclear factor kappa B ligand (RANKL), which acts as the primary signal to promote bone removal (15). Sipuleucel-T is a vaccine containing activated antigen-presenting cells from the patient that is approved by the FDA for treatment of CRPC (124).

2.3. Disease and treatment related clinical outcomes for men with prostate cancer

The following section of this chapter provides a background for the four projects conducted as part of this PhD – and thus specifically focuses on clinical outcomes for men already diagnosed with prostate cancer. Further details on the rationale for each project are also provided in the following chapters.

2.3.1. Serum biomarkers and secondly diagnosed primary tumours in men with prostate cancer

One of the biggest fears cancer survivors face is the diagnosis of a new cancer. In the United States of America Surveillance, Epidemiology and End Results program (SEER) second primary tumours (SPTs) or subsequent cancers represent 16% of incident cancers (125).

Several studies have specifically focused on prostate cancer and the risk of SPTs (126-128). For instance, it has been shown in a cohort of American men, that those men with prostate cancer who underwent orchiectomy had a 40% higher risk of developing colorectal cancer (129). Moreover, it has been reported that men with prostate cancer show a higher risk of developing bladder cancer, although this finding may be due to detection bias (130). A study performed using a Swiss cohort concluded that the overall risk of SPTs is higher in prostate cancer men, and this risk was even higher after 10 years of receiving radiotherapy (131).

Smoking, obesity and insulin resistance are some of the well-known risk factors for the development of first primary tumours (132-137). Nonetheless, whether these risk factors can increase the risk of developing SPTs is less clear. Evidence indicates that SPTs may be the result of genetic and hormonal risk factors (138-140), as well as late effects of chemo and radiotherapy (125, 141). In the context of prostate cancer, it has been suggested that the IGF-1 could play a role in the development of secondary malignancies given that IGF-1 has been associated with first primary prostate cancer

(142, 143). Even though epidemiological evidence observes an association between prostate cancer and SPTs, whether there are convergent biological pathways remains unknown. Thus, it is important to further investigate aetiological influences that increase risk of SPTs, so that we can better define high-risk groups for targeted preventive and interventional clinical strategies (144).

As part of this PhD project I aimed to investigate how serum biomarkers of the glucose, liver and lipid metabolism measured before prostate cancer diagnosis are associated with patterns of SPTs. For instance, there has been growing evidence for a role of the glucose and lipid metabolism in tumour development. ROS are examples of mechanisms through which these metabolic abnormalities might be connected to cancer risk (145). Diabetes and its treatment, as well as serum levels of glucose, are the most commonly used markers of the glucose metabolism in the context of cancer (146). Another possible link between oxidative stress and cancer development is suggested in recently published epidemiological studies that showed a link between gamma-glutamyltransferase (GGT), a metabolic marker of liver dysfunction, and cancer risk (147). GGT is a central enzyme of the metabolism of glutathione, which plays an important role in maintaining tissue oxidant/antioxidant balance, cellular defence, proliferation, and death (147). Increased levels of GGT appear to reflect high levels of glutathione turnover in response to intracellular oxidative stress. The persistent production of ROS as a result of increased GGT expression in tumour cells may contribute to genetic instability and to tumour progression (148). Also, inflammatory markers are suggested to be linked to cancer development through increased oxidative stress (149), which may potentially link dyslipidemia to carcinogenesis. Dyslipidemia is associated with a state of low-grade chronic inflammation, infiltrating macrophages within adipose tissue, and elevated concentrations of pro-inflammatory molecules (150). In addition, there has been consistent evidence of lipid oxidation (of low-density lipoprotein phospholipids) playing a role in many metabolic disorders, including obesity and several forms of cancer (150).

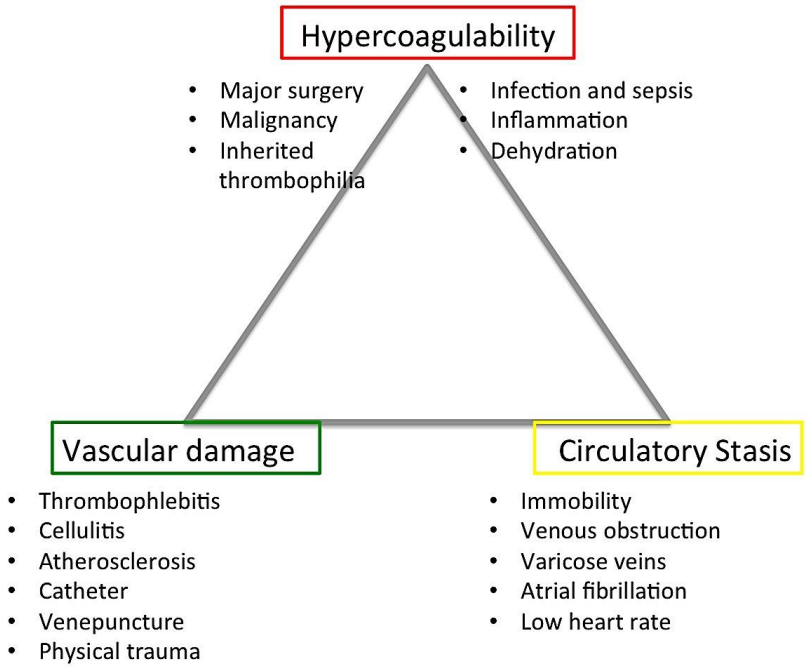
Hence, the first project of this PhD project (Chapter III) aimed to increase our understanding of possible indicators for cancer prevention in men with prostate cancer.

2.3.2. Radiotherapy and the risk of thromboembolic disease

Thromboembolic disease (TED) is a condition in which a thrombus either arises spontaneously or is caused by clinical conditions as listed below. Venous thrombi usually form in the valve pockets of the legs veins, where there is a low blood flow and are mainly made of fibrin, red blood cells and platelets. Deep venous thrombosis (DVT) and pulmonary embolism (PE) are TED manifestations (151).

In 1884, Rudolph Virchow proposed that thrombosis was the result of the presentation of one or more of the following conditions: vascular endothelial damage, stasis of blood flow and hypercoagulability of blood. Through time, it was found that these conditions were present in cancer, prolonged periods of immobilisation, major surgery, hip fracture, spinal cord injury, prior thromboembolic events, hereditary coagulopathies amongst others as listed in figure 10 (152). In the last decades, metabolic syndrome has also been found to be a risk factor for TED, as several of its components contribute to the Virchow's triad (153-155).

Figure 10 Virchow's triad of risk factors for venous thromboembolism (adapted from (156))



Even though RT has been associated with an increased risk of TED for other cancers (i.e. lung, uterus), no studies have been published on this association for prostate cancer (157, 158). It is thus not known whether RT is a risk factor for TED or whether TED is a side effect of RT. However, it is of interest to investigate this link, as there are biological findings that suggest a possible association.

It has, for instance, been reported that RT can induce structural changes in arteries in patients who have previously undergone RT for lymphoma, breast cancer, and head and neck cancer (159). Furthermore, according to experimental studies, RT can activate nuclear factors that promote inflammation status in the vascular wall cells, a key step in in the coagulation cascade (160).

Based on the above, the aim of the second project in this PhD (Chapter IV) was to quantify the risk of TED after RT for prostate cancer.

2.3.3. Drugs for metabolic conditions and prostate cancer death in men on GnRH agonists

The risk of type 2 diabetes, as well as other components of the metabolic syndrome (MetS), following initiation ADT for prostate cancer, has been studied and analysed in several cohorts. Overall, it is thought that ADT is associated with an increased risk of diabetes as well as MetS (161). Several definitions have been proposed for MetS. In general, the following symptoms are involved: central obesity, raised triglycerides or specific treatment for this lipid abnormality, reduced high-density lipoprotein (HDL) cholesterol or specific treatment for this lipid abnormality, raised blood pressure or treatment of previously diagnosed hypertension, or raised fasting plasma glucose (162).

However, less attention has been given to how being diagnosed and getting treatment for MetS components before prostate cancer diagnosis may also affect ADT response in terms of prostate cancer survival. Only two recent studies have investigated this scenario, with one suggesting that having metabolic syndrome at time of ADT initiation may shorten time to castrate resistant prostate cancer and overall survival (163). The other study found no statistically significant associations between baseline metabolic syndrome or its components and risk of prostate cancer specific death (164).

GnRH agonists aim to deprive prostate cancer cells from the growth stimuli provided by testosterone. However, it has been established that not all prostate cancer cells need testosterone to grow and survive; moreover those known as castrate resistant cancers are more aggressive and progress more rapidly. Metabolic syndrome and its individual components are associated with low circulating testosterone levels (165). For instance, in the EPIC study men with diabetes had 26% lower risk of prostate cancer and lower levels of circulating concentrations of androstenedione, total testosterone and insulin-like growth factor binding protein-three than those who did not have diabetes (166). This could thus potentially explain why men who present with metabolic syndrome at time of ADT initiation may do worse.

To further evaluate how metabolic components and their related drugs have an impact on prostate cancer death, the third study of this PhD project aimed to evaluate how a variety of treated metabolic syndrome components diagnosed before prostate cancer diagnosis are associated with prostate cancer death in men who started on primary ADT.

2.3.4. Anti-androgens versus GnRH agonists in relation to prostate cancer death

In Europe, both GnRH agonists and AAs are currently approved as ADT monotherapies for high-risk locally advanced prostate cancer (167). However, the European Urology Association (EUA) does not recommend the use of AA as monotherapy based on the results of a 2014 Cochrane systematic review and meta-analysis (17). Nonetheless, in the Cochrane review it was shown in the subgroup analysis of non-metastatic disease that there was no significant difference between AA and castration in terms of overall survival (168). Two previous RCTs looking at AA vs. GnRH agonists and overall survival and prostate cancer specific survival had similar results to the subgroup analysis of the Cochrane review (169, 170). Yet, more recently it has been accepted that "real-world" data is more likely to better reflect clinical practice experiences rather than RCTs in which patients are highly selected.

Therefore, considering that GnRH agonists are associated with a number of negative metabolic effects including bone loss with increased risk of fractures, increased risk of cardiovascular disease, diabetes mellitus type 2 and possibly dementia (171-174) and in order to assess the external validity of these early RCTs, I performed a nationwide, population-based observational study on AA monotherapy versus GnRH agonists and overall survival and prostate cancer specific survival.

3. Chapter III: Disease-related outcomes

3.1. Serum biomarkers and secondly diagnosed primary tumours in men with prostate cancer- results from the AMORIS database

The findings of this study were presented as an abstract at the NCRI Conference in 2015 (Appendix I). The manuscript is currently under review with BMC Cancer.

3.1.1. Rationale

As already mentioned, due to advances in detection and treatment, the prevalence of prostate cancer is increasing (125). These trends translate into more men living longer with prostate cancer diagnosis and consequently being at risk of getting diagnosed with a second primary tumour. Travis *et al*. suggested grouping of second primary malignancies based on leading etiological causes: treatment-related, syndromic, and those due to shared etiologic factors (125). Most studies to date have focused on primary cancer treatment related second primary malignancies (175, 176) and little is known about other causes of these tumours. However, it was recently suggested that radiotherapy is only responsible for <10% of second primary malignancies, implying a larger role for lifestyle, genetic or biochemical related factors in their development (177). There are several definitions for second primary malignancies, and although most definitions contemplate the fact that it cannot be a metastasis of a first primary tumour, there is an implicit notion that second primary malignancies must have started developing after the first primaries. This implicit notion may be accurate for those second malignancies in which the lag time between treatment of the first primary tumour or long-term exposure to certain factors has taken place before the occurrence of the second primary malignancy. However, for those tumours diagnosed within a relatively short period of time after diagnosis of first primary malignancies, these factors are less likely to play a role in the development of a second primary malignancy. Here, I refer to second primary tumours as those primary tumours (non-metastatic) of any organ/tissue diagnosed after diagnosis of prostate cancer. “Second” does not refer to when the tumour started developing, it refers to when it was diagnosed in relation to prostate cancer diagnosis. Therefore, to emphasize this

concept, below I will refer to these tumours as “secondly diagnosed primary tumours” (SDPTs).

Abnormal metabolism in cancer cells has been well studied and continues to be of high interest as a potential target for drug development (178). It is therefore of interest to investigate serum biomarkers measured before a first primary cancer as these could activate mechanisms leading to other cancer development or be a consequence of common alterations that will lead to a second cancer (179).

Studies based on the Swedish AMORIS study have reported that the interplay between serum lipids, glucose and gamma-glutamyl transferase (GGT) increases the risk of prostate cancer (180-182). Since cancer development following exposure to risk factors may take several decades (183), we hypothesized that these elevated serum levels of glucose, total cholesterol, triglycerides, fructosamine and GGT measured before prostate cancer diagnosis may also be associated with development of SDPTs – either because these biomarkers activate a shared carcinogenic mechanism or because they are the consequence of a common underlying alteration (179, 184, 185) . I therefore conducted a hypothesis-generating study to evaluate how serum biomarkers of common metabolisms are associated with development of prostate cancer and subsequent other cancers.

3.1.2. Methods

3.1.2.1. Data source: AMORIS

The AMORIS (Apolipoprotein-related MOrtality RISk) cohort has been described in detail elsewhere (52, 186-188). During the period 1985–96, the Central Automation Laboratory (CALAB), Stockholm, Sweden was a leading centre for analyses of blood and urine samples from health screenings and primary health care in Sweden. The AMORIS cohort was initiated by Ingmar Jungner, one of the founders of CALAB, and Göran Walldius, the medical advisor. This AMORIS cohort was originally set up to test if levels of apolipoprotein (apo) B (atherogenic) and apoA-I (atheroprotective) were more closely related to fatal myocardial infarction and stroke than conventional lipids,

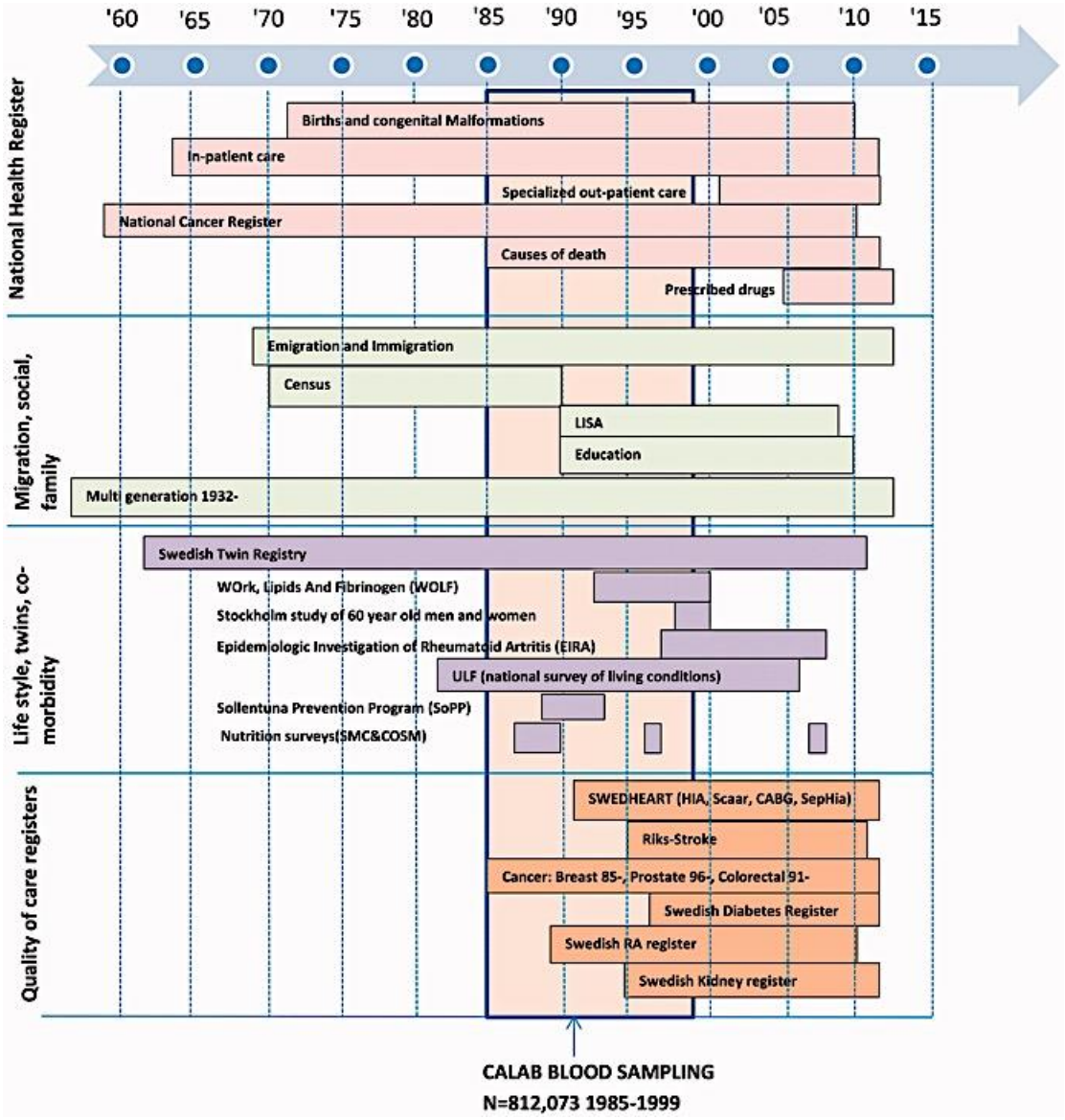
especially low-density lipoprotein (LDL)-cholesterol. In 2012, Ingmar Jungner donated the CALAB database to the Karolinska Institutet, Stockholm, Sweden, for research purposes in general and in the short term to support the on-going research project 'Epidemiologic studies of metabolic factors and inflammation in relation to chronic diseases'. This database includes 812,073 Swedish men and women with blood and/or urine samples. Individuals recruited were either healthy and having laboratory testing as part of a general check-up or outpatients referred for laboratory testing. None of the participants were inpatients at the time of sampling (189, 190). The CALAB database was further updated in 2012–14 in connection with the above-mentioned research project by record linkages to 24 Swedish national health registers, quality of care registers and research cohorts. This included the Swedish National Cancer Register, the Hospital Discharge Register, the Cause of Death Register, the Swedish Prostate Cancer Register, the consecutive Swedish Censuses during 1970–1990, and the National Register of Emigration using the Swedish 10-digit personal identity number (these registers are further described in chapter IV) (191-193) (Figure 11). Previous studies based on the AMORIS cohort focusing on prostate cancer can be found in Table 7.

Table 7. AMORIS studies focused on prostate cancer

Author	Cohort	Biomarkers	Results
Van Hemelrijck et al.[194]	69,735	LDL, HDL, ApoB, ApoA-I	Low HDL and ApoA-I and increased lipid ratios are associated with higher risk of prostate cancer
Van Hemelrijck et al.[195]	196,022	Calcium, Albumin	Weak negative association between Calcium and prostate cancer risk likely due competing risks
Van Hemelrijck et al.[149]	34,891	C-Reactive Protein, Haptoglobin, Albumin, Leukocytes	No association between inflammatory markers and prostate cancer
Van Hemelrijck et al.[187]	200,660	TG,TC, Glucose	Negative association between glucose and prostate cancer risk for the second, third, and fourth quartiles compared with the first. Positive association between hypertriglyceridemia and prostate cancer risk, in combination with high glucose levels. No association was found for hypercholesterolemia.

LDL: Low density lipoprotein, HDL: High-density lipoprotein, ApoA: Apolipoprotein A, ApoB: Apolipoprotein B, TG: triglycerides, TC: total cholesterol.

Figure 11 The AMORIS cohort linkages to national health registers, quality of care registers and research cohorts in 2012-14.



3.1.2.2. Study population

From the AMORIS cohort, I selected all men diagnosed with prostate cancer that had at least one of the five biomarkers of interest (glucose, total cholesterol, triglycerides, fructosamine and GGT) measured before prostate cancer diagnosis. To obtain information on stage and treatment of prostate cancer, I used a linkage of the AMORIS cohort with the National Prostate Cancer Register (NPCR), resulting in a total of 14,021 prostate cancer cases (54). As carcinogenesis may initiate several years before diagnosis, I divided the time between blood measurement and prostate cancer diagnosis into five periods to exhibit this potential lag time: 0-5, 5-10, 10-15, 15-20 years and more than 20 years before prostate cancer diagnosis. For the current study, I focused on those measurements taken 10-15 and 15-20 years prior to prostate cancer diagnosis (n= 10,791 cases) (Figure 12). Excluding the most recent periods limits the potential effects of reverse causation. Moreover, prostate cancer is known to have a long natural history (196). If men had more than one measurement taken within the period studied, the measurement closest to the mid-point of the interval was selected. Follow-up started at the time of prostate cancer diagnosis and ended at time of occurrence of a SDPT, emigration, death or end of the study (December 31st 2011), whichever came first.

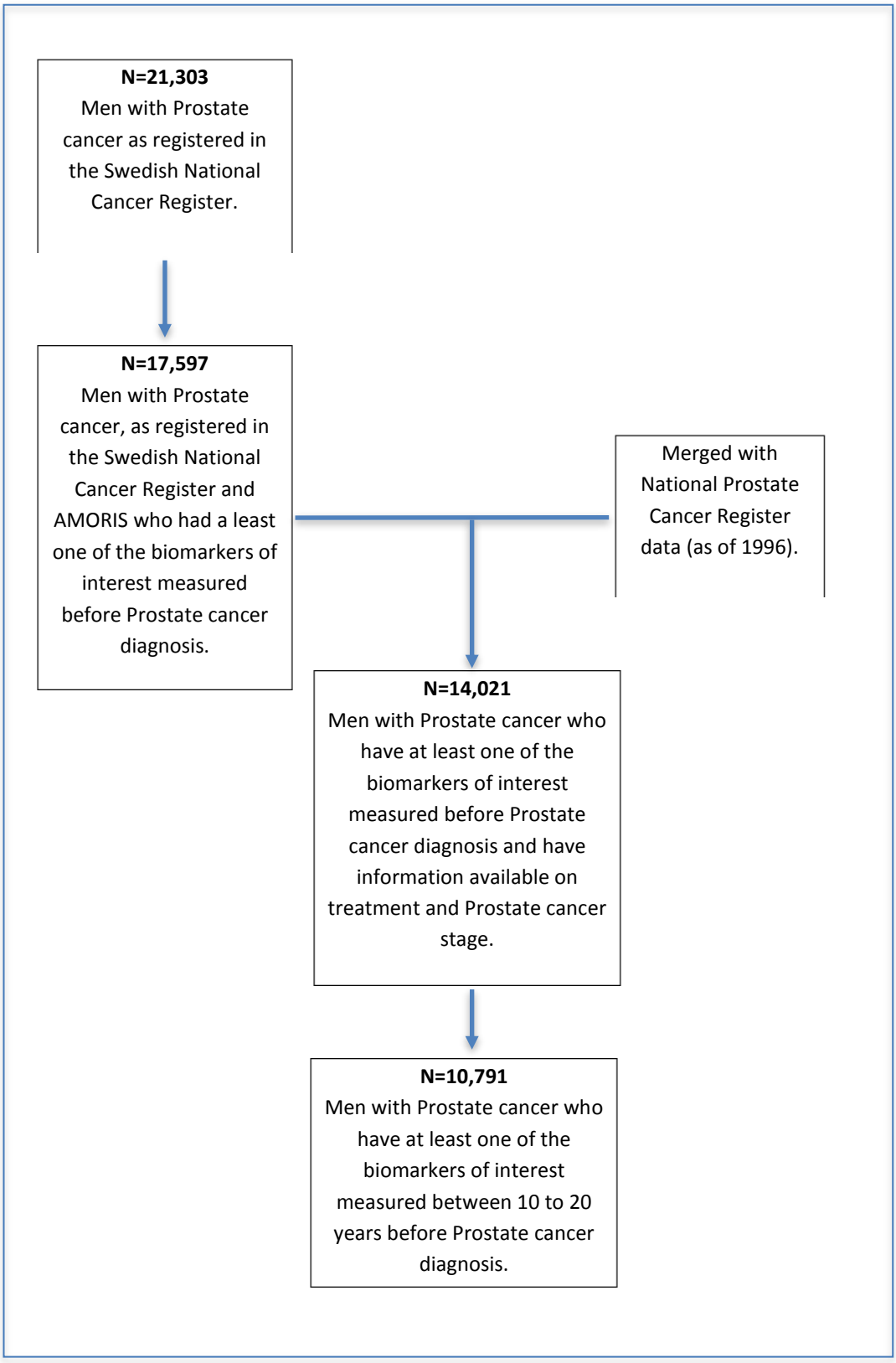
Information on educational level was retrieved from the Population and Housing Census. Using information from the National Patient Register, I calculated a baseline Charlson comorbidity index (CCI), which includes 19 diseases, with each disease category assigned a weight. The sum of an individual's weights was used to create a score, resulting in four comorbidity levels ranging from no comorbidity to severe comorbidity (0, 1, 2, and ≥3) (197).

3.1.2.3. Exposure variables

The main exposure variables of interest were the above-mentioned five biomarkers. I used both quartiles and medical cut-offs for the analyses. Medical cut-offs for glucose (6.11 mmol/L), total cholesterol (6.5 mmol/L) and triglycerides (1.71 mmol/L) were based on the National Cholesterol Education Program guidelines (198). Fructosamine and GGT have less consistently established clinical cut-off levels, therefore these were

defined based on the laboratory cut-offs used by CALAB which have also been applied in other recent studies (2.5 mmol/L and 36 IU/L respectively) (181, 199).

Figure 12 Selection of men with Prostate cancer from the AMORIS cohort to study the association between metabolism markers and risk of Second Primary Tumours



Although these biomarker measurements were part of regular health check-up and missing data could therefore be considered missing at random, I used the most general approach to the problem of missing data: multiple imputation. More specifically, I applied multivariate imputation using chained equations (MICE), also known as imputation using fully conditional specifications (200). The MICE method imputes multiple variables sequentially using univariate fully conditional specifications.

Glucose was measured enzymatically with a glucoseoxidase/peroxidase method. Total cholesterol was determined with the cholesterol oxidase/peroxidase assay and triglycerides with the glycerol phosphate oxidase/peroxidase assay (191, 192). GGT levels measurement was performed with an enzymatic colorimetric test using L-c-glutamyl-3-carboxy-4-nitroanilide as donor substrate at a temperature of 37^0C, which is the reference method recommended by the International Federation of Clinical Chemistry and Laboratory Medicine (201). Fructosamine was measured by the Nitroblue Teterazolium colorimetric technique based on the reducing ability of fructosamine in an alkaline solution (202).

All methods were fully automated with automatic calibration and performed at one and the same accredited laboratory (CALAB) (191).

3.1.2.4. Outcome definition

The main outcome of interest was the occurrence of cancer diagnosed after prostate cancer diagnosis. SDPTs were defined as any non-benign and non-metastatic tumour, and grouped according to the International Classification of Diseases 7th revision (ICD7) codes (203) which are the codes used to enter the data in the register. Based on previous evidence (204), rectal SDPTs were grouped together with genitourinary tumours for anatomic reasons to account for possible effects of radiotherapy and health seeker bias (as with urologic cancers) in prostate cancer.

3.1.2.5. Statistical Analysis

Baseline cohort characteristics were compared using descriptive statistics (Student's t-test, Wilcoxon Rank Sum test, and Chi squared test). Multivariate Cox proportional hazards regression analysis with age as a timescale was used to determine hazard ratios (HR) and 95% confidence intervals (95% CI) of risk of SDPTs. I adjusted for the remaining biomarkers, fasting status, prostate cancer treatment, CCI at prostate cancer diagnosis, diabetes mellitus at prostate cancer diagnosis, time between blood test and prostate cancer diagnosis and education. Even though information on grade and treatment were both available, I only adjusted for cancer treatment to avoid collinearity – treatment is a well-accepted indication of disease severity (205).

Furthermore, I ran an analysis according to the type of SDPT for those cancer groups that had at least 60 events leaving out other types of cancers (i.e. haematological). The assumption of proportionality of the Cox model covariates was tested by plotting Schoenfeld residuals on several of the imputed datasets (206). To address the potential effects of prostate cancer treatment I performed both a stratified analysis as well as an additional adjustment for prostate cancer treatment.

The study complied with the Declaration of Helsinki and was approved by the Ethics Review Board of the Karolinska Institutet (Dnr 2010/1:7).

Statistical Analysis Software (SAS) release 9.4 (SAS Institute, Cary, NC) was used for data management; Stata Statistical Software: Release 14 (College Station, TX: StataCorp LP) was used for imputation and data analysis.

3.1.3. Results

Study population baseline characteristics for prostate cancer cases with and without a SDPT are described in Table 7. A total of 811 SDPTs (7.5 %) were diagnosed during a mean follow-up time of 4.98 (SD: 3.36) years.

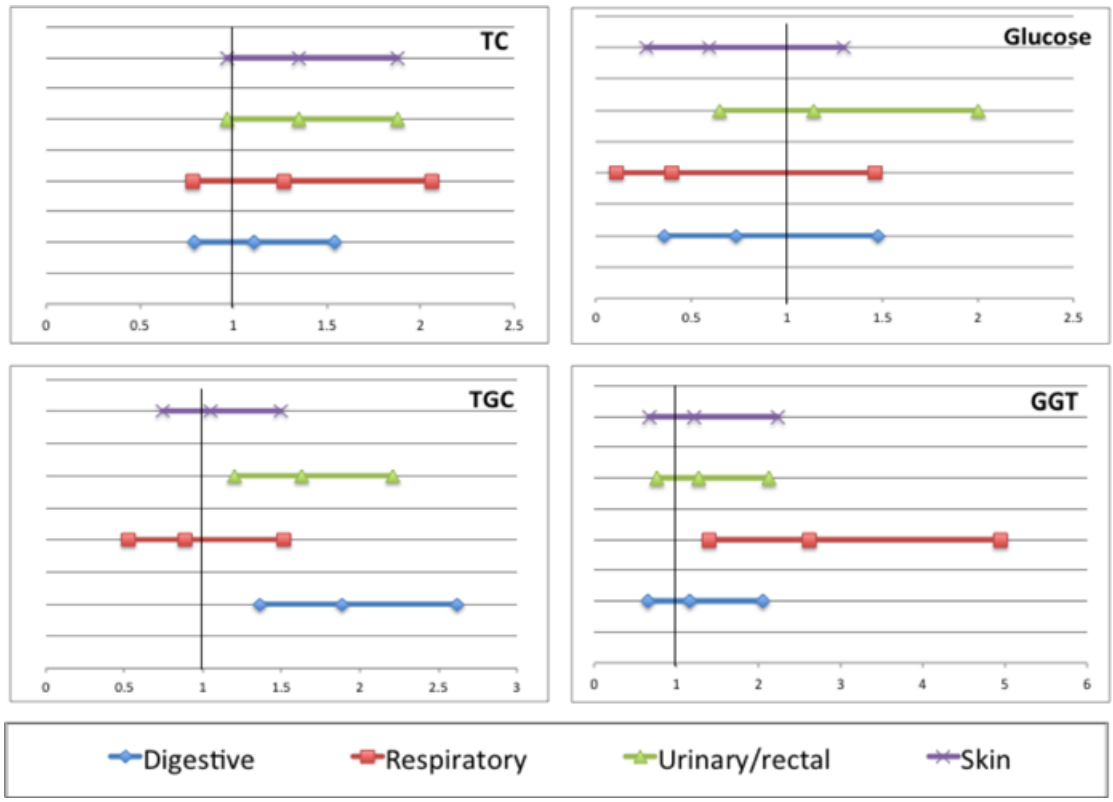
Multivariate analysis including all biomarkers studied showed a higher risk of SDPTs for those with high serum levels (based on the above established clinical cut-offs) of

triglycerides (HR: 1.37, 95%CI: 1.17-1.60), total cholesterol (HR: 1.22, 95%CI: 1.04-1.42) and GGT (HR: 1.32, 95%CI: 1.02-1.71), as compared to the normal levels (Table 9). When looking at quartiles, only those in the 4th quartile of triglycerides were at higher risk of SDPTs, as compared to the first quartile. A weaker positive association with risk of developing a SDPT was also observed for those in the 4th quartiles of total cholesterol and GGT, as compared to the first quartile (table 9).

Cancer treatment stratified analysis did not substantially change these associations (table 10).

The risk of SDPTs of digestive organs, peritoneum, genitourinary and rectum was also higher for those with elevated blood levels of triglycerides. Furthermore, high levels of GGT were associated with SDPTs of the respiratory system. Total cholesterol levels were borderline significant for the risk of skin and genitourinary and rectum SDPTs (Figure 13).

*Figure 13 Hazard ratios and 95%CI (X-axis) for risk of specific types of SDPTS by levels of total cholesterol, glucose, triglycerides, and GGT based on their medical cut-off**



*6.5 mmol/L, 6.11 mmol/L, 1.71 mmol/L and 36 IU/L respectively. All models were adjusted for Education, diabetes mellitus at Prostate cancer diagnosis, age, CCI, Fasting Status, time between date of blood test and Prostate cancer diagnosis date, Prostate cancer treatment: Hormonal, Radiotherapy, Radical prostatectomy.

Table 8. Baseline characteristics of study population of men diagnosed with prostate cancer in the Swedish AMORIS cohort.

	SDPTs n=811	%	No-SDPTs n=9983	%
Age				
Mean (SD)	69.83 (7.07)		68.06 (7.89)	
<63	133	16.40	2693	26.98
63-67.99	191	23.55	2417	24.21
68-73.99	253	31.20	2542	25.46
>74	234	28.85	2331	23.35
Education				
High	226	27.87	3132	31.37
Middle	352	43.40	4088	40.95
Low/no	222	27.37	2619	26.23
Missing data	11	1.36	144	1.44
Glucose (mmol/L)				
Mean (SD)	5.10 (1.28)		5.09 (1.19)	
>6.11	54	6.66	661	6.62
<6.11	724	89.27	8672	86.87
Missing data	33	4.07	650	6.51
Triglycerides (mmol/L)				
Mean (SD)	1.64 (1.26)		1.53 (1.06)	
>1.71	270	33.29	2751	27.56
<1.71	530	65.35	7028	70.40
Missing data	11	1.36	204	2.04
Cholesterol (mmol/L)				
Mean (SD)	6 (1.02)		5.91 (1.05)	
>6.5	275	33.91	2797	28.02
<6.5	526	64.86	7016	70.28
Missing data	10	1.23	170	1.70
Fructosamine (mmol/L)				
Mean (SD)	2.11 (0.32)		2.11 (0.26)	
>2.5	31	3.82	296	2.97
<2.5	575	70.90	7307	73.19
Missing data	205	25.28	2380	23.84
GGT (IU/L)				
Mean (SD)	36.98 (97.36)		32.30 (35.29)	
>36	69	8.51	701	7.02
<36	688	84.83	8424	84.38
Missing data	54	6.66	858	8.59
CCI at PCa diagnosis				
0	606	74.72	7705	77.18
1	131	16.15	1451	14.53
2	45	5.55	466	4.67
3+	29	3.58	361	3.62
Diabetes at PCa diagnosis				
Yes	42	5.18	418	4.19
No	769	94.82	9565	95.81

FUT	3.77 (2.83)		5.28 (3.22)	
Treatment				
RT	101	12.45	1040	10.42
RP	175	21.58	2936	29.41
HT	206	25.40	2211	22.15
DT (AS WW)	231	28.48	2332	23.36
Unspecified	55	6.78	823	8.24
Missing data	43	5.30	641	6.42
Stage group				
Low risk	205	25.28	3140	31.45
Intermediate risk	251	30.95	2791	27.96
High risk	225	27.74	2220	22.24
Regionally metastatic	41	5.06	567	5.68
Distant metastases	59	7.27	928	9.30
Missing data	30	3.70	336	3.37

Abbreviations: GGT: gamma glutamyl transferase, Prostate cancer: prostate cancer, SDPTs: secondly diagnosed primary tumours, CCI: Charlson comorbidity index, FUT: follow up time; RT: Radiotherapy, RP: radical prostatectomy, HT: hormonal treatment, DT: deferred treatment

Table 9. Univariate and multivariate Cox proportional hazards regression analysis for risk of SDPTs by levels of serum biomarkers (Glucose, triglycerides, total cholesterol, GGT, and fructosamine).

		Univariate		Multivariate		p-value/trend
	Variables	**HR**	**95%CI**	**HR**	**95%CI**	
Clinical cut-offs	Glucose (6.11 mmol/L)	1.11	0.84-1.46	0.87	0.64-1.19	0.42
	TG (1.71 mmol/L)	1.37	1.18-1.58	1.37	1.17-1.6	<0.001
	TC (6.5 mmol/L)	1.33	1.15-1.54	1.22	1.04-1.42	0.01
	GGT (36 IU/L)	1.37	1.07-1.76	1.32	1.02-1.71	0.02
	FAMN (2.5 mmol/L)	1.27	0.88-1.82	0.91	0.59-1.39	0.64
Quartiles	Gluc-q1(<4.6mmol/L)	1	ref	1	ref	0.56
	Gluc-q2 (4.6-5mmol/L)	1.29	1.06-1.57	1.25	1.03-1.52	
	Gluc-q3 (5-5.4mmol/L)	1.09	0.89-1.33	1.01	0.83-1.24	
	Gluc-q4(>5.4mmol/L)	1.15	0.95-1.39	0.98	0.8-1.19	
	TC-q1(<5.2mmol/L)	1	ref	1	ref	0.09
	TC-q2(5.2-5.8 mmol/L)	1.1	0.88-1.36	1.05	0.84-1.3	
	TC-q3(5.8-6.6 mmol/L)	1.06	0.87-1.3	0.98	0.8-1.49	
	TC-q4(>6.6 mmol/L)	1.38	1.14-1.67	1.21	0.99-1.49	
	Tg-q1 (<0.9 mmol/L)	1	ref	1	ref	0.004
	Tg-q2 (0.9-1.3 mmol/L)	0.95	0.76-1.18	0.94	0.75-1.17	
	Tg-q3(1.3-1.9 mmol/L)	1.11	0.91-1.34	1.08	0.88-1.31	
	Tg-q4(>1.9 mmol/L)	1.35	1.12-1.62	1.32	1.08-1.61	
	Ggt-q1(<16.79 IU/L)	1	ref	1	ref	0.27
	Ggt-q2(16.79-23.39 IU/L)	1.07	0.87-1.32	1.05	0.86-1.29	
	Ggt-q3 (23.39-35.99 IU/L)	1.05	0.86-1.29	0.99	0.8-1.22	
	Ggt-q4 (>35.99)	1.24	1.01-1.52	1.15	0.93-1.42	
	Famn-q1 (<2 mmol/L)	1	ref	1	ref	0.26
	Famn-q2 (2-2.11 mmol/L)	0.88	0.69-1.11	0.84	0.66-1.07	
	Famn-q3 (2.11-2.25 [illegible])	0.97	0.79-1.21	0.91	0.73-1.14	
	Famn-q4 (>2.25 mmol/L)	0.99	0.8-1.24	0.85	0.67-1.07	

Abbreviations: Gluc: glucose, TC: total cholesterol, TG: triglycerides, GGT: gamma glutamyl transferase, FAMN: fructosamine, Prostate cancer: prostate cancer, SDPTs: secondly diagnosed primary tumours, HR: Hazard ratios, CI: Confidence intervals

**Adjusted for: Education, diabetes mellitus at Prostate cancer diagnosis, age, CCI, Fasting Status, time between date of blood test and Prostate cancer diagnosis date, Prostate cancer treatment: Hormonal, Radiotherapy, Radical prostatectomy

Table 10. Treatment stratified analysis according to biomarkers blood levels clinical cut offs and quartiles

		RT		RP		HT		DT	
	Variables	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Clinical cut offs	Glucose (6.11 mmol/L)	0,64	(0.24-1.72)	1,16	(0.56-2.41)	1,06	(0.6-1.88)	1,62	(1.01-2.6)
	TG (1.71 mmol/L)	1,46	(0.91-2.33)	1,14	(0.78-1.66)	1,02	(0.71-1.46)	1,64	(1.2-2.25)
	TC (6.5 mmol/L)	1,71	(1.08-2.7)	1,31	(0.90-1.91)	1,31	(0.94-1.83)	1,17	(0.86-1.6)
	GGT (36 IU/L)	1,20	(0.64-2.27)	1,70	(1.05-2.75)	1,32	(0.81-2.15)	0,99	(0.62-1.57)
	FAMN (2.5 mmol/L)	1,27	(0.47-3.4)	0,75	(0.29-1.96)	0,68	(2.73-1.69)	0,57	(0.23-1.42)
Quartiles	Gluc-q1(<4.6mmol/L)	ref	ref	ref	ref	ref	ref	ref	ref
	Gluc-q2 (4.6-5mmol/L)	1,38	(0.76-2.49)	1,59	(1.02-2.48)	1,10	(0.72-1.69)	0,88	(0.58-1.33)
	Gluc-q3 (5-5.4mmol/L)	1,09	(0.58-2.04)	1,08	(0.66-1.75)	0,81	(0.52-1.27)	0,99	(0.68-1.46)
	Gluc-q4(>5.4mmol/L)	1,27	(0.69-2.33)	1,64	(1.03-2.61)	1,15	(0.76-1.74)	1,05	(0.72-1.53)
	TC-q1(<5.2mmol/L)	ref	ref	ref	ref	ref	ref	ref	ref
	TC-q2(5.2-5.8 mmol/L)	1,01	(0.48-2.12)	0,95	(0.57-1.59)	0,82	(0.53-1.28)	0,84	(0.55-1.3)
	TC-q3(5.8-6.6 mmol/L)	1,42	(0.74-2.71)	1,12	(0.7-1.79)	0,52	(0.33-0.81)	1,07	(0.74-1.55)
	TC-q4(>6.6 mmol/L)	1,85	(0.96-3.58)	1,44	(0.89-2.32)	1,07	(0.71-1.61)	1,07	(0.71-1.6)
	Tg-q1 (<0.9 mmol/L)	ref	ref	ref	ref	ref	ref	ref	ref
	Tg-q2 (0.9-1.3 mmol/L)	0,89	(0.44-1.8)	1,01	(0.62-1.66)	0,97	(0.61-1.53)	0,86	(0.55-1.36)
	Tg-q3(1.3-1.9 mmol/L)	0,72	(0.39-1.36)	0,98	(0.61-1.55)	1,19	(0.78-1.81)	1,08	(0.73-1.59)
	Tg-q4(>1.9 mmol/L)	1,17	(0.65-2.2)	1,16	(0.73-1.85)	0,99	(0.62-1.58)	1,76	(1.19-2.61)
	Ggt-q1(<16.79 IU/L)	ref	ref	ref	ref	ref	ref	ref	ref
	Ggt-q2(16.79-23.39 IU/L)	0,85	(0.43-1.69)	1,44	(0.91-2.26)	1,05	(0.68-1.62)	0,93	(0.64-1.36)
	Ggt-q3 (23.39-35.99 IU/L)	1,55	(0.85-2.84)	1,02	(0.62-1.68)	1,18	(0.77-1.8)	0,70	(0.47-1.06)
	Ggt-q4 (>35.99)	1,36	(0.74-2.49)	1,24	(0.77-2.02)	0,96	(0.61-1.52)	0,96	(0.64-1.43)
	Famn-q1 (<2 mmol/L)	ref	ref	ref	ref	ref	ref	ref	ref
	Famn-q2 (2-2.11 mmol/L)	0,84	(0.45-1.56)	0,81	(0.52-1.27)	0,86	(0.55-1.33)	0,88	(0.59-1.29)
	Famn-q3 (2.11-2.25 mmol/L)	0,95	(0.51-1.74)	0,59	(0.37-0.94)	1,02	(0.67-1.53)	0,85	(0.58-1.25)
	Famn-q4 (>2.25 mmol/L)	0,80	(0.42-1.49)	0,56	(0.34-0.90)	0,80	(0.5-1.27)	0,80	(0.53-1.22)

Abbreviations: Gluc: glucose, TC: total cholesterol, TG: triglycerides, GGT: gamma glutamyl transferase, FAMN: fructosamine, RT: radiotherapy, RP: radical prostatectomy, HT: hormonal treatment, DT: deferred treatment, HR: Hazard ratios, CI: Confidence intervals **Adjusted for: Education, diabetes mellitus at Prostate cancer diagnosis, age, CCI, Fasting Status, time between date of blood test and Prostate cancer diagnosis date

3.1.4. Discussion

To the best of my knowledge, this is the first study investigating an association between serum markers of lipids, glucose and GGT and development of SDPTs in men with prostate cancer. High levels of cholesterol, triglycerides and GGT measured on average 16 years prior to prostate cancer diagnosis were associated with an increased risk of developing a SDPT. When looking at specific types of SDPTs, I found an increased risk of SDPTs of digestive organs, peritoneum, genitourinary and rectum for those with elevated levels of triglycerides. High levels of GGT were also associated with an increased risk of SDPTs of the respiratory system.

Carcinogenesis is a complex process that can require several components to act/occur before irreversible disease develops. This concept implies the temporality of the component causes, meaning that the factors involved in the development of the disease do not necessarily happen at the same time. The present paper illustrates these well-described characteristics of the sufficient-cause model (25). By analysing how biomarkers of different metabolism measured before PCa diagnosis are associated to the occurrence of a secondly diagnosed primary tumour-while accounting for all possible confounders given the available data- we have been able to establish a possible component cause for these tumours. Which other components need to occur (either as cause or prevention i.e. taking any medication that may reduce the risk of cancer) and when escapes the scope of this study and further research to establish other component causes is needed. Whether the strength of the association would be wakened by including in the model other potential component causes that we haven't been able to account for would not disregard the potential etiological significance of this study results.

My findings for SDPTs were consistent with our and other previously published findings for these biomarkers and primary prostate cancer (181, 184, 207, 208). Interestingly, results from a recent nested case-control study on the association between circulating fatty acids and prostate cancer showed that those who had been diagnosed 10 or more years after blood collection had stronger associations than those diagnosed less than 10 years after blood collection. These findings support the

hypothesis that metabolic factors may play a role several years before disease occurrence and detection (209).

Below I describe my findings in the context of other epidemiological studies as well as the hallmarks of cancer (183, 210).

Lipid metabolism

Elevated levels of cholesterol and triglycerides are associated with a higher risk of prostate cancer, gastrointestinal and renal cancer (211, 212). In the AMORIS study these associations varied by levels of glucose (187, 208, 213). Our findings for risk of SDPTs corroborate these observations, suggesting that prostate cancer, gastrointestinal and genitourinary cancers may share a common lipid metabolism phenotype. In the context of melanoma, few epidemiological studies have investigated links with lipid metabolic alterations (214).

Cholesterol is necessary to build cell membrane and preclinical studies suggest that low levels of cholesterol cause cell cycle arrest. Furthermore, high levels of cholesterol induce a chronic inflammatory state, and thus potentially cell proliferation (215). Recent experimental data also suggest that statins, a commonly used cholesterol-lowering drug, may impair cell proliferation and induce apoptosis (216-218).
This excess of lipids may not only be part of the tumour phenotype of uncontrolled cell proliferation, but it may also be involved in the altered cell signaling activated cascades that are characteristic of cancer cells (219).

GGT

My results show that elevated levels of GGT measured before prostate cancer diagnosis are associated with higher overall SDPTs risk and more specifically with SDPTS of digestive organs and lung cancer. Epidemiological studies have established an association between GGT and several primary cancers. For instance, elevated levels of GGT are associated with increased risk of cancer in men (220), specifically prostate cancer (181).

Several mechanisms have been suggested explaining the role of GGT in cancer cell proliferation and survival. Some of these processes include the recovery of essential aminoacids like glutamic acid and cysteine and balancing the reactive oxygen species ROS levels and facilitating the biosynthesis of the macromolecules and organelles required for assembling new cells (221).

Glucose and fructosamine

In contrast to previous findings in the AMORIS study (182), these results did not support a link between markers of glucose metabolism and development of SDPTs in men with prostate cancer. This discrepancy in findings could be due to glucose measurements being potentially more sensitive to the time window before carcinogenesis. Our previous study focused on primary tumours and hence the time window between glucose measurement and risk of cancer was shorter than in the current study (182).

Summary

Studying the common aetiology of SDPTs is broad and difficult to implement. Using AMORIS, and its linkages to well-documented registries, allowed me to establish a stable association between the biomarkers of interest and development of SDPTS – which informs future hypotheses for understanding the process of carcinogenesis.

Unmeasured confounding can compromise the validity of observational studies. Usually propensity scores, regression and matching are used to reduce potential confounding of known variables. However, they cannot account for unknown or unmeasured confounders. Instrumental variables analysis is a method used for controlling for unmeasured confounding that requires a valid instrumental variable which must be independent of the unmeasured confounding; must affect the treatment; and can affect the outcome indirectly through its effect on the treatment. Genes as such can be used as instrumental variables in what is known as Mendelian randomization because it makes use of the random assignment of genetic variants conditional on parents' genes (222). Although this method is appealing to improve the

current study, gene data was not available for this study and therefore adjusted Cox proportional hazard models were used.

Further strengths of the present study are the large number of men with prospective measurements of biomarkers measured at the same clinical laboratory with a clearly documented methodology. Missing data, for the biomarkers studied, was limited and multiple imputations were used to address this. Not enough data was available to perform longitudinal analysis including repeated biomarker measurements, a study design that would benefit future studies aiming to evaluate the effect of serum biomarkers on development of prostate cancer and subsequent cancers. Use of national health registers provided complete follow-up for each person as well as detailed information on cancer diagnosis, time of death and emigration (223). Subjects included in the study were mainly healthy at baseline as these measurements were to a large extent part of routine health check-ups in the occupational setting. The selection of biomarkers followed findings in previous AMORIS studies (180-182), but could be widened in future studies.

Prostate cancer treatment is unlikely to impact on this study as a confounder. Although cancer treatment potentially increases risk of SDPTs, the induction period is usually >5 years (224, 225). The current follow-up time was on average five years, suggesting that SDPTs captured were probably already being developed by the time of prostate cancer diagnosis.

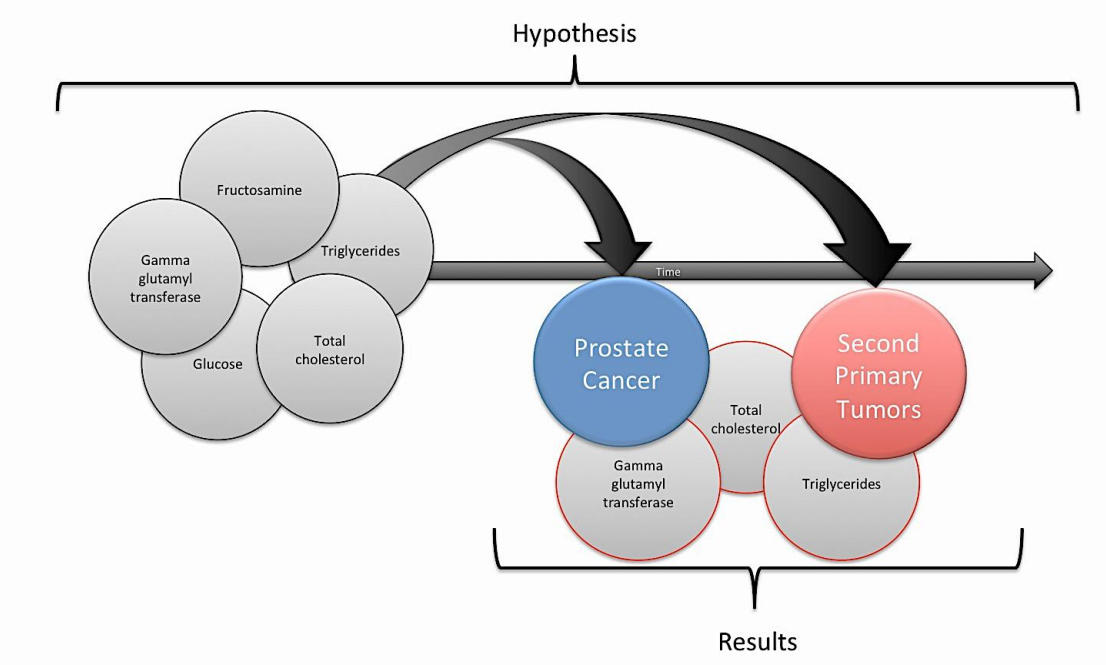
Limited data on lifestyle factors was available, however all models were adjusted for CCI, which indirectly accounts for effects of lifestyle (e.g., smoking-related diseases). However, the biomarkers themselves can also be considered a downstream of some lifestyle habits. Another limitation is the lack of information on drug prescriptions related to the biomarkers studied (e.g. anti-diabetes drugs). The Prescribed Drug Register only starts recording in Sweden in July 2015 (226). Adjustment for CCI can therefore be considered as a crude proxy for the potential use of these drugs. Detection bias (i.e. those with higher levels of the biomarkers were more closely

followed up by their physicians and therefore secondary tumours would have been detected more promptly) is not affecting this study, as we did not focus on biomarkers measured after prostate cancer diagnosis. Detection bias of urological cancers is plausible due to closer specialist follow-up, however again this does not discard a possible biological link.

3.1.5. Conclusion

Biomarkers of lipid metabolism as well as GGT measured before prostate cancer diagnosis were associated with a higher risk of developing a SDPT. In the AMORIS cohort, lipids and GGT (180, 181) have been previously linked with higher risk of prostate cancer and I can therefore suggest that SDPTs and prostate cancer could share common biological components, involving the metabolism or potential effect of lipids or GGT – a hypothesis which will require future pre-clinical as well as longitudinal studies to corroborate (Figure 14).

Figure 14 Study hypothesis and results



4. Chapter IV: Treatment-related outcomes – results from PCBaSe Sweden

The following three projects are all based on data from PCBaSe Sweden, so that the first section of this chapter describes this data resource in more detail. Next, the rationale, study population, research methods, and results for each specific project are explained.

4.1. Data source: PCBaSe

The National Prostate Cancer Register (NPCR) was formed in 1996 when several Swedish regional prostate cancer registers joined together. The primary aim of the NPCR is to provide data for quality assurance, bench marking of patient care and clinical research. NPCR captures >96% of all newly diagnosed, biopsy-confirmed prostate cancers registered in the Swedish National Cancer Register, to which registration is compulsory and mandated by law. Reports to the Cancer Register are obtained from the treating clinician and the pathology department that made morphological diagnosis.

All new incident cases of prostate adenocarcinomas are reported to the respective regional register, which are also regularly linked to each Regional Cancer Register, and data on cases not reported to NPCR are requested from each reporting clinical unit. Data are validated and checked for completeness at each Regional Cancer Centre before being entered to the online IT platform Information Network for CAncer care (INCA).

Prostate cancer database Sweden PCBaSe is a linkage of the records from the NPCR with several Swedish nationwide registers (Table 11). It was first described in 2008 and later on updated in 2012 and 2016 (53, 54, 227). In the following sections I only describe those registers relevant to my projects.

Table 11. PCBaSe included registers

Register	Data content
Swedish Cancer Register (1958)	Notification of cancer diagnosis, site and date. Reporting mandated by law from clinician and pathology department
Patient Register (Patient register regionally since 1964 and national since 1987)	In-patient and Out-patient Registers, with diagnostic and surgical codes
Cause of Death Register (1961)	Date and underlying and contributing causes of death coded according to ICD-10
Register of the Total Population and Changes (1968)	PIN for all Swedish residents, country of birth,
Registers of Immigration and Emigration	Date of immigration and emigration
Sweden Household Census	Demographics collected 1960–90 including e.g. profession
Longitudinal database on socioeconomic factors (LISA) (1990)	Extensive set of socio-economic factors with annual update including data on annual income, marital status, profession and income
The Prescribed Drug Register (2005)	All non-hospital prescribed and dispensed drugs for all Swedish residents since July 2005
The Multi-Generation Register (1932)	Data on all residents born after 1932 with information on identity for father, mother, brothers, sisters and offspring
National Diabetes Register	Details on diabetes diagnosis and metabolic factors for diabetes in Sweden
Hernia Register (1992)	>95% of all hernia surgeries performed since 1992
Riks-HIA/Swede Heart	Details on cardiac diagnoses and treatment since 1995 Patients treated at coronary care units and/or undergoing cardiac intervention

4.1.1. The Patient Register

As of 1987, the National Patient Register collects information regarding in-patient care nationwide. Prior to this the Patient Register operated on a regional level since 1964. From 2001 the register also includes all specialized outpatient care. Each record contains medical information on surgical procedures, hospital department and discharge diagnoses coded according to International Classification of Diseases (ICD-9 or ICD-10).

4.1.2. The Cancer Register

The Swedish National Cancer Register was founded in 1958. All incident cases of cancer in Sweden must be separately reported to the cancer register by the responsible clinician as well as the respective pathologist/cytologist.

4.1.3. The Cause of Death Register

The National Cause of Death Register originates since 1953. It is maintained by the National Board of Health and Welfare and shows underlying as well as contributory cause of death coded according to ICD-10 since 1997 and ICD9 before 1997.

4.1.4. Population and housing census

During the period 1960-1990 mandatory national censuses were performed in Sweden. This has yielded information on the individual, their household and housing, such as demographics, occupation, earnings, number of people per household, etc. Based on this information for PCBaSe, a socio-economic index can be constructed using five categories based on occupation: blue-collar workers, farmers, self-employed, lower white-collar workers and higher white-collar workers.

4.1.5. The Total Population Register

The Total Population Register provides information on country of origin and emigration. Moreover, socio-economic characteristics in PCBaSe be defined by information on education level, annual family income and marital status, available from linkage with the Longitudinal integration database for health insurance and labour market studies (LISA by its Swedish acronym) The database integrates existing data from the labour market and educational and social sectors.

4.1.6. The Prescribed Drug Register

The Swedish Prescribed Drug Register includes all prescriptions dispensed in Swedish pharmacies from July 2005. Information on the prescribed item includes amount and dose, and age, sex and place of residence of the patient, as well as date of prescribing and dispensing. Drugs administered in hospital are not recorded.

4.1.7. Comparison cohort

The comparison cohort for these men consists of men free of prostate cancer who were randomly selected according to the eligibility criteria as described for each of the studies below. Briefly, follow-up started at time of prostate cancer diagnosis for men with prostate cancer (index cases) and ended at time of death, emigration or study closing date. For all men with prostate cancer registered between 1996 and 2009 five men free of prostate cancer from the same county and birth year were selected. Thus, for each man with prostate cancer, PCBaSe contains five matched men without prostate cancer. Each man with prostate cancer has a unique set of men without prostate cancer in the comparison cohort.

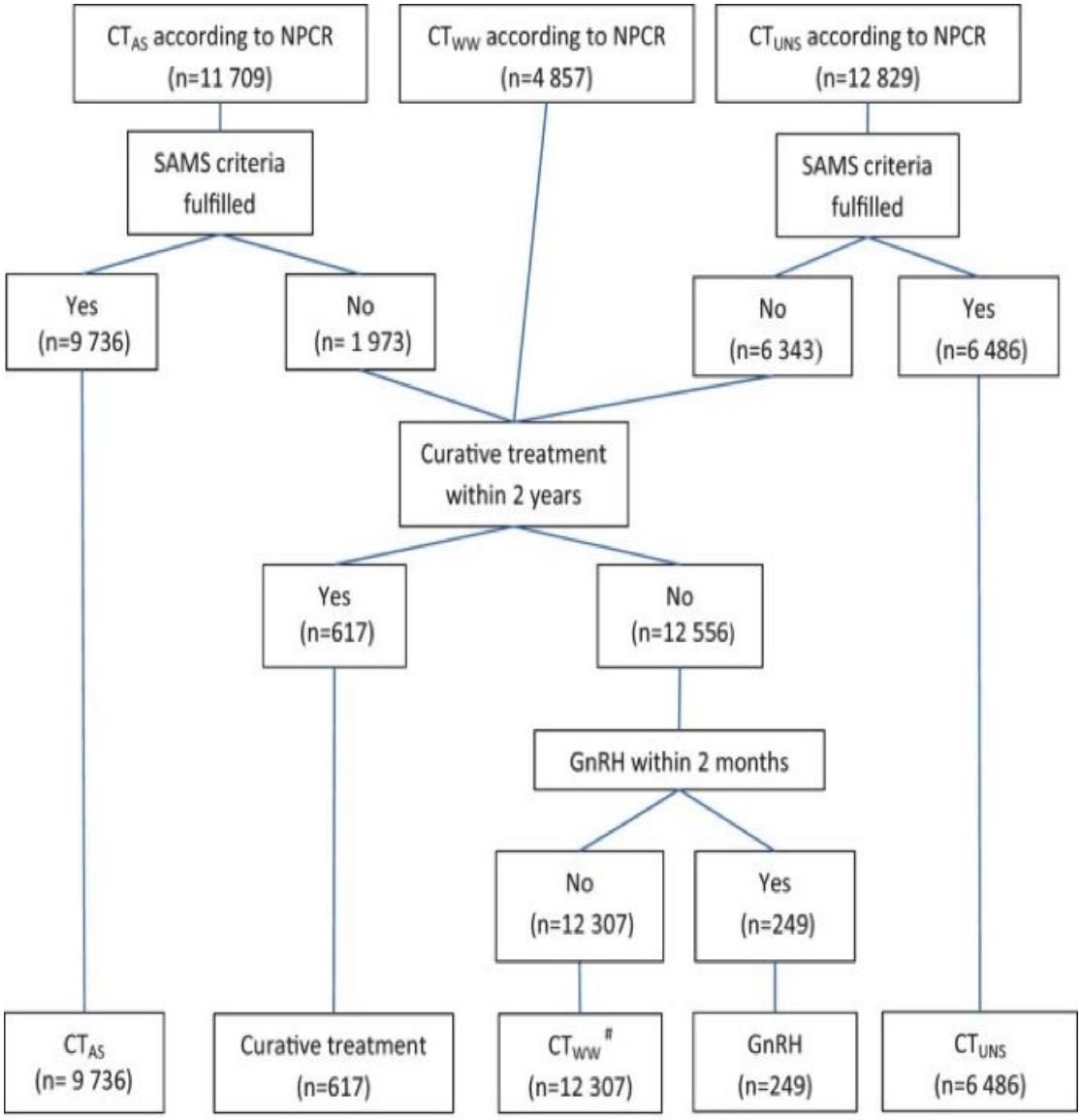
4.1.8. Strengths

NPCR captures virtually all men diagnosed with prostate cancer in Sweden since 1998. Including more than 110 000 cases, NPCR is the world's largest clinical database on prostate cancer with data available on clinical stage, specific tumour differentiation according to the Gleason grading and serum PSA levels in an unselected patient population from an entire nation.

4.1.9. PCBaSe traject

In the latest database update of PCBaSe ($PCBaSe^{Traject}$), prostate cancer treatment trajectory is delineated by use of data on primary treatment in the NPCR, verified by data obtained by linkages with the Prescribed Drug Registry (i.e. use of ADT) and the National Patient Registry (i.e. surgical procedures and hospital admissions). In addition, data were collected on radiotherapy (RetroRad) from oncology information systems and local databases at radiotherapy departments for treatments performed before 2008 (53). The order of treatments is the following: conservative treatment (CT) → radical prostatectomy → radiotherapy → anti-androgens (AA) → gonadotropin-releasing hormone (GnRH) agonists, in which any treatment may be omitted (example of conservative treatment flow chart in Figure 15).

Figure 15 Flow chart of data collection for conservative treatment in PCBaSe Traject (53, 228)



#285 curative treatments performed later than 2 years from diagnosis recorded as non-standard procedures according to registered initial CT$_{WW}$ treatment

Abbreviations: NPCR: National Prostate Cancer Register, CTWW: watchful waiting, CTAS: active surveillance, CTUNS: Unspecified conservative treatment, SAMS: Study on Active Monitoring in Sweden

4.2. Radiotherapy and the risk of thromboembolic disease

The findings of this section were presented as an abstract at the NCRI Conference in 2016 (Appendix II) and published in the International Journal of Radiation Oncology, Biology, and Physics (Appendix III) (229).

4.2.1. Rationale

Cancer increases risk of TED as tumour cells can activate the coagulation system (230). Previously, it has been shown in a PCBaSe study, that men with prostate cancer are at higher risk of TED (231) and this risk is especially high for those who have undergone prostate cancer-related surgeries, whilst receiving ADT (232).

No large epidemiological study has yet investigated the association between radiotherapy and risk of TED. It has been suggested that veins are less susceptible to radiation effects, however there are several case reports of arterial thrombosis for patients who received radiotherapy for breast, lung or uterine cancer (233-235). There is also a considerable body of experimental and epidemiological evidence showing that radiotherapy causes damage to endothelial cells in the arteries via different mechanism (236). For instance, the association between radiotherapy for breast cancer and higher risk of myocardial infarction and coronary heart disease is well established (237, 238). Based on this evidence, endothelial damage to veins is possible. According to a systematic review TED annual incidence rates ranges from 0.75 to 2.69 per 1000 individuals in the population, with the incidence in most of the studies ranging between 1.07 and 1.83. These rates increase with age and one of the main risk factors is cancer (239). Considering that men with localised prostate cancer have different treatment options (mainly AS, radical prostatectomy or RT) with curative intent evaluating life threatening possible treatment side effects is of high interest. Therefore, quantifying the risk of TED after radiotherapy is of relevance.

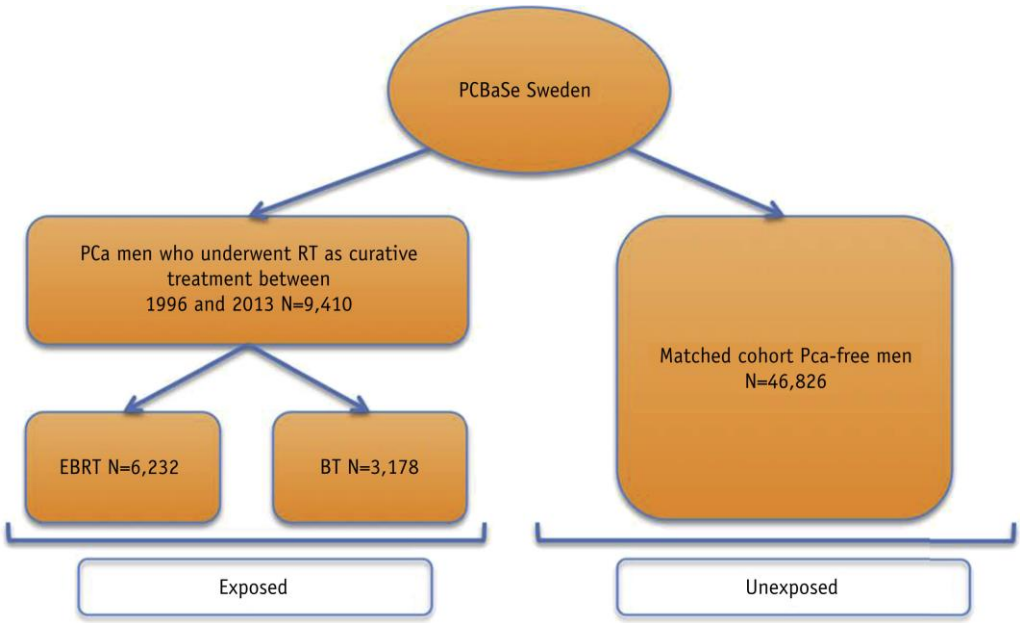
In this study, I investigated the association between curative radiotherapy given with contemporary standards for prostate cancer and risk of TED in a nationwide population-based cohort in Sweden.

4.2.2. Methods

4.2.2.1. Study population

All men with prostate cancer who received curative radiotherapy for prostate cancer between 1996 and 2013, as registered in PCBaSe (n=9,178) (Figure 16). These men were matched by county of residence and birth year with an index case. For the current study, we selected 46,826 men free of prostate cancer. This comparison with a non-prostate cancer cohort has been successfully applied previously in PCBaSe when investigating the risk of TED, cardiovascular disease or diabetes following androgen deprivation therapy or surgery (54, 231, 240-242).

Figure 16 Selection of study population from Prostate Cancer Database Sweden



Abbreviations: EBRT: external beam radiotherapy, BT brachytherapy, PCa prostate cancer RT: radiotherapy, PCBaSe: prostate cancer database Sweden

4.2.2.2. Exposure definition

Radiotherapy data was obtained from the NPCR as well as from RetroRadioTherapy, a separate retrospective data collection at radiation units in Sweden. For this register data on treatment type, timing, total dose and fractionation was retrieved directly from the verification/oncology information systems and local databases of the radiotherapy departments in Sweden. Men were followed up starting on the day of RT until the end of the study, death, immigration or loss of follow up. Prostate cancer free

men inherited a RT date according to their matched prostate cancer men. The Research Ethics Board at Umea University approved this study (228).

The following information on potential confounders was also obtained. Based on information from the National Patient Register, comorbidities were measured using CCI, as previously described in chapter III. Information on age, serum PSA, treatment at time of diagnosis, tumour grade and stage, educational level, and history of TED was also used. Prostate cancer risk category was defined according to a modification of the National Comprehensive Cancer Network Guideline (243): low risk: T1–2, Gleason score 2–6 and PSA < 10 ng/mL; intermediate risk: T1–2, Gleason score 7 and/or PSA 10– 20 ng/mL; high risk: T3 and/or Gleason score 8–10 and/or PSA 20–50 ng/mL; regionally metastatic/locally advanced: T4 and/or N1 and/or PSA 50–100 ng/mL in the absence of distant metastases (M0 or MX); and distant metastases: M1 and/or PSA > 100 ng/mL. Information on surgeries was taken from the National Patient Register, and included transurethral resection of the prostate (TURP), open or laparoscopic radical prostatectomy, pelvic lymph node dissection, and orchiectomy (232). Information on filled prescriptions of anti-androgens and GnRH agonists was obtained from the National Prescribed Drug Register, in which all filled prescriptions have been registered since 1st of July 2005. This allowed us to create a time-updated covariate for adjuvant and neoadjuvant ADT. Disease progression was defined by using the following proxy variables as time-dependent covariates: ADT starting 9 months after RT, TURP indicating infravesical obstruction; palliative radiotherapy indicating a rise in serum PSA level or skeletal pain; and use of nephrostomy indicating overgrowth on the ureter. This is consistent with previously published work on the association between ADT and TED (241).

4.2.2.3. Outcome definition

The main outcomes were DVT (International Classification of Diseases [ICD]-10 code: I80-82) and PE (ICD-10: I26) as primary diagnoses in the National Inpatient Register and National Outpatient Register or Cause of Death Register. All three registers were used to avoid underestimation of severe cases of PE that may have only been captured as fatal in the Cause of Death Register (231).

4.2.2.4. Statistical analysis

First, univariate Cox proportional hazards models were conducted to evaluate the association between known clinical risk factors (i.e., lymph node dissection, palliative radiotherapy, ADT due to disease progression, hydronephrostomy, non-prostate cancer related surgeries) and TED. This then confirmed the need to take these factors into account as time-updated covariates in our multivariate models. To further justify the choice for time-updated covariates related to prostate cancer only, a sensitivity analysis was performed in which these events were censored (e.g. androgen deprivation therapy for disease progression) or used delayed entry (e.g. one year following lymph node dissection). The results were virtually the same as for the adjusted models. Univariate and multivariate Cox proportional hazards models with age as a time-scale were then conducted to determine the hazard ratios (HR) and 95% confidence intervals (CI) for risk of DVT and PE by types of radiotherapy (brachytherapy (BT) and external beam radiotherapy (EBRT). The assumption of proportionality of the Cox model covariates was tested by plotting Schoenfeld residuals (206). The multivariate analyses were conducted stepwise, allowing the identification of the effect of each confounder: CCI, education, prostate cancer risk categories, prostate cancer-related surgeries, history of TED, disease progression markers, other surgeries, adjuvant and neoadjuvant ADT. Exposure to surgeries, neoadjuvant and adjuvant ADT and markers of disease progression were incorporated as time-updated covariates. Due to the rather small sample size for BT, only an additional stratified analysis by time since radiotherapy for EBRT: 0-6 months, 6-12 months, 1-2 y, >2y was performed.

Data management was done using SAS version 9.3 (SAS Institute, Cary, NC, USA) and data analysis was conducted with R version 2.13.2 (R Foundation for Statistical Computing, Wien, Austria).

4.2.3. Results

Between 1996 and 2013, 9,410 men received curative radiotherapy as registered in PCBaSe Sweden out of which 6,232 underwent EBRT and 3,178 BT. The latter group

consisted of patients receiving either high-dose-rate BT (HDR-BT) to the prostate (n=2,452), combined with EBRT in the majority of the patients or low-dose-rate BT (LDR-BT) via implanted radioactive seeds (n=726). There were 144 TED events in the exposed groups (43 in the BT group and 101 in the EBRT group) and 483 in the comparison cohort. Baseline characteristics of the study cohort are presented in Table 12.

Univariate analyses for the association between known TED risk factors and PE and DVT are presented in Table 13, confirming the need for time-updated covariates in the multivariate analyses.

There was a positive association between EBRT and BT and the risk of PE, although after adjusting for CCI, prostate cancer risk category, prostate cancer-related surgeries, previous TED, disease progression markers, other surgeries, education, adjuvant ADT and neoadjuvant ADT it was no longer statistically significant (HR: 1.05, 95% C.I.: 0.61-1.79 and HR: 0.97, 95%C.I.: 0.29-1.44 respectively) (Table 14). In the stratified analysis, the highest HR was observed for the first period (0 to 6 months), however after adjustment for the named covariates it remained not statistically significant. No associations between EBRT or BT and the risk of DVT were found. Residual plots for all covariates versus time at risk showed the residuals centred around zero, indicating no violation of the hazards proportionality assumption.

Table 12. Radiotherapy and TED study participant's baseline characteristics

	BT		EBRT		PCa free men	
	#	%	#	%	#	%
Total number of men	3178	100	6232	100	46826	100
Age						
<60	490	15.4	566	9.1	5299	11.3
60-64	772	24.3	1179	18.9	9678	20.7
65-74	1747	55.0	3827	61.4	27706	59.2
75+	169	5.3	660	10.6	4143	8.8
CCI						
0	2574	81.0	4632	74.3	35975	76.8
1	382	12.0	935	15.0	5751	12.3
2	158	5.0	436	7.0	2944	6.3
3+	64	2.0	229	3.7	2156	4.6
Stage Group						
No PCa	0	0.0	0	0.0	46826	100.0
1. Low risk	864	27.2	900	14.4	0	
2. Intermediate risk	1059	33.3	2387	38.3	0	
3. High risk	1106	34.8	2503	40.2	0	
4. Regionally metastatic	126	4.0	391	6.3	0	
6. Missing data	23	0.7	51	0.8	0	
Prior DVT						
0	3171	99.8	6190	99.3	46529	99.4
1	7	0.2	38	0.6	140	0.3
2+	0	0.0	4	0.1	157	0.3
Prior PE						
0	3151	99.2	6157	98.8	46497	99.3
1	26	0.8	65	1.0	146	0.3
2+	1	0.0	10	0.2	183	0.4
Neo adjuvant ADT						
No ADT	1029	32.4	2463	39.5	46826	100.0
Anti androgens	200	6.3	309	5.0	0	
GnRH	1949	61.3	3460	55.5	0	
Educational level						
Low	869	27.3	2279	36.6	16861	36.0
Middle	1333	41.9	2525	40.5	18684	39.9
High	959	30.2	1388	22.3	10652	22.7
Missing	17	0.5	40	0.6	629	1.3
Follow-up time (SD)						
	5.1 (2.1)		4.6 (2.1)		4.7 (2.2)	

Adjuvant ADT: BR group (AA=222 GnRH=134) EBRT group (AA=484, GnRH=678)
Abbreviations: CCI: Charlson Comorbidity Index, ADT: Androgen Deprivation therapy; DVT: Deep Venous Thrombosis, PE; Pulmonary Embolism, GnRH: Gonadotropin releasing hormone, EBRT: external beam radiotherapy, BT: brachytherapy, SD: standard deviation, ADT: androgen deprivation therapy

Table 13. Univariate hazard ratios (HR) and 95% confidence intervals (CI) for risk of DVT and PE based on known clinical risk factors for TED.

TED known risk factors		Number of events		Univariate			
				PE		DVT	
		BT	EBRT	HR	95% C.I.	HR	95% C.I.
PCa men	Lymph node dissection (LND within last 12 months versus no LND within last 12 months)	759	1166	2.03	0.82-4.99	3.44	0.80-14.76
	Palliative RT	25	90	1.68	0.23-12.06	17.72	4.16-75.47
	AA due to disease progression vs. no AA	181	665	1.09	0.50-2.58	2.64	0.92-7.56
	GnRH due to disease progression	183	537	2.46	1.30-4.65	9.41	3.83-23.06
	Hydronephrostomy	4	24	7.56	1.03-55.44	NA*	NA
Non-PCa related surgeries**		427	863	7.83**	4.88-12.56	5.04**	1.86-13.62

*No events

**PCa-free men included for this variable (Nr of events=5106)

TED: Thromboembolic diseases, BT: Brachytherapy, EBRT: External beam radiotherapy, PE: Pulmonary embolism, DVT; Deep venous thrombosis, HR: Hazard ratio, CI: confidence intervals, NA: non applicable, AA: Anti-androgens, GnRH: Gonadotropin releasing hormone agonists, LND: lymph node dissection.

Table 14. Multivariate analysis hazard ratios (HR) and 95% confidence intervals (CI) for risk of DVT and PE.

		DVT		PE	
		HR	**95%CI**	**HR**	**95%CI**
Ref	**No RT**	1.00	Ref.	1.00	Ref.
Unadjusted	**BT**	0.60	0.26-1.36	1.47	1.05-2.07
	EBRT	1.09	0.68-1.74	1.73	1.35-2.2
Adjusted*	**BT**	0.34	0.08-1.11	0.97	0.29-1.44
	EBRT	0.44	0.14-1.4	1.05	0.61-1.79

*Charlson comorbidity index ,PCa risk category, PCa-related surgeries, previous thromboembolic events, TED known risk factors as determined in table 13, education, adjuvant ADT and neoadjuvant ADT

4.2.4. Discussion

The current study shows that in a cohort of Swedish men with prostate cancer, curative radiotherapy for prostate cancer was not associated with an increased risk of TED. This study's analyses compare men with prostate cancer receiving RT with matched men from the general population, so that these results cannot disentangle the effects of RT and the tumour itself on development of TED. The observed lack of an association between RT and TED when comparing with the general population can be explained by one of the following reasons: (1) RT is truly not associated with risk of TED;(2) Men on RT are heavily selected based on their TED risk factors so that a potential increased risk of TED from RT is at most as big as the risk reduction due to the selection. However, as cancer itself is a risk factor for TED, this indicates that the second explanation is unlikely.

To the best of my knowledge, no large study to date has investigated the association between RT for prostate cancer and TED. Experimental data shows that radiotherapy can induce changes in artery walls, sinusoids and capillaries (236). The different layers of the wall vessels can suffer several alterations after radiation exposure such as endothelial cell damage, neointima lipid deposit, necrosis, fibrosis rupture and thrombosis (236, 244). Moreover, EBRT to the pelvis has been found to increase the risk of bleeding in men who were on an anticoagulant scheme before receiving RT (245). Less evidence has been found for large veins (246), except for hepatic and large intestine veins, which radiotherapy frequently affects. Little is known regarding the biological mechanisms for this lesser impact of RT in large veins, although it has been suggested that large veins that do get affected by RT were probably invaded by the neoplasm prior to RT (246). Our results suggest that large veins from the pelvic area of patients who received RT for prostate cancer do not seem to suffer enough alterations that can lead to a short-term thromboembolic event. However most of the reported RT changes in the arteries and heart seem to happen several years after receiving RT, and our mean follow up time was 5 years, so that the present study may not be sensitive for long-term events.

Men who undergo radical prostatectomy are at a slightly increased risk of TEDs (231). Moreover, results from a recent observational showed that ADT also increases the risk of TED (241). In my analysis, I included adjuvant and neoadjuvant ADT as potential confounders, however this adjustment did not alter the final point estimates for the association.

A major strength of this study is the use of comprehensive data in PCBaSe Sweden, a large nationwide population-based register from which information on complete follow-up, prostate cancer treatment, prostate cancer stage, surgeries, disease progression, ADT, comorbidities and socio-economic status can be retrieved, which allowed me to adjust for known TED risk factors. Additionally, the use of a prostate cancer-free, age and residence matched comparison cohort allowed for accurate risk estimation. The availability of data regarding delivered radiotherapy doses for this large cohort is another strength of this study. It allowed us to confirm that the selected patients had received radiation doses with curative potential to the prostate.

Detailed information on irradiated volumes was lacking which excluded the possibility to examine dose-volume effects on TED. Even though we had data on type and dosage of EBRT, it was not possible to divide this further into subtypes due to the low number of TED events. However, it is unlikely that we have missed strong associations as none of our findings suggested any indication of a positive trend. Additional limitations include lack of information on lifestyle factors and residual confounding, which could not be accounted for (247, 248). However, adjustment for CCI and history of TED served as proxies for lifestyle and health status at initiation of RT. Furthermore, as it can be observed baseline characteristics amongst EBRT, BT and the comparison cohort are very similar, reducing the amount of residual confounding.

4.2.5. Conclusion

These results indicate that curative radiotherapy for prostate cancer is not associated with the risk of developing PE or DVT within the first 5 years since treatment.

4.3. Drugs for metabolic conditions and prostate cancer death in men on GnRH agonists

The findings of this section were published in the BJU International (Appendix IV) (249).

4.3.1. Rationale

A recent meta-analysis estimated that the risk of prostate cancer is 1.54 times (95%CI: 1.23-1.94) higher for those with MetS, as compared to those without MetS (250). Recent studies also suggest that the presence of MetS or some of its features is associated with higher grade disease in men with prostate cancer and can lead to more rapid progression (163, 251). Moreover, in a review on the urological aspects of MetS authors found evidence linking the following conditions to prostate cancer: Increased fasting plasma insulin level, higher BMI, increased body weight, increased waist measurement, increased hip measurement, increased waist: hip ratio, type 2 diabetes, faster growing BPH, treated hypertension, higher systolic blood pressure, higher diastolic blood pressure, lower serum HDL-cholesterol level, increased serum triglycerides levels (252).

In contrast, some drugs used to treat conditions that are part of the MetS definition (e.g. metformin for diabetes or statins for dyslipidemia) have also been associated with a reduced risk and progression of prostate cancer (253-256) although results are not conclusive. For instance, in a study looking at metformin vs. sulfonylureas and their association with cancer incidence no differences were found(257). Nonetheless, the underlying biological mechanisms for these observations have not been fully elucidated (258).

GnRH agonists are associated with an increased risk of type 2 diabetes as well as other components of the MetS in men with prostate cancer who are treated with ADT (161). Moreover, one recent study found that that having MetS may shorten time to castrate resistant prostate cancer and overall survival (163), whereas another study did not find any statistically significant associations between baseline MetS and prostate cancer death (164).

Given this complex interaction between MetS, its related drugs, and prostate cancer progression, the current study aimed to evaluate how use of drugs for metabolic conditions (below referred to as “metabolic drugs”) at time of GnRH agonist initiation may affect response to treatment by studying time to prostate cancer death.

4.3.2. Methods

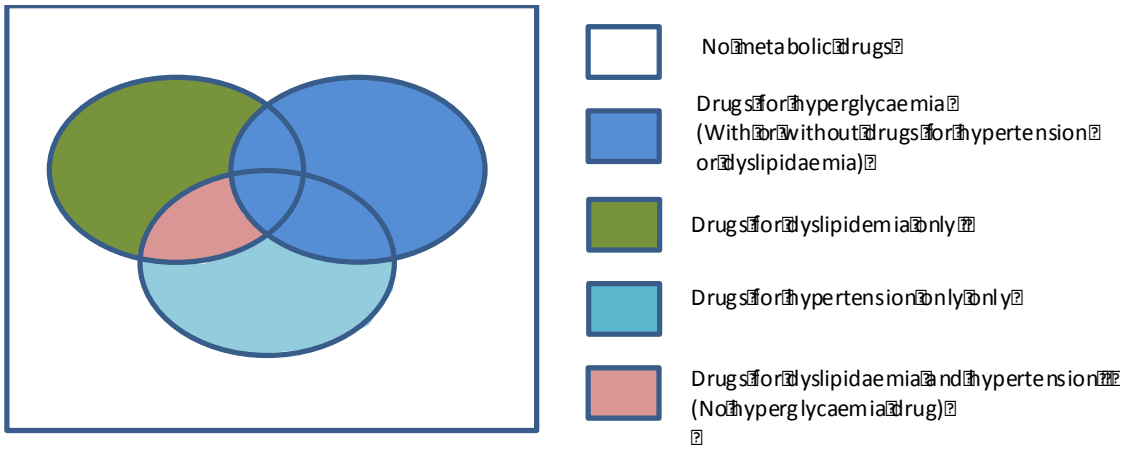
4.3.2.1. Study population

All men with prostate cancer who received primary GnRH agonists between 2007 and 2013 (n=9,267), as registered in PCBaSe Traject (228) were selected.

4.3.2.2. Exposure definition

The main exposure variables for this study were newly filled prescriptions, prescribed before GnRH agonist initiation, for treatment of diabetes (metformin, sulphonylurea, insulin), dyslipidaemia (statins), hypertension (angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta blockers, calcium channel blockers, or thiazide and thiazide-like diuretics), or anti-obesity agents in the Prescribed Drug Register. As only 20 men received anti-obesity agents, exposure to these drugs was not considered as part of the analysis a priori. As many men often take drugs for more than one of the metabolic conditions listed above, we looked at each metabolic drug group individually as well as the most common combinations: dyslipidaemia only (n=241), hyperglycaemia only (n=67 ;38 on insulin and 29 on metformin or sulphonylurea), hypertension only (n=2,933), dyslipidaemia and hyperglycaemia (n=41), dyslipidaemia and hypertension (n=1,996), hyperglycaemia and hypertension (n=333), and treated for more than two metabolic conditions (n=651). The analyses focused on the four most common groups of drugs: hypertension only, hypertension and dyslipidaemia, any hyperglycaemia, dyslipidaemia only (Figure 17).

Figure 17 Combinations of metabolic drugs studied



The following information on potential covariates was also obtained: age, tumour grade and stage and educational level. Prostate cancer risk category was defined according to a modification of the National Comprehensive Cancer Network Guideline (243): low risk: T1–2, Gleason score 2–6 and PSA < 10 ng/mL; intermediate risk: T1–2, Gleason score 7 and/or PSA 10– 20 ng/mL; high risk: T3 and/or Gleason score 8–10 and/or PSA 20–50 ng/mL; regionally metastatic/locally advanced: T4 and/or N1 and/or PSA 50–100 ng/mL in the absence of distant metastases (M0 or MX); and distant metastases: M1 and/or PSA > 100 ng/mL. In addition, we collected information on history of CVD, defined by any CVD hospital admission (ICD-10 codes: I20-I25, I50, I60-I69, I70-I79) as primary diagnoses in the National Patient Register. Comorbidities were also measured using CCI. Individuals were grouped into CCI categories for final scores of 0, 1, 2 or 3+. History of CVD and CCI were included for descriptive purposes, as careful assessment of the causal pathway did not indicate that these covariates need to be included in multivariate models (Figure 18) (259).

4.3.2.3. Outcome definition

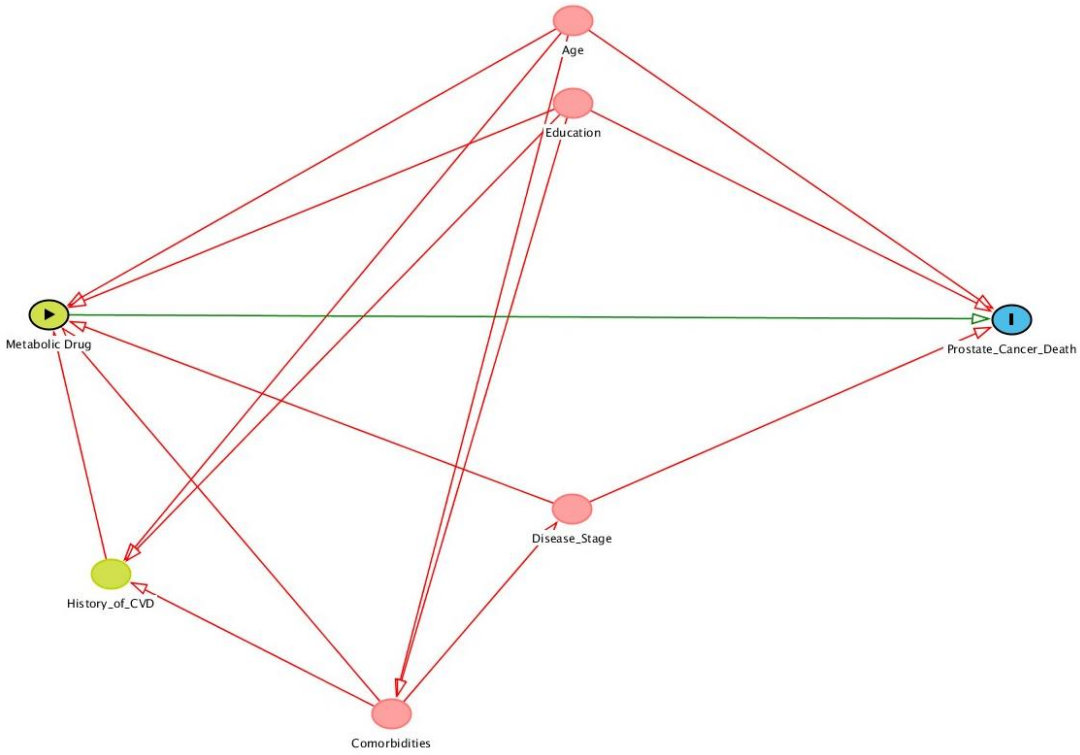
Based on the underlying causes of death registered in the Cause of Death Register, the following main outcomes were defined for this study: death from prostate cancer (ICD-10:C61), death from cardiovascular disease (CVD) (ICD-10: I10 to I99), as well as other deaths (remaining ICD 10 codes), and overall mortality (231).

4.3.2.4. *Statistical analysis*

Univariate and multivariate Cox proportional hazard regression models were conducted to assess the association between individual metabolic drugs as well as common combinations (Figure 18) and death from prostate cancer, CVD and overall mortality. Multivariate models were adjusted for age, education level, disease stage and civil status. Adjustment for age was done using natural cubic splines with four degrees of freedom. To consider competing risks, we repeated the analyses using Fine and Grays competing risk regression (260).

To further illustrate the associations between metabolic drugs and causes of death, stacked cumulative incidence proportion functions for all-cause, CVD, and prostate cancer -specific death were displayed by categories of metabolic drug use.

Figure 18 Directed acyclic graph for the association between metabolic drugs and prostate cancer death in men who start on GnRH agonists.



Data management was performed using SAS version 9.3 (SAS Institute, Cary, NC, USA) and data analysis was conducted with R version 2.13.2 (R Foundation for Statistical Computing, Wien, Austria).

4.3.3. Results

A total of 6,322 (68%) men used at least one drug for a metabolic condition at the time they started GnRH agonists. The majority of these men were on antihypertensive drugs only (46%), followed by men on drugs for dyslipidemia and hypertension (32%). About 10% of men were on drugs for more than two metabolic conditions. Table 15 shows the baseline characteristics of all men included in the study based on the type of metabolic drugs they were taking at time of GnRH agonist initiation.

Multivariate Cox proportional hazards regression adjusted for age, education, and prostate cancer risk category showed that use of most metabolic drugs were associated with an increased risk of CVD death and hence also overall death (Table 16). For instance, those men on anti-hypertensive drugs only were 1.87 times more likely to die of CVD than men not taking any metabolic drugs (95%CI: 1.56-2.24) and this increased to 2.46 times if these men were also taking drugs for dyslipidaemia (95%CI: 2.03-2.98). With respect to prostate cancer death, a small increased risk was observed for those on drugs for hypertension only or any hyperglycaemia (HR: 1.12 (95%CI: 1.03-1.23) and 1.19 (95%CI: 1.06-1.35), respectively).

Given the strong association with death from CVD, competing risk regression showed little impact on the association between metabolic drugs and death from CVD (Table 17). However, the associations observed for prostate cancer death disappeared for those on drugs for hypertension and dyslipidaemia, (HR: 0.92 (95%CI: 0.83-1.02)).

Figure 19 illustrates these findings using stacked cumulative incidence proportions. The largest proportion of CVD-deaths (red) can be observed amongst those on metabolic drugs, with the biggest proportion for those who are on drugs for both hypertension and statins. The proportion of prostate cancer -deaths (blue) was fairly similar across all the groups studied.

Table 15. Baseline characteristics of all men included in the study based on the type of metabolic drugs they were taking at time of GnRH agonist initiation.

	All men (n=9267)		Only Dyslipidaemia (n=241)		Only Hyperglycaemia (n=67)		Only Hyperglycaemia with insulin (n=38)		Only Hyperglycaemia with Metformin/SU (n=29)		Only Hypertension (n=2,933)		Dyslipidaemia and Hyperglycaemia (n=41)		Dyslipidaemia and Hypertension (n=1,996)		Hyperglycaemia and Hypertension (n=333)		≥2 Metabolic conditions (n=651)	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Age																				
<65	896	(9.7)	15	(6.2)	7	(10.4)	2	(5.3)	5	(17.2)	17	(6.0)	5	(12.2)	112	(5.6)	19	(5.7)	40	(6.1)
65-74	259	(28.0)	76	(31.5)	28	(41.8)	18	(47.4)	10	(34.5)	65	(22.2)	12	(29.3)	548	(27.5)	76	(22.8)	217	(33.3)
75-84	420	(45.4)	133	(55.2)	29	(43.3)	17	(44.7)	12	(41.4)	13	(46.6)	21	(51.2)	1068	(53.5)	173	(52.0)	319	(49.0)
85+	156	(16.9)	17	(7.1)	3	(4.5)	1	(2.6)	2	(6.9)	73	(25.2)	3	(7.3)	268	(13.4)	65	(19.5)	75	(11.5)
CCI																				
0	575	(62.1)	150	(62.2)	33	(49.3)	24	(63.2)	9	(31.0)	18	(63.3)	17	(41.5)	827	(41.4)	124	(37.2)	179	(27.5)
1	168	(18.2)	55	(22.8)	18	(26.9)	8	(21.1)	10	(34.5)	54	(18.6)	12	(29.3)	607	(30.4)	77	(23.1)	151	(23.2)
2	983	(10.6)	25	(10.4)	10	(14.9)	5	(13.2)	5	(17.2)	31	(10.7)	6	(14.6)	278	(13.9)	57	(17.1)	133	(20.4)
3+	846	(9.1)	11	(4.6)	6	(9.0)	1	(2.6)	5	(17.2)	21	(7.4)	6	(14.6)	284	(14.2)	75	(22.5)	188	(28.9)
Educational																				
High	141	(15.2)	52	(21.6)	9	(13.4)	4	(10.5)	5	(17.2)	42	(14.5)	9	(22.0)	283	(14.2)	30	(9.0)	78	(12.0)
Low	455	(49.1)	104	(43.2)	34	(50.7)	22	(57.9)	12	(41.4)	15	(51.4)	21	(51.2)	999	(50.1)	194	(58.3)	333	(51.2)
Middle	317	(34.2)	81	(33.6)	24	(35.8)	12	(31.6)	12	(41.4)	96	(32.7)	9	(22.0)	699	(35.0)	104	(31.2)	229	(35.2)
Missing	133	(1.4)	4	(1.7)	0	(0.0)	0	(0.0)	0	(0.0)	41	(1.4)	2	(4.9)	15	(0.8)	5	(1.5)	11	(1.7)
Civil status,																				
Married	566	(61.1)	157	(65.1)	33	(49.3)	17	(44.7)	16	(55.2)	17	(60.6)	28	(68.3)	1284	(64.3)	183	(55.0)	411	(63.1)
Not married	360	(38.9)	84	(34.9)	34	(50.7)	21	(55.3)	13	(44.8)	11	(39.4)	13	(31.7)	712	(35.7)	150	(45.0)	240	(36.9)
Risk category																				
Low risk	98	(1.1)	6	(2.5)	1	(1.5)	1	(2.6)	0	(0.0)	33	(1.1)	2	(4.9)	26	(1.3)	2	(0.6)	9	(1.4)
Intermediate	778	(8.4)	33	(13.7)	5	(7.5)	4	(10.5)	1	(3.4)	26	(9.0)	1	(2.4)	200	(10.0)	29	(8.7)	68	(10.4)
High risk	297	(32.1)	80	(33.2)	22	(32.8)	14	(36.8)	8	(27.6)	10	(34.7)	17	(41.5)	708	(35.5)	122	(36.6)	223	(34.3)
Regionally	143	(15.5)	32	(13.3)	14	(20.9)	6	(15.8)	8	(27.6)	44	(15.1)	4	(9.8)	309	(15.5)	63	(18.9)	91	(14.0)
Distant	397	(42.9)	90	(37.3)	25	(37.3)	13	(34.2)	12	(41.4)	11	(40.1)	17	(41.5)	753	(37.7)	117	(35.1)	260	(39.9)

Abbreviations: CCI: Charlson comorbidity index

Table 16. Cox proportional Hazard Ratios and Competing risks regression Ratios with 95% confidence intervals (CI) for the associations between drugs for metabolic conditions and prostate cancer death, other deaths, CVD deaths and overall death.

		Univariate Cox		Multivariate Cox*		Competing risk regression*	
	No of events	HR	95% CI	HR	95% CI	HR	95% CI
Prostate cancer death							
No metabolic drugs	1117	1.00	Ref	1.00	Ref	1.00	Ref
Only Hypertension	986	0.97	0.89 - 1.06	1.12	1.03-1.23	1.03	0.94-1.13
Hypertension+ Dyslipidaemia	578	0.81	0.74 - 0.90	1.02	0.93-1.14	0.92	0.83-1.02
Hyperglycaemia	336	0.93	0.82 - 1.05	1.19	1.06-1.35	1.00	0.89-1.14
Only Statins	73	0.78	0.62 - 0.99	1.06	0.83-1.34	1.01	0.80-1.29
Other death							
No metabolic drugs	294	1.00	Ref	1.00	Ref	1.00	Ref
Only Hypertension	394	1.48	1.27 - 1.72	1.23	1.05 - 1.43	1.12	0.96 - 1.31
Hypertension+ Dyslipidaemia	238	1.29	1.09 - 1.53	1.20	1.01 - 1.43	1.07	0.90 - 1.27
Hyperglycaemia	185	1.97	1.64 - 2.37	1.85	1.53 - 2.22	1.59	1.32 - 1.91
Only Statins	31	1.26	0.87 - 1.82	1.23	0.85 - 1.79	1.19	0.83 - 1.72
CVD Death							
No metabolic drugs	174	1.00	Ref	1.00	Ref	1.00	Ref
Only Hypertension	385	2.45	2.05 - 2.93	1.87	1.56 - 2.24	1.71	1.43 - 2.05
Hypertension+ Dyslipidaemia	283	2.59	2.14 - 3.13	2.46	2.03 - 2.98	2.26	1.87 - 2.74
Hyperglycaemia	149	2.69	2.16 - 3.35	2.53	2.03 - 3.16	2.15	1.72 - 2.69
Only Statins	15	1.03	0.61 - 1.74	1.06	0.63 - 1.80	1.01	0.59 - 1.71
Overall Death							
No metabolic drugs	1585	1.00	Ref	1.00	Ref	1.00	Ref
Only Hypertension	1765	1.23	1.15 - 1.31	1.22	1.14-1.31	N/A	N/A
Hypertension+ Dyslipidaemia	1099	1.10	1.02 - 1.18	1.23	1.14-1.33	N/A	N/A
Hyperglycaemia	670	1.32	1.20 - 1.44	1.49	1.36-1.63	N/A	N/A
Only Statins	119	0.90	0.74 - 1.08	1.08	0.89-1.30	N/A	N/A

*Adjusted for education, prostate cancer risk category, civil status, and age.

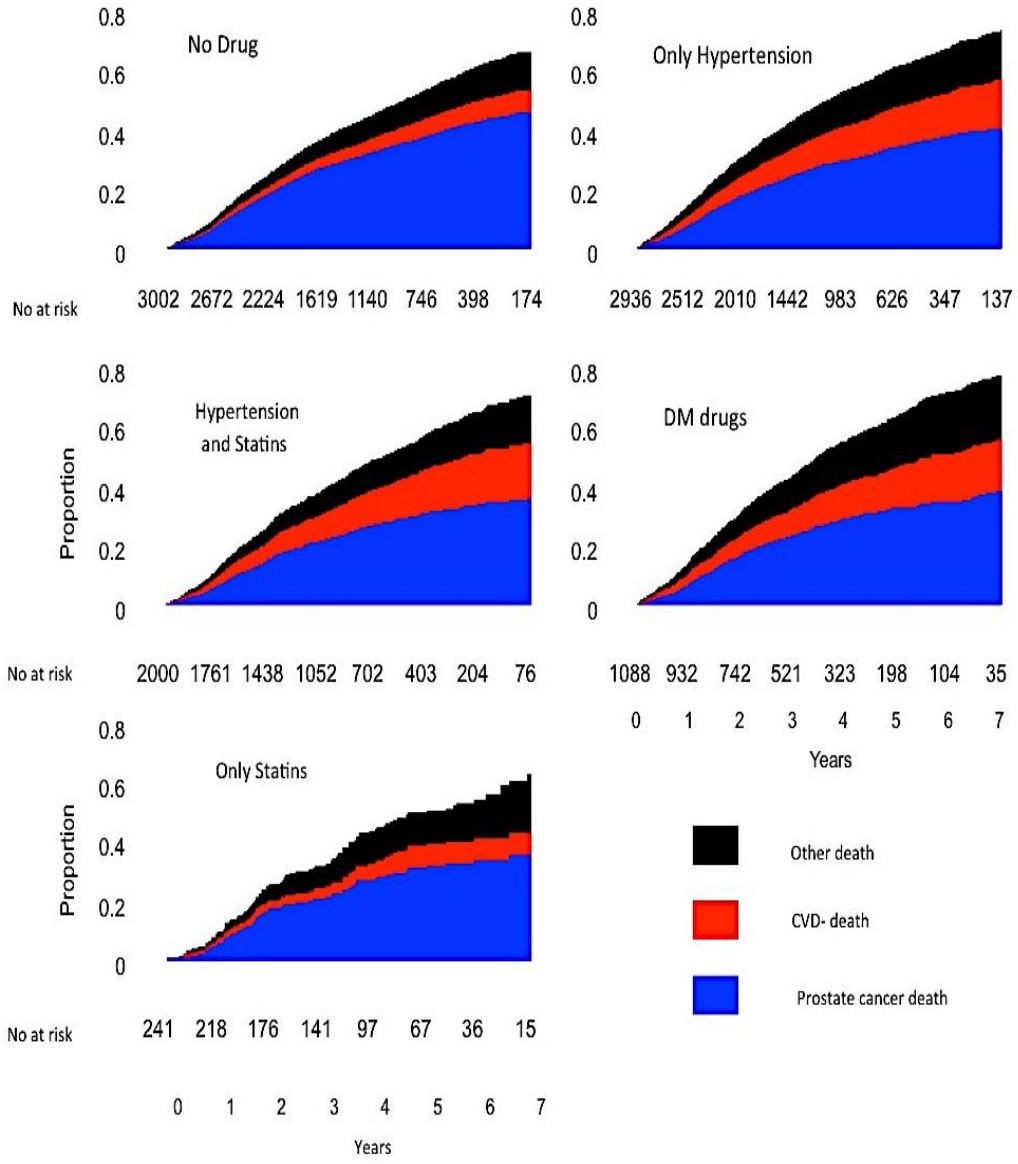
Table 17. Cox proportional Hazard Ratios and Competing risks regression Ratios with 95% confidence intervals (CI) for the associations between drugs for metabolic conditions and prostate cancer death, other deaths, CVD deaths and overall death – stratified by M1.

		Univariate Cox		Multivariate Cox*		Competing risk regression*	
METASTATIC DISEASE ONLY							
	No of events	HR	95% CI	HR	95% CI	HR	95% CI
Prostate cancer death							
No metabolic drugs	820	1.00	Ref	1.00	Ref	1.00	Ref
Only Hypertension	614	1.12	1.01 - 1.24	1.07	0.96 - 1.2	0.99	0.88 - 1.1
Hypertension+ Dyslipidaemia	361	0.98	0.87 - 1.11	1.01	0.89 - 1.14	0.91	0.81 - 1.04
Hyperglycaemia	204	1.13	0.97 - 1.32	1.16	0.99 - 1.36	0.97	0.83 - 1.14
Only Statins	48	1.07	0.8 - 1.43	1.11	0.83 - 1.49	1.05	0.78 - 1.42
Other death							
No metabolic drugs	105	1.00	Ref	1.00	Ref	1.00	Ref
Only Hypertension	124	1.78	1.37 - 2.30	1.41	1.08 - 1.84	1.30	0.99 - 1.70
Hypertension+ Dyslipidaemia	69	1.48	1.09 – 2.00	1.34	0.98 - 1.82	1.22	0.90 - 1.67
Hyperglycaemia	50	2.20	1.57 - 3.09	1.95	1.39 - 2.75	1.63	1.16 - 2.30
Only Statins	0	1.40	0.68 - 2.86	1.28	0.62 - 2.63	1.18	0.58 - 2.43
CVD Death							
No metabolic drugs	59	1.00	Ref	1.00	Ref	1.00	Ref
Only Hypertension	118	2.99	2.19 - 4.09	2.06	1.50 - 2.83	1.90	1.38 - 2.61
Hypertension+ Dyslipidaemia	78	2.97	2.12 - 4.16	2.58	1.83 - 3.64	2.38	1.69 - 3.35
Hyperglycaemia	50	3.90	2.67 - 5.68	3.31	2.26 - 4.85	2.77	1.90 - 4.06
Only Statins	6	1.86	0.80 - 4.32	1.64	0.71 - 3.81	1.57	0.69 - 3.54
Overall Death							
No metabolic drugs	984	1.00	Ref	1.00	Ref	1.00	Ref
Only Hypertension	856	1.30	1.19 - 1.43	1.18	1.07 - 1.29	N/A	N/A
Hypertension+ Dyslipidaemia	508	1.15	1.04 - 1.28	1.14	1.03 - 1.28	N/A	N/A
Hyperglycaemia	304	1.41	1.24 - 1.61	1.40	1.23 - 1.59	N/A	N/A
Only Statins	62	1.15	0.89 - 1.49	1.16	0.90 - 1.50	N/A	N/A
NON-METASTATIC DISEASE ONLY							
Prostate cancer death							
No metabolic drugs	297	1.00	Ref	1.00	Ref	1.00	Ref
Only Hypertension	372	1.16	1.00 - 1.36	1.22	1.05 - 1.43	1.14	0.97 - 1.32
Hypertension+ Dyslipidaemia	217	0.96	0.81 - 1.15	1.08	0.9 - 1.29	0.96	0.81 - 1.14
Hyperglycaemia	132	1.14	0.93 - 1.40	1.26	1.03 - 1.55	1.09	0.89 - 1.34
Only Statins	25	0.79	0.53 - 1.20	0.96	0.64 - 1.44	0.95	0.64 - 1.42
Other death							
No metabolic drugs	189	1.00	Ref	1.00	Ref	1.00	Ref

Only Hypertension	270	1.32	1.10 - 1.59	1.15	0.95 - 1.38	1.04	0.86 - 1.26
Hypertension+ Dyslipidaemia	169	1.17	0.95 - 1.44	1.14	0.92 - 1.40	1.00	0.81 - 1.23
Hyperglycaemia	135	1.81	1.45 - 2.26	1.78	1.43 - 2.22	1.55	1.25 - 1.94
Only Statins	23	1.15	0.74 - 1.77	1.20	0.77 - 1.85	1.17	0.76 - 1.79
CVD Death							
No metabolic drugs	115	1.00	Ref	1.00	Ref	1.00	Ref
Only Hypertension	267	2.15	1.73 - 2.68	1.77	1.42 - 2.21	1.63	1.30 - 2.03
Hypertension+ Dyslipidaemia	205	2.33	1.86 - 2.93	2.37	1.88 - 2.98	2.19	1.73 - 2.76
Hyperglycaemia	99	2.19	1.67 - 2.86	2.22	1.69 - 2.91	1.90	1.45 - 2.50
Only Statins	9	0.74	0.38 - 1.46	0.84	0.43 - 1.67	0.79	0.40 - 1.57
Overall death							
No metabolic drugs	601	1.00	Ref	1.00	Ref	1.00	Ref
Only Hypertension	909	1.40	1.26 - 1.55	1.30	1.17 - 1.44	N/A	N/A
Hypertension+ Dyslipidaemia	591	1.29	1.15 - 1.45	1.35	1.20 - 1.51	N/A	N/A
Hyperglycaemia	366	1.55	1.36 - 1.77	1.62	1.42 - 1.84	N/A	N/A
Only Statins	57	0.90	0.68 - 1.18	1.02	0.78 - 1.34	N/A	N/A

*Adjusted for education, PCa risk category, civil status, and age.

Figure 19. Stacked cumulative incidence of prostate cancer-specific, CVD, and other deaths based on exposure to metabolic drugs.



4.3.4. Discussion

Traditional Cox proportional hazards model indicated a weak increased risk of prostate cancer death in men who are on drugs for hypertension or hyperglycaemia at the time they start GnRH agonists. However, upon taking into account competing risk from CVD death none of the drugs for metabolic conditions were associated with an increased risk of prostate cancer death – and a trend towards an inverse association was observed for those who were on drugs for both hypertension and dyslipidaemia.

To my knowledge, few studies have investigated the effect of drugs for metabolic conditions in relation to response to treatment for men on GnRH agonists (261). Most studies to date have explored the effect of single drugs, predominantly metformin, statins, or beta-blockers, in relation to prostate cancer death (218, 254, 262-268). Moreover, these existing observational studies have found contradicting results – and they did not specifically study those men on GnRH agonists, a drug that in itself is also associated with an increased risk of metabolic conditions (161). Some reasons for these contrasting findings have been summarised previously (256), but by investigating several drugs for metabolic conditions in a specifically defined group of men with prostate cancer, the current study aims to improve our understanding of possible associations to metabolic drugs in the context of prostate cancer progression.

These results are in line with a small study (n=273) investigating the effect of metabolic syndrome (without looking at the related drugs) on prostate cancer death in men on ADT using data from the Health Professionals Follow-up Study and the Veteran's Administration (164). The authors concluded that there was no association of prostate cancer-specific death and metabolic syndrome, but the latter was associated with an increased risk of death from all causes. However, there was a weak positive association between hypertension only and prostate cancer-specific death (HR: 1.59 (95%CI: 0.89-2.84)). Even though the exposure assessment in this study is different from what I have done here, none of the studies support a strong association between metabolic aberrations and prostate cancer -specific death in men on GnRH agonists. The latter is relevant from a clinical point of view and could inform future studies looking into how development and treatment of metabolic syndrome following initiation of GnRH

agonists (as an adverse event) may affect response to hormonal treatment or prostate cancer progression.

Some of the medications used in this study as proxies for metabolic syndrome components have been associated with reduced overall and CVD mortality when compared to no treatment or placebo. Therefore, the drugs could potentially impact on mortality differently than the syndrome itself. In my study, no beneficial effect was observed for any of the metabolic scores. Although it is difficult to distinguish whether these findings are the result of the drugs or the diseases on the outcomes, the lack of inverse associations may reflect that our observations are indeed the effect of the diseases rather than the drugs. Another point to consider is the short follow-up time, which could also be preventing us from seeing the effects of the medications on overall/CVD mortality.

A major strength of our study is the use of comprehensive data in PCBaSe Sweden. As with other currently published studies, our follow-up was rather short and a follow-up study might provide more power to investigate risk of prostate cancer death in a competing risk setting. Another limitation of the current study is the lack of direct measures of metabolic syndrome (e.g. serum glucose and cholesterol levels (256)) and information on lifestyle factors

4.3.5. Conclusion

Despite the suggested complex interaction between metabolic syndrome, metabolic drugs, and prostate cancer progression, the current study did not find any evidence for a better or worse response to GnRH agonists in men who were also on drugs for hypertension, dyslipidaemia, or hyperglycaemia. These findings suggest that treatment of metabolic syndrome is important in men on GnRH agonists, but not a contraindication for their prostate cancer treatment.

4.4. Anti-androgens versus GnRH agonists in relation to prostate cancer death

The findings of this section were submitted as an abstract for the European Urology Association Annual Congress 2018 and the manuscript is under review with Annals of Oncology.

4.4.1. Rationale

Currently, the recommended treatment for men with advanced, non-metastatic prostate cancer is radiotherapy with adjuvant hormonal therapy (17). However, a substantial number of men with non-metastatic prostate cancer start primary hormonal therapy without radiotherapy, especially men with PSA levels above 50 ng/mL and/or locally advanced prostate cancer (clinical local stage T3-4) (269). Moreover, around 20% of men diagnosed with localised prostate cancer who received primary curative treatment will require hormonal therapy within 10 years (270).

The two main types of ADT for advanced, non-metastatic prostate cancer are medical castration by use of GnRH agonists and non-steroidal anti-androgen monotherapy (AA)(17). Presently, both types of medications are approved as monotherapies in Europe. Nonetheless, the European Urology Association, does not recommend AA in their prostate cancer guidelines based on a Cochrane systematic review results (168). However, the subgroup analysis of the Cochrane results showed no statistical differences in overall survival between bicalutamide and medical castration for non-metastatic disease and for bicalutamide 150mg/daily. No analysis was performed for disease stage or dose for cancer specific survival due to lack of data.
Therefore, large-scale evidence comparing AA vs GnRH for men with locally advanced non-metastatic disease in terms of overall survival and cancer specific survival is lacking.

Previously, two RCTs have compared the efficacy as well as the adverse events profile between AA monotherapy and GnRH agonists. Briefly, these RCTs compared AA in the form of bicalutamide 150 mg/daily and GnRH agonists or maximal androgen blockade (GnRH agonist combined with continuous AA) (169, 170). Although the statistical

requirement for non-inferiority was not met, survival was similar between AA and GnRH agonists, and AA with bicalutamide 150 mg/daily, which is in line with the Cochrane review subgroup analysis, and was subsequently approved for use in men with advanced, non-metastatic prostate cancer by the European Medicines Agency (167).

In addition to RCTs, it has now been widely accepted that observational data, i.e. real world data, are important in clinical decision making (271). It has been shown repeatedly that patients in RCTs are highly selected and have a lower risk profile than real-world populations, with the frequent exclusion of elderly patients and patients with co-morbidities (272-275). Supplementing RCT evidence with data generated from observational settings (e.g. registry data) can also improve the external validity of oncology drug trials, such that physicians treating patients in real-world settings have the appropriate evidence on which to base their clinical decisions (272, 274, 275).

As AAs present with less severe adverse effects than GnRH agonists, comparing their results in terms of prostate cancer specific survival and overall survival is important. Thus, in order to assess the external validity of these early RCTs on AA monotherapy versus GnRH agonists, we performed a nationwide, population-based observational study.

4.4.2. Methods

4.4.2.1. Study population

Men diagnosed with high-risk or regionally metastatic prostate cancer in 2006-2012, i.e. clinical local stage T3 or higher and/or prostate specific-antigen (PSA) 20 ng/mL or higher and/or Gleason Grade Group 4-5 and/or N1 and M0/X, who received AA or GnRH agonists as primary hormonal therapy were included in the study.

4.4.2.2. Exposure definition

The main exposure, i.e. type of hormonal therapy, as well as conversion from AA to GnRH agonists were verified by use of data on filled prescriptions in the Prescribed

Drug Registry. To mimic the RCT setting, men older than 90 years and men with a history of a previous cancer diagnosis were excluded.

4.4.2.3. Outcome definition

Follow-up was calculated from date of prostate cancer diagnosis until death, emigration, or date of censoring, whichever event came first. Date of censoring was 31 December 2013 for analysis of prostate cancer mortality and 31 December 2014 for all-cause mortality.

4.4.2.4. Statistical methods

Given that the study population of interest was men with M0 prostate cancer, it was important to evaluate those with missing data for M stage. Several other covariates had limited missing data, so therefore we first performed multiple imputation using the MICE package (200) based on data from men with intermediate-risk, high-risk, regionally metastatic and metastatic prostate cancer (i.e. four distinct risk categories) treated with primary hormonal therapy (Table 17). A series of univariate marginal models were specified to impute each of the variables with missing data: education (1.0-1.8%; range of messiness over the four risk categories), mode of detection (2.7-3.4% missing), PSA (0. -1.9% missing), Gleason Grade Groups (5.3-9.0% missing), percentage of positive biopsies (19.5-28.9% missing), T stage (0.8-1.2% missing), N stage (0.2-0.4% missing) and M stage (30.8-60.1% missing as diagnostic imaging was not performed). The model also included all the complete variables: age at diagnosis, year of diagnosis, civil status, cause of death, censoring and time-to-event i.e. death. The number of multiple imputations was set to five with 50 iterations, and convergence was diagnosed (data not shown). All subsequent analyses were conducted on the imputed data sets including men originally categorized with high-risk or regionally metastatic prostate cancer, but excluding men with imputed M1 disease (as per inclusion criteria described above).

The median follow-up was calculated with the reverse Kaplan-Meier method.

As there is a potential to convert to GnRH agonists after primary AA, we first used the cumulative incidence proportions to quantify conversion to GnRH agonists, considering death from prostate cancer and other causes and conversion to GnRH as competing events. Cumulative incidence proportions of death from prostate cancer and death from other causes for men on both treatments was calculated considering these as competing events.

Next, uni- and multivariable Cox proportional hazards regression analyses were conducted for death of prostate cancer and death from all causes. Age was used as timescale, whilst adjusting for year of diagnosis (continuous), mode of detection (categorical), T stage (categorical), Gleason Grade Groups (categorical), proportion positive biopsy cores (modelled as an interaction with T stage in men not diagnosed following TUR-P with two spine knots), PSA at diagnosis (categorical), bone imaging performed (dichotomized), CCI (categorical), marital status (categorical) and education level (categorical). Results are presented as Hazard ratios (HR) with 95% CIs.

Then, in order to mimic a potential target trial by conducting additional Cox model analysis based on propensity score matching for type of hormonal therapy. Propensity score matching was done with the MatchIt package for R using a caliper of 0.1 and included the covariates enumerated above. Subsequent multivariate Cox proportional hazards regression analyses were performed adjusting for the covariates used to perform the propensity score matching.

Finally, Kaplan-Meier estimates of prostate cancer-specific and overall survival were calculated for the propensity score matched groups and used to assess the cumulative deaths from prostate cancer and all causes.

The Research Ethics Board at Umeå University Hospital approved the study.

Statistical analysis was performed with R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

4.4.3. Results

The study population consisted of 2,078 men on AA and 4,878 men on GnRH agonists as primary hormonal therapy. The median follow-up was 4.7 years, representing a total of 28,315 person-years. Men treated with AA were younger, diagnosed in more recent calendar years, had less adverse cancer characteristics and had higher education level, compared to men treated with GnRH agonists (Table 19).
Virtually all men (99%) managed with AA received bicalutamide 150 mg/daily. In total, 765 men converted from AA to GnRH agonists, with a median time of exposure to AA of 4.3 years (95% CI 4.1-4.6) (Figure 20).

The 5-year cumulative incidence of prostate cancer mortality for men on AA was lower than men on GnRH agonists (AA 16% [95% CI 15-18%], GnRH agonists 22% [95% CI 21-24%]). The 5-year cumulative incidence of other causes mortality than prostate cancer was also lower for men on AA than men on GnRH agonists (AA 17% [95% CI 15-19%] and GnRH agonists 27% [95% CI 25-28%]) (Figure 21).

In multivariable analyses, men who received GnRH agonists had a similar risk of death from prostate cancer as men on AA, HR 1.08 (95% CI 0.95-1.23), but a higher risk of death from all causes, HR 1.23 (95% CI 1.13-1.34), compared to men on AA, Table 20. Stratification by prostate cancer risk category revealed similar results, with the exception of no difference in death from any causes in men with regional metastatic prostate cancer, HR 1.09 (95% CI 0.94-1.26). Symptoms at diagnosis, high T stage, high Gleason Grade Group and imaging at diagnosis were all associated with increased risk of death from prostate cancer and all causes, while presence of comorbidities and not married status were associated with increased risk of death from all causes, Table 21.

Following propensity score matching, a total of 1,972-1,976 men were identified in each treatment group in the 5 imputed datasets. Similar to the results of the traditional multivariable Cox analyses, men on GnRH agonists had a similar risk of death from prostate cancer as men on AA, HR 1.09 (95% CI 0.94-1.27), but a higher risk of death from all causes, HR 1.25 (95% CI 1.14-1.37), Table 22. Stratification by prostate cancer risk category revealed similar results, again with the exception of men

with regionally metastatic prostate cancer for whom there was no difference in deaths from all causes.

Following propensity score matching, there was no statistically significant difference in 5-year prostate cancer-specific mortality in men on AA (19% [95% CI 17-21%]) and men on GnRH agonists (21% [95% CI 19-24]) whereas the 5-year overall mortality was lower for men on AA (32% [95% CI 30-35%]) than for men on GnRH agonists (42% [95% CI 39-45%)]. Figure 21 shows cumulative deaths from prostate cancer and deaths from all causes in the propensity score matched groups.

Table 18. Baseline characteristics of men in PCBaSe 3.0 diagnosed with intermediate, high-risk, regionally metastatic, or distant metastatic prostate cancer in 2006-2012 and treated with primary anti-androgen monotherapy (AA) or GnRH agonists.

	Intermediate-risk				High-risk				Regionally metastatic				Distant metastatic			
	AA n = 692		GnRH n = 918		AA n = 1 552		GnRH n = 3 262		AA n = 526		GnRH n = 1 616		AA n = 428		GnRH n = 4 654	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Year of diagnosis																
2006-2007	144	(20.8)	337	(36.7)	371	(23.9)	1105	(33.9)	171	(32.5)	587	(36.3)	103	(24.1)	1356	(29.1)
2008-2010	195	(28.2)	288	(31.4)	435	(28.0)	1008	(30.9)	124	(23.6)	468	(29.0)	107	(25.0)	1384	(29.7)
2011-2012	353	(51.0)	293	(31.9)	746	(48.1)	1149	(35.2)	231	(43.9)	561	(34.7)	218	(50.9)	1914	(41.1)
Age at diagnosis, years																
<70	69	(10.0)	88	(9.6)	184	(11.9)	365	(11.2)	144	(27.4)	334	(20.7)	150	(35.0)	1529	(32.9)
70-74	169	(24.4)	203	(22.1)	301	(19.4)	471	(14.4)	113	(21.5)	280	(17.3)	97	(22.7)	800	(17.2)
75-79	303	(43.8)	321	(35.0)	549	(35.4)	939	(28.8)	139	(26.4)	348	(21.5)	77	(18.0)	866	(18.6)
80-84	116	(16.8)	220	(24.0)	376	(24.2)	971	(29.8)	94	(17.9)	396	(24.5)	69	(16.1)	885	(19.0)
85-90	35	(5.1)	86	(9.4)	142	(9.1)	516	(15.8)	36	(6.8)	258	(16.0)	35	(8.2)	574	(12.3)
Mode of detection																
Screening*	246	(35.5)	208	(22.7)	433	(27.9)	640	(19.6)	129	(24.6)	293	(18.2)	92	(21.5)	622	(13.4)
LUTS	311	(44.9)	511	(55.7)	755	(48.7)	1932	(59.2)	261	(49.7)	904	(56.0)	198	(46.3)	1912	(41.1)
Symptoms	109	(15.8)	146	(15.9)	311	(20.1)	594	(18.2)	120	(22.9)	372	(23.1)	127	(29.7)	1996	(42.9)
Missing	26	(3.8)	53	(5.8)	52	(3.4)	95	(2.9)	15	(2.9)	44	(2.7)	11	(2.6)	124	(2.7)
Clinical tumour category																
T1a	3	(0.4)	10	(1.1)	3	(0.2)	10	(0.3)	0	(0.0)	3	(0.2)	3	(0.7)	9	(0.2)
T1b	15	(2.2)	23	(2.5)	22	(1.4)	55	(1.7)	7	(1.3)	11	(0.7)	0	(0.0)	22	(0.5)
T1c	261	(37.7)	342	(37.3)	297	(19.1)	465	(14.3)	77	(14.6)	175	(10.8)	50	(11.7)	341	(7.3)
T2	413	(59.7)	543	(59.2)	503	(32.4)	1200	(36.8)	136	(25.9)	434	(26.9)	119	(27.8)	946	(20.3)
T3	-	-	-	-	711	(45.8)	1492	(45.7)	226	(43.0)	715	(44.2)	201	(47.0)	2288	(49.2)

T4	-	-	-	-	-	-	-	-	76	(14.4)	262	(16.2)	49	(11.4)	933	(20.0)
Missing	-	-	-	-	16	(1.0)	40	(1.2)	4	(0.8)	16	(1.0)	6	(1.4)	115	(2.5)
N stage																
N0	52	(7.5)	49	(5.3)	115	(7.4)	179	(5.5)	35	(6.7)	83	(5.1)	42	(9.8)	199	(4.3)
N1	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	66	(12.5)	211	(13.1)	22	(5.1)	282	(6.1)
NX	639	(92.3)	869	(94.7)	1434	(92.4)	3074	(94.2)	423	(80.4)	1318	(81.6)	364	(85.0)	4164	(89.5)
Missing	1	(0.1)	0	(0.0)	3	(0.2)	9	(0.3)	2	(0.4)	4	(0.2)	0	(0.0)	9	(0.2)
M stage																
M0	326	(47.1)	267	(29.1)	902	(58.1)	1300	(39.9)	364	(69.2)	776	(48.0)	169	(39.5)	701	(15.1)
M1	-	-	-	-	-	-	-	-	-	-	-	-	192	(44.9)	2979	(64.0)
MX	366	(52.9)	651	(70.9)	650	(41.9)	1962	(60.1)	162	(30.8)	840	(52.0)	67	(15.7)	974	(20.9)
Gleason Grade Group																
GGG1	113	(16.3)	171	(18.6)	208	(13.4)	265	(8.1)	61	(11.6)	117	(7.2)	25	(5.8)	142	(3.1)
GGG2	307	(44.4)	371	(40.4)	316	(20.4)	434	(13.3)	96	(18.3)	218	(13.5)	65	(15.2)	379	(8.1)
GGG3	244	(35.3)	308	(33.6)	292	(18.8)	562	(17.2)	117	(22.2)	284	(17.6)	99	(23.1)	714	(15.3)
GGG4	0	(0.0)	0	(0.0)	415	(26.7)	875	(26.8)	132	(25.1)	398	(24.6)	101	(23.6)	1275	(27.4)
GGG5	0	(0.0)	0	(0.0)	238	(15.3)	923	(28.3)	90	(17.1)	454	(28.1)	104	(24.3)	1600	(34.4)
Missing	28	(4.0)	68	(7.4)	83	(5.3)	203	(6.2)	30	(5.7)	145	(9.0)	34	(7.9)	544	(11.7)
Percentage positive biopsy cores																
0-49%	231	(33.4)	248	(27.0)	373	(24.0)	479	(14.7)	79	(15.0)	144	(8.9)	37	(8.6)	275	(5.9)
50-74%	193	(27.9)	227	(24.7)	385	(24.8)	575	(17.6)	74	(14.1)	202	(12.5)	66	(15.4)	469	(10.1)
75-100%	149	(21.5)	176	(19.2)	491	(31.6)	1318	(40.4)	242	(46.0)	803	(49.7)	234	(54.7)	2647	(56.9)
Missing	119	(17.2)	267	(29.1)	303	(19.5)	890	(27.3)	131	(24.9)	467	(28.9)	91	(21.3)	1263	(27.1)
PSA at diagnosis, ng/ml																
<3	10	(1.4)	14	(1.5)	21	(1.4)	32	(1.0)	1	(0.2)	12	(0.7)	1	(0.2)	26	(0.6)
3-10	264	(38.2)	290	(31.6)	222	(14.3)	431	(13.2)	16	(3.0)	52	(3.2)	11	(2.6)	129	(2.8)
10-20	405	(58.5)	593	(64.6)	352	(22.7)	768	(23.5)	35	(6.7)	78	(4.8)	22	(5.1)	171	(3.7)
20-50	-	-	-	-	942	(60.7)	1968	(60.3)	51	(9.7)	183	(11.3)	48	(11.2)	419	(9.0)

50+	-	-	-	-	-	-	-	-	423	(80.4)	1277	(79.0)	345	(80.6)	3822	(82.1)
Missing	13	(1.9)	21	(2.3)	15	(1.0)	63	(1.9)	0	(0.0)	14	(0.9)	1	(0.2)	87	(1.9)
Charlson comorbidity index																
0	375	(54.2)	463	(50.4)	884	(57.0)	1771	(54.3)	333	(63.3)	931	(57.6)	294	(68.7)	2886	(62.0)
1	184	(26.6)	248	(27.0)	394	(25.4)	781	(23.9)	112	(21.3)	372	(23.0)	85	(19.9)	1030	(22.1)
2	77	(11.1)	107	(11.7)	164	(10.6)	386	(11.8)	44	(8.4)	191	(11.8)	30	(7.0)	391	(8.4)
3+	56	(8.1)	100	(10.9)	110	(7.1)	324	(9.9)	37	(7.0)	122	(7.5)	19	(4.4)	347	(7.5)
Marital status																
Married	479	(69.2)	572	(62.3)	1036	(66.8)	2080	(63.8)	352	(66.9)	977	(60.5)	268	(62.6)	2773	(59.6)
Not married	213	(30.8)	346	(37.7)	516	(33.2)	1182	(36.2)	174	(33.1)	639	(39.5)	160	(37.4)	1881	(40.4)
Education level																
High	131	(18.9)	113	(12.3)	301	(19.4)	458	(14.0)	91	(17.3)	221	(13.7)	95	(22.2)	769	(16.5)
Middle	249	(36.0)	310	(33.8)	531	(34.2)	1039	(31.9)	185	(35.2)	551	(34.1)	154	(36.0)	1590	(34.2)
Low	304	(43.9)	488	(53.2)	705	(45.4)	1717	(52.6)	243	(46.2)	815	(50.4)	171	(40.0)	2226	(47.8)
Missing	8	(1.2)	7	(0.8)	15	(1.0)	48	(1.5)	7	(1.3)	29	(1.8)	8	(1.9)	69	(1.5)

*Screening defined in NPCR as PSA testing as a part of a health care check-up in a symptomless ma

Table 19. Baseline characteristics of men in PCBaSe 3.0 diagnosed with high-risk and regionally metastatic prostate cancer in 2006-2012 and treated with anti-androgen monotherapy or GnRH agonists.

	Raw				Imputed*				Propensity score matched			
	Anti-androgens N = 2,078		GnRH agonists N = 4,878		Anti-androgens N = 2,060		GnRH agonists N = 4,740		Anti-androgens N = 1,975		GnRH agonists N = 1,975	
	N	%	N	%	N	%	N	%	N	%	N	%
Year of diagnosis												
2006-2007	542	(26.1)	1692	(34.7)	538	(26.1)	1638	(34.6)	527	(26.7)	547	(27.7)
2008-2010	559	(26.9)	1476	(30.3)	548	(26.6)	1415	(29.9)	532	(26.9)	532	(26.9)
2011-2012	977	(47.0)	1710	(35.1)	974	(47.3)	1687	(35.6)	916	(46.4)	896	(45.4)
Age at diagnosis, years												
<70	328	(15.8)	699	(14.3)	327	(15.9)	681	(14.4)	311	(15.7)	357	(18.1)
70-74	414	(19.9)	751	(15.4)	409	(19.9)	735	(15.5)	382	(19.3)	342	(17.3)
75-79	688	(33.1)	1287	(26.4)	682	(33.1)	1247	(26.3)	654	(33.1)	548	(27.7)
80-84	470	(22.6)	1367	(28.0)	464	(22.5)	1323	(27.9)	454	(23.0)	485	(24.6)
85-90	178	(8.6)	774	(15.9)	178	(8.6)	754	(15.9)	174	(8.8)	243	(12.3)
Mode of detection												
Screening	562	(27.1)	933	(19.1)	575	(27.9)	936	(19.7)	536	(27.1)	510	(25.8)
LUTS	1016	(48.9)	2836	(58.2)	1045	(50.7)	2852	(60.2)	1021	(51.7)	1058	(53.6)
Symptoms	431	(20.8)	966	(19.8)	440	(21.4)	952	(20.1)	418	(21.2)	407	(20.6)
Missing	67	(3.2)	139	(2.9)								
Clinical tumour category												
T1a	3	(0.1)	13	(0.3)	3	(0.1)	13	(0.3)	3	(0.2)	2	(0.1)
T1b	29	(1.4)	66	(1.4)	30	(1.5)	66	(1.4)	30	(1.5)	28	(1.4)
T1c	374	(18.0)	640	(13.1)	375	(18.2)	639	(13.5)	342	(17.3)	335	(17.0)
T2	639	(30.8)	1634	(33.5)	645	(31.3)	1612	(34.0)	629	(31.8)	652	(33.0)

T3	937	(45.1)	2207	(45.2)	932	(45.2)	2157	(45.5)	897	(45.4)	882	(44.7)
T4	76	(3.7)	262	(5.4)	75	(3.6)	253	(5.3)	74	(3.7)	76	(3.8)
TX	20	(1.0)	56	(1.1)								
N stage												
N0	150	(7.2)	262	(5.4)	150	(7.3)	263	(5.5)	145	(7.3)	133	(6.7)
N1	66	(3.2)	211	(4.3)	66	(3.2)	207	(4.4)	66	(3.3)	64	(3.2)
NX	1857	(89.4)	4392	(90.0)	1844	(89.5)	4270	(90.1)	1764	(89.3)	1778	(90.0)
Missing	5	(0.2)	13	(0.3)								
Gleason Grade Group												
GGG1	269	(12.9)	382	(7.8)	274	(13.3)	394	(8.3)	254	(12.9)	221	(11.2)
GGG2	412	(19.8)	652	(13.4)	444	(21.6)	725	(15.3)	415	(21.0)	385	(19.5)
GGG3	409	(19.7)	846	(17.3)	433	(21.0)	908	(19.2)	410	(20.8)	410	(20.8)
GGG4	547	(26.3)	1273	(26.1)	564	(27.4)	1308	(27.6)	552	(27.9)	568	(28.8)
GGG5	328	(15.8)	1377	(28.2)	345	(16.7)	1405	(29.6)	344	(17.4)	391	(19.8)
Missing	113	(5.4)	348	(7.1)								
Percent positive biopsy cores												
0-49%	452	(21.8)	623	(12.8)	570	(27.7)	838	(17.7)	528	(26.7)	507	(25.7)
50-74%	459	(22.1)	777	(15.9)	562	(27.3)	1049	(22.1)	526	(26.6)	499	(25.3)
75-100%	733	(35.3)	2121	(43.5)	928	(45.0)	2853	(60.2)	921	(46.6)	969	(49.1)
Missing	434	(20.9)	1357	(27.8)								
PSA at diagnosis, ng/ml												
<3	22	(1.1)	44	(0.9)	23	(1.1)	43	(0.9)	23	(1.2)	18	(0.9)
03-Oct	238	(11.5)	483	(9.9)	238	(11.6)	477	(10.1)	223	(11.3)	230	(11.6)
Oct-20	387	(18.6)	846	(17.3)	388	(18.8)	840	(17.7)	376	(19.0)	376	(19.0)
20-50	993	(47.8)	2151	(44.1)	988	(48.0)	2111	(44.5)	936	(47.4)	936	(47.4)
50+	423	(20.4)	1277	(26.2)	423	(20.5)	1269	(26.8)	417	(21.1)	415	(21.0)
Missing	15	(0.7)	77	(1.6)								
CCI												

0	1217	(58.6)	2702	(55.4)	1206	(58.5)	2618	(55.2)	1148	(58.1)	1168	(59.1)
1	506	(24.4)	1153	(23.6)	501	(24.3)	1124	(23.7)	486	(24.6)	459	(23.2)
2	208	(10.0)	577	(11.8)	206	(10.0)	565	(11.9)	199	(10.1)	205	(10.4)
3+	147	(7.1)	446	(9.1)	147	(7.1)	433	(9.1)	142	(7.2)	143	(7.2)
Marital status												
Married	1388	(66.8)	3057	(62.7)	1375	(66.7)	2969	(62.6)	1315	(66.6)	1335	(67.6)
Not married	690	(33.2)	1821	(37.3)	685	(33.3)	1771	(37.4)	660	(33.4)	640	(32.4)
Education level												
High	392	(18.9)	679	(13.9)	394	(19.1)	670	(14.1)	367	(18.6)	335	(17.0)
Middle	716	(34.5)	1590	(32.6)	717	(34.8)	1564	(33.0)	682	(34.5)	689	(34.9)
Low	948	(45.6)	2532	(51.9)	949	(46.1)	2506	(52.9)	926	(46.9)	951	(48.2)
Missing	22	(1.1)	77	(1.6)								

*Results obtained after imputation of missing values and following matching on propensity score. Results from the first imputed dataset presented

Table 20. Risk of death from prostate cancer or death from all causes in men on primary anti-androgen monotherapy or GnRH agonists.

	All men with high-risk and regionally metastatic prostate cancer				Men with high-risk*prostate cancer				Men with regionally metastatic**prostate cancer			
	Death from prostate cancer		Death from all causes		Death from prostate cancer		Death from all causes		Death from prostate cancer		Death from all causes	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Anti-androgen monotherapy	1.00	(Ref)	1.00	(Ref)								
GnRH agonists												
Crude model using age as time scale	1.39	(1.23-1.57)	1.45	(1.34-1.57)	1.45	(1.24-1.69)	1.50	(1.37-1.65)	1.16	(0.96-1.42)	1.26	(1.09-1.46)
Adjustment												
T stage	1.37	(1.21-1.55)	1.45	(1.34-1.57)	1.44	(1.23-1.68)	1.51	(1.37-1.66)	1.16	(0.95-1.41)	1.26	(1.09-1.46)
+ Gleason Grade Group	1.21	(1.07-1.37)	1.37	(1.27-1.48)	1.25	(1.07-1.47)	1.44	(1.30-1.58)	1.06	(0.87-1.29)	1.19	(1.03-1.37)
+ PSA***	1.19	(1.05-1.34)	1.36	(1.25-1.47)	1.24	(1.06-1.46)	1.43	(1.30-1.57)	1.04	(0.85-1.27)	1.18	(1.02-1.36)
+ Proportion positive biopsy cores****	1.15	(1.01-1.30)	1.34	(1.23-1.45)	1.19	(1.01-1.39)	1.40	(1.27-1.55)	1.02	(0.84-1.25)	1.17	(1.01-1.35)
+ Imaging performed	1.11	(0.98-1.25)	1.29	(1.19-1.40)	1.15	(0.98-1.34)	1.36	(1.23-1.50)	0.96	(0.79-1.18)	1.11	(0.96-1.29)
+ Time between diagnosis and start of treatment	1.11	(0.98-1.26)	1.29	(1.19-1.40)	1.15	(0.98-1.35)	1.36	(1.23-1.50)	0.97	(0.79-1.18)	1.11	(0.95-1.28)
+ Mode of detection	1.10	(0.97-1.25)	1.28	(1.18-1.39)	1.13	(0.96-1.33)	1.34	(1.21-1.48)	0.96	(0.78-1.18)	1.10	(0.95-1.28)
+ Year of diagnosis	1.09	(0.96-1.23)	1.27	(1.17-1.38)	1.12	(0.95-1.31)	1.33	(1.20-1.47)	0.96	(0.78-1.17)	1.10	(0.95-1.28)
+ CCI	1.08	(0.96-1.23)	1.25	(1.15-1.36)	1.11	(0.95-1.31)	1.30	(1.18-1.43)	0.96	(0.78-1.17)	1.10	(0.95-1.27)
+ Marital status	1.08	(0.96-1.23)	1.24	(1.14-1.35)	1.11	(0.95-1.31)	1.29	(1.17-1.43)	0.96	(0.78-1.17)	1.09	(0.94-1.26)
+ Education	1.08	(0.95-1.23)	1.23	(1.13-1.34)	1.11	(0.94-1.31)	1.28	(1.16-1.41)	0.96	(0.78-1.17)	1.09	(0.94-1.26)

Hazard ratios calculated by use of Cox regression analyses
* High-risk prostate cancer: T3 and/or PSA 20 ng/ml or higher and lower than 50 ng/ml and/or Gleason Grade Group 4-5
** Regionally metastatic prostate cancer: T4 and/or PSA 50 ng/ml or higher and lower than 100 ng/ml or N1
*** Modelled using a linear spline with knots in PSA 3, 10, 20 and 50
**** Modelled as an interaction with T stage in men not diagnosed following TURP

Table 21. Risk of death from prostate cancer or death from all causes in men treated with primary anti-androgen monotherapy or GnRH agonists.

	All men with high-risk and regional metastatic prostate cancer				Men with high-risk prostate cancer				Men with regional metastatic prostate cancer			
	Death from all causes		Death from prostate cancer		Death from all causes		Death from prostate cancer		Death from all causes		Death from prostate cancer	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Treatment												
Anti-androgen monotherapy	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
GnRH agonists	1.23	(1.14-1.34)	1.09	(0.96-1.24)	1.12	(0.95-1.32)	1.28	(1.16-1.42)	0.96	(0.79-1.18)	1.09	(0.94-1.27)
Clinical tumuor category												
T1a/ T1b	0.99	(0.74-1.32)	1.68	(1.14-2.48)	1.64	(1.04-2.58)	0.99	(0.71-1.39)	1.79	(0.78-4.11)	1.11	(0.58-2.13)
T1c	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
T2	1.01	(0.91-1.13)	1.13	(0.93-1.37)	1.06	(0.84-1.34)	1.00	(0.88-1.14)	1.26	(0.90-1.76)	1.09	(0.87-1.35)
T3	1.14	(1.02-1.27)	1.28	(1.06-1.55)	1.28	(1.01-1.62)	1.13	(0.99-1.28)	1.30	(0.93-1.82)	1.17	(0.95-1.45)
T4	1.48	(1.24-1.77)	1.91	(1.46-2.50)	NA	NA	NA	NA	1.32	(0.87-1.98)	1.28	(0.98-1.68)
Gleason Grade Group												
GGG 1	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
GGG 2	1.11	(0.96-1.29)	1.43	(1.08-1.90)	1.48	(1.02-2.17)	1.03	(0.86-1.22)	1.36	(0.89-2.07)	1.38	(1.05-1.82)
GGG 3	1.29	(1.12-1.49)	1.72	(1.32-2.25)	1.67	(1.15-2.42)	1.13	(0.95-1.33)	1.72	(1.14-2.59)	1.76	(1.34-2.31)
GGG 4	1.39	(1.22-1.60)	2.34	(1.79-3.06)	2.50	(1.74-3.60)	1.23	(1.04-1.44)	2.17	(1.46-3.23)	1.91	(1.47-2.49)
GGG 5	1.75	(1.53-2.02)	3.44	(2.63-4.50)	3.89	(2.68-5.64)	1.56	(1.32-1.83)	2.92	(1.97-4.32)	2.36	(1.82-3.08)
Proportion positive biopsy cores *												
<50%	0.90	(0.80-1.02)	0.76	(0.63-0.92)	0.74	(0.58-0.94)	0.93	(0.82-1.06)	0.84	(0.53-1.31)	0.83	(0.62-1.10)
50-74%	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
75-89%	1.00	(0.84-1.18)	1.01	(0.78-1.31)	1.03	(0.78-1.37)	1.06	(0.88-1.27)	0.95	(0.57-1.56)	0.87	(0.66-1.15)
90+%	1.07	(0.95-1.20)	1.16	(0.98-1.37)	1.18	(0.94-1.47)	1.09	(0.96-1.25)	1.09	(0.81-1.48)	0.97	(0.78-1.19)
PSA at diagnosis, ng/mL												
<3	1.47	(1.05-2.05)	1.91	(1.20-3.04)	1.73	(0.97-3.06)	1.26	(0.85-1.88)	1.93	(0.79-4.72)	2.01	(0.95-4.26)

3-10	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
10-20	0.98	(0.86-1.12)	0.96	(0.77-1.19)	0.99	(0.78-1.27)	1.00	(0.87-1.15)	0.77	(0.47-1.25)	0.83	(0.56-1.21)
20-50	1.07	(0.95-1.20)	1.01	(0.84-1.22)	1.07	(0.87-1.32)	1.10	(0.97-1.24)	0.74	(0.48-1.14)	0.68	(0.48-0.97)
50+	1.16	(1.02-1.31)	1.27	(1.03-1.55)	1.46	(0.34-6.25)	1.13	(0.30-4.23)	0.54	(0.36-0.82)	0.68	(0.49-0.94)
Mode of detection												
Screening	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
LUTS	1.28	(1.16-1.41)	1.44	(1.22-1.70)	1.42	(1.15-1.76)	1.28	(1.14-1.44)	1.43	(1.11-1.85)	1.24	(1.05-1.46)
Symptoms	1.37	(1.22-1.53)	1.51	(1.25-1.83)	1.40	(1.10-1.79)	1.34	(1.17-1.55)	1.61	(1.21-2.15)	1.40	(1.16-1.70)
Bone imaging performed												
No	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
Yes	0.87	(0.81-0.94)	0.86	(0.77-0.97)	0.86	(0.74-1.01)	0.88	(0.80-0.97)	0.75	(0.62-0.91)	0.80	(0.70-0.92)
Year of diagnosis, By one year increase	0.97	(0.95-0.99)	0.95	(0.92-0.98)	0.95	(0.91-0.99)	0.97	(0.94-0.99)	0.98	(0.93-1.02)	0.98	(0.95-1.01)
Time from diagnosis to treatment initiation, by six month delay	1.00	(0.99-1.01)	1.01	(1.00-1.02)	1.00	(0.99-1.02)	1.00	(0.99-1.01)	1.00	(0.99-1.02)	1.00	(0.99-1.01)
Charlson comorbidity index												
0	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
1	1.21	(1.12-1.32)	0.96	(0.84-1.10)	0.91	(0.76-1.08)	1.18	(1.07-1.31)	1.06	(0.87-1.30)	1.26	(1.09-1.45)
2	1.46	(1.31-1.62)	0.82	(0.68-1.00)	0.89	(0.70-1.12)	1.45	(1.27-1.64)	0.73	(0.52-1.02)	1.47	(1.21-1.78)
3+	1.88	(1.68-2.09)	1.08	(0.88-1.32)	1.16	(0.91-1.48)	2.06	(1.81-2.33)	0.99	(0.68-1.42)	1.49	(1.19-1.87)
Marital status												
Married	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
Not married	1.08	(1.00-1.15)	1.00	(0.89-1.11)	1.00	(0.87-1.15)	1.08	(0.99-1.17)	1.04	(0.87-1.23)	1.09	(0.97-1.23)
Education level												
High	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref	1.00	Ref
Middle	1.18	(1.06-1.30)	1.03	(0.88-1.19)	1.02	(0.84-1.24)	1.22	(1.08-1.38)	1.05	(0.82-1.34)	1.07	(0.90-1.28)
Low	1.07	(0.96-1.19)	0.98	(0.84-1.15)	0.98	(0.80-1.20)	1.12	(0.98-1.27)	1.00	(0.77-1.28)	0.99	(0.82-1.18)

Hazard ratios calculated by use of Cox regression analyses.

* Modelled as an interaction with diagnosis following TURP.

Table 22. Risk of death from prostate cancer or death from all causes for men on primary GnRH agonists or anti-androgen monotherapy (AA, reference in analyses) following propensity score matching.

	Including all men with a match						
	Number of men in group*	No of events		Crude		Adjusted	
		AA	GnRH	HR	95% CI	HR	95% CI
High-risk and regionally metastatic prostate cancer							
Death from prostate cancer	1975	348	371	1.09	(0.94-1.26)	1.05	(0.90-1.23)
Death from all causes	1975	702	858	1.25	(1.13-1.38)	1.23	(1.11-1.36)
High-risk prostate cancer**							
Death from prostate cancer	1436	209	239	1.15	(0.91-1.45)	1.12	(0.88-1.42)
Death from all causes	1436	473	619	1.33	(1.18-1.50)	1.29	(1.14-1.46)
Regionally metastatic prostate cancer***							
Death from prostate cancer	506	132	123	0.96	(0.72-1.28)	0.93	(0.69-1.43)
Death from all causes	506	215	237	1.11	(0.89-1.37)	1.12	(0.89-1.40)

Hazard ratios calculate by use of Cox regression analyses. The median time from diagnosis to start of treatment was 16 days longer for men on AA compared to men on GnRH agonists
*Number of men in the first of the imputed dataset
** High-risk prostate cancer: T3 and/or PSA 20 ng/ml or higher and lower than 50 ng/ml and/or Gleason Grade Group 4-5
** *Regionally metastatic prostate cancer: T4 and/or PSA 50 ng/ml or higher and lower than 100 ng/ml or N1

Figure 20 Cumulative incidence of PCa-death and other death

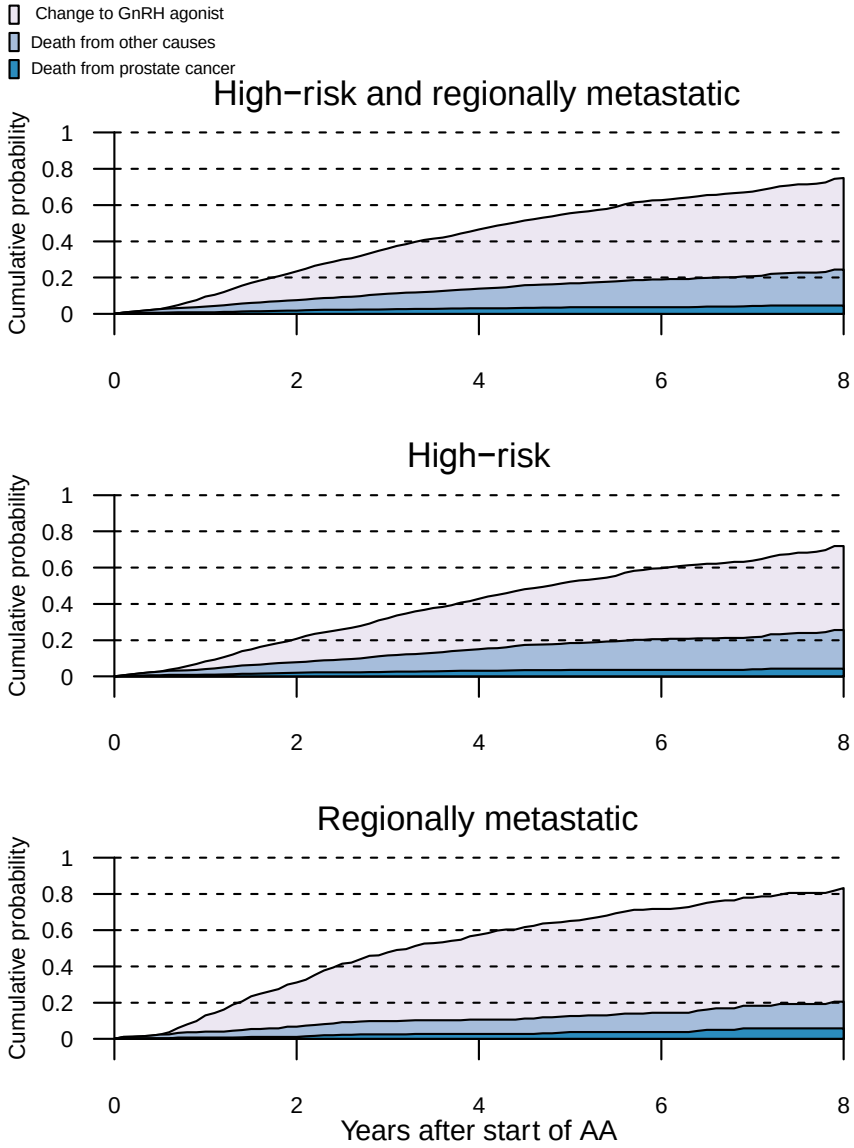


Figure 21 Cumulative incidence by AA-GnRH and Risk group

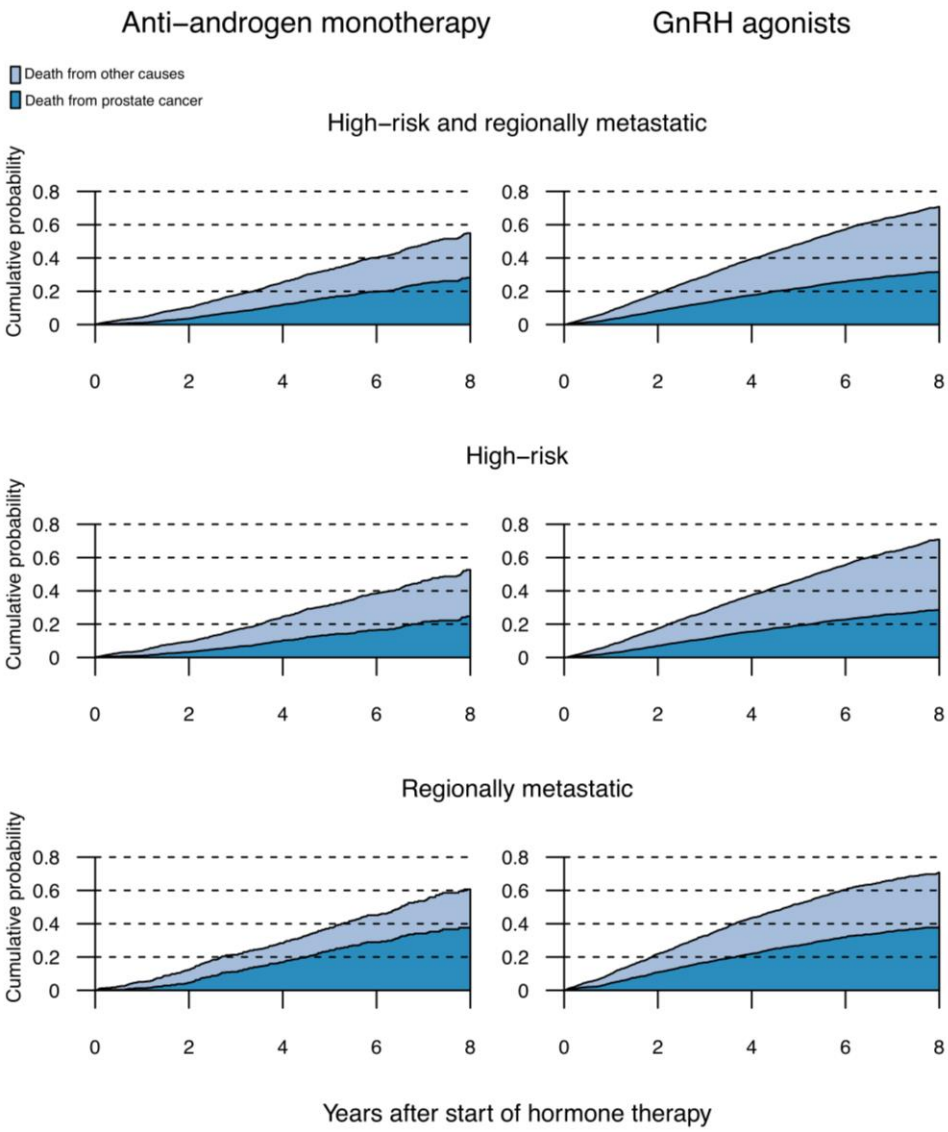
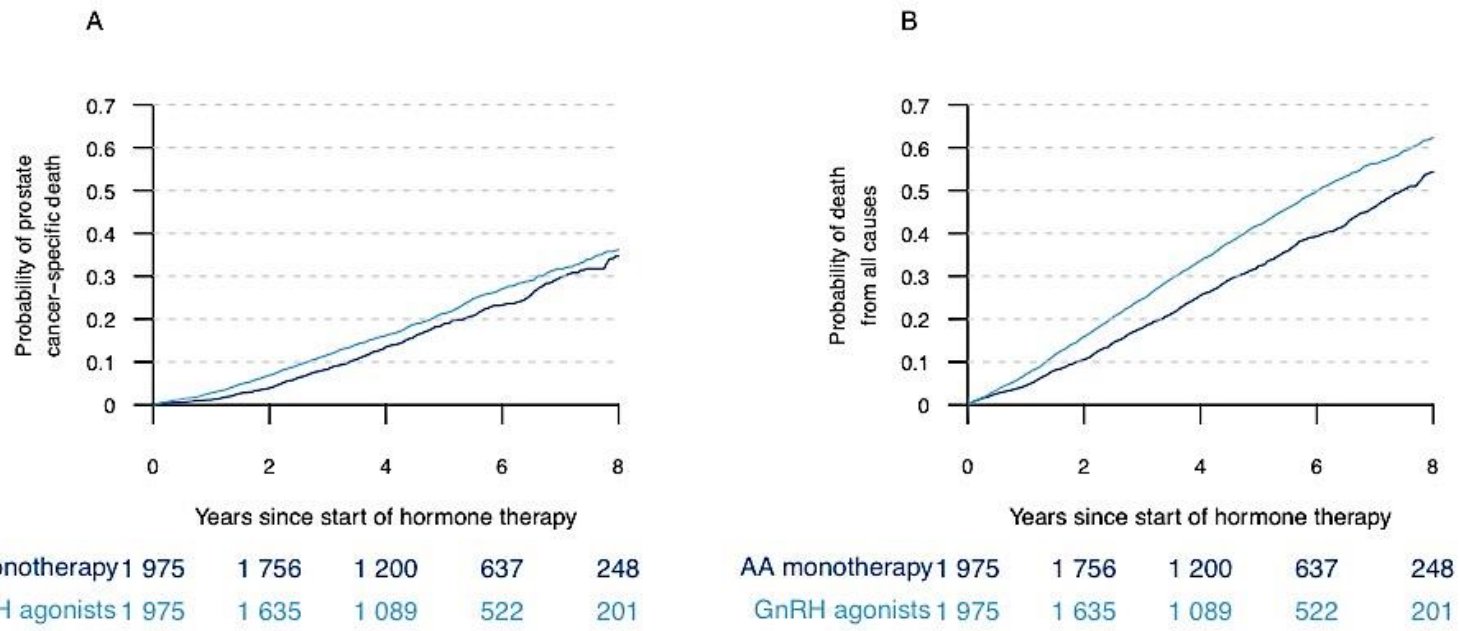


Figure 22 One minus Kaplan-Meier, PCa-death and other death



4.4.4. Discussion

In this register-based, observational study of men with advanced, non-metastatic prostate cancer treated with primary hormonal therapy, men on AA had similar prostate cancer mortality and lower all-cause mortality than men on GnRH agonists.

As previously mentioned two RCTs conducted in the 1990s showed similar risk of prostate cancer death in men with advanced, non-metastatic prostate cancer on AA and men on GnRH agonists (169, 170). Even though RCTs are considered the gold standard for comparisons of treatments, when it comes to show clinical effectiveness observational studies are more accurate (273). The guidelines from the European GetReal consortium ("incorporating real-life data into drug development") specifically recommend considering evidence from pragmatic trials and non-randomised studies to improve applicability of treatment effect estimates, inform disconnected or scarce networks of evidence, identify patient populations that will likely receive the drug after launch, and to improve relevant to decision/policy makers and patients (276). Given that including more patients in an observational study may be associated with a low cost, usually these studies count with large number of participants who may be more representative of the general population. For instance, in the RCTs mentioned before there were 320 patients recruited in one and 480 in the other versus 6,956 from this study. Furthermore, some of the RCTs limitations include: high selectivity of participants enrolled which results in an underrepresentation of individuals seen in practice in certain characteristics like age, comorbidities, and socioeconomic status (277, 278). In the current register-based, observational study, all men with relevant cancer characteristics were included regardless of other characteristics, with the exception of very high age (>90 years) and a previous cancer diagnosis.

A general limitation of observational studies comparing the outcome of treatment is the possibility of channelling bias or confounding by indication. Confounding by indication may occur if the factors associated with the indication for treatment are also associated with the outcome under study. In the current study, men managed with AA were younger, diagnosed in more recent calendar years and had less adverse cancer characteristics compared to men treated with GnRH agonists. All these factors could

be associated with a lower mortality. We adjusted for these differences in the traditional Cox regression analyses and found that the results were in line with previous RCTs with similar risk of prostate cancer death in men on AA and men on GnRH agonists (169, 170). However, lower all-cause mortality among men treated with AA was also observed. Unaccounted confounders are likely to explain some of this difference, as it can be hypothesised that in a clinical setting, results from treatment with AA will be better than for GnRH agonists because men on AA have smaller cancer burden and lower comorbidity resulting in a lower risk of death from all causes.

In order to account for differences between treatment groups that could cause confounding by indication propensity scores were calculated for the propensity of AA treatment. These scores were used to match groups of men on AA and men on GnRH agonists respectively. One limitation propensity score matching has in common with traditional multivariable Cox regression analyses is the inability to adjust for unmeasured confounders. However, a matched propensity score analysis excludes exposed participants who have no comparable unexposed participant and vice versa (279). Propensity score matching does not assume linearity in the relationship between the propensity and outcome and allows for simple, transparent analyses. It provides a better balance of covariates between exposed and unexposed groups compared to other matching strategies in datasets with many covariates (280). Interestingly, the propensity score matched analyses revealed similar results as the traditional multivariable Cox regression analyses.

Considering that we have applied methods used to reduce confounding and make the comparison groups as similar as possible in baseline characteristics, our results suggest that the found differences are likely to be due to the drugs mechanism of action and side effects. More specifically, antiandrogens are known to block the androgen receptor, with androgen levels remaining normal, whereas GnRH agonists lower androgen levels. Low levels of androgens have been associated with increased levels of LDL, triglycerides and insulin, all risk factors of CVD (281). Furthermore, testosterone may have a protective effect against the development of atheroma through coronary artery dilation(282). Further effects via the immune system have also been described.

The GnRH receptor is expressed on T lymphocytes, which can be found in atheroma. T-cell activation upon application of GnRH agonists has been observed in experimental studies. Plaque instability can increase the risk of thrombo-embolic complications (283-285).

As mentioned above, a main limitation of the present study is the non-random allocation to type of hormonal therapy, with ensuing possible confounding by indication and illustrated by younger and healthier men with less advanced cancer to receive AA. However, as previously discussed use of propensity score matching did not alter the main findings. In a recent study using STAMPEDE data, authors found that high-risk non-metastatic prostate cancer two-year survival was 96% (95% CI, 93%-97%) and 2-year failure free survival was 77% (95% CI, 73%-81%). Median failure free survival was 63 months. Therefore, a potential further limitation of the present study may be a short follow up period (286).

Strengths of this study include the nationwide, population-based cohort of men with comprehensive data from a clinical cancer register with documented high data quality as well as several other high-quality health care registers (287, 288) a setting that thus provides strong real-world data.

4.4.5. Conclusion

In men with advanced, non-metastatic prostate cancer, prostate cancer-specific mortality was similar and all-cause mortality was lower in men primarily treated with AA compared to men treated with GnRH agonists. Our data provide further support for the use of AA as primary hormonal therapy in men with advanced, non-metastatic prostate cancer.

5. Chapter 5: Conclusion and future directions

When I started this thesis, I wanted to investigate the impact of a prostate cancer diagnosis and its treatments on a man's quality and quantity of life, by looking at less understood and studied exposures and outcomes. Overall, the findings of all projects involved helped generate hypotheses and ideas for future studies. In the following section, I give a brief summary of each of my projects results followed by proposals for follow-up projects to be carried out in the near future.

To the best of my knowledge, my findings in the AMORIS study of an association between elevated lipid levels and GGT and SDPTs are the first of its kind and call for further investigation. However, it is important to highlight that variation in lipid levels and GGT may be a proxy for other exposures, such as lifestyle factors, which were only taken into account by use of the Charlson Comorbidity Index. Hence, the current findings may reflect the association between any other unknown biological changes and the risk of SDPTs. Whether these biomarkers alterations caused further changes that led to carcinogenesis or are a common underlying alteration remains unknown. Hence, pre-clinical studies are necessary to support the findings and related generated hypothesis.

The findings from the radiotherapy and thromboembolic events project are also the first of its kind. As mentioned before, it is important to acknowledge study limitations, such as the impossibility to present subgroup analysis according to the type of external beam radiotherapy or brachytherapy and the rather short follow up period. Therefore, a follow-up of this study is of interest to further establish if type of radiotherapy, dose of radiotherapy, or longer time after exposure affect the current findings.

My study on 'metabolic drugs' and prostate cancer death and overall mortality amongst men treated with GnRH agonists is also one of the few studies currently investigating this question. So far, most studies focused on the use of metabolic drugs after GnRH agonists initiation in the context of adverse effects. Therefore, my results show that receiving treatment for symptoms of metabolic syndrome is not a

contraindication for GnRH agonists. However, as this was an observational study, it remains difficult to tease out whether I studied the metabolic symptoms or their related drugs.

My findings in the last project provided support that antiandrogens might be an alternative to GnRH agonists. When physicians and patients have the option to use different medications, understanding how they compare in terms of efficacy and adverse effects is key. Given the diversity of drugs available for men with advanced prostate cancer, it would be of interest to design studies where all these drugs are compared with a focus on both cancer specific (prostate cancer death, progression) and treatment-related outcomes (side effects).

In conclusion, the current thesis provides more insight into how prostate cancer and its treatments affect the quality and quantiy of life of men with prostate cancer. For each of the projects the following are potential follow-up studies:

(1) Longitudinal studies using the lifecourse approach to get a better understanding of how different metabolisms are associated with development of different cancers
(2) Assessment of TED risk comparing different types and dosages of radiotherapy
(3) Window-of-opportunity trials to identify how drugs for metabolic components or the metabolic factors themselves are biologically affecting prostate cancer progression in the context of other prostate cancer treatments
(4) Inclusion of anti-androgen monotherapy as a treatment arm in future RCTs for men with advanced prostate cancer

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7. Appendix I

Metabolism biomarkers measured before prostate cancer diagnosis and second primary tumours: a prospective study in the Swedish AMORIS cohort.

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BACKGROUND

- Due to advances in detection and treatment, the number of men living with prostate cancer **(PCa)** is increasing.
- Men with PCa are at an increased risk of a second primary tumour **(SPT)**.
- Little is known about the potential biochemical mechanisms linking PCa with the occurrence of SPTs.
- **HYPOTHESIS**: Elevated serum levels : **glucose (Glu), fructosamine (FAMN), triglycerides (TGC), total cholesterol (TC), and gamma-glutamyl transferase (GGT)** measured before PCa diagnosis may be associated with development of SPTs – either because these biomarkers activate a shared carcinogenic mechanism or because they are the consequence of a common underlying alteration (Figure 1).

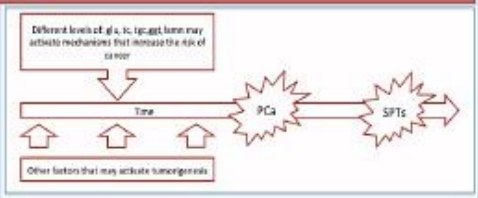


Figure 1. Potential biological mechanisms linking PCa and SPTs.

METHODS

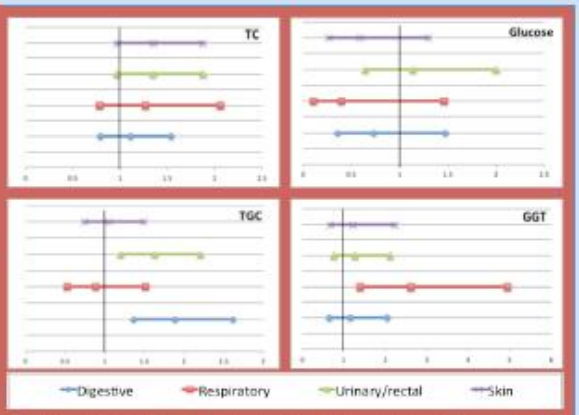
- From the Swedish AMORIS cohort, we selected all men diagnosed with PCa between 1996 and 2011 who had at least one of the following biomarkers measured before PCa diagnosis (n=10,794): **GLU, FAMN, TGC, TC, GGT.**
- Multivariate Cox proportional hazards models were used to determine the hazard ratios (HR) for risk of SPTs by levels of the five biomarkers of interest (according to set medical cut-offs). Subtypes of SPTs were also evaluated.

RESULTS

- 811 SPTs were diagnosed during a median follow-up time of 5 years.
- There was a positive association with SPTs for triglycerides, total cholesterol, and GGT when comparing elevated levels (according to clinical cut-offs) with normal levels (HR: 1.37 (95%CI: 1.17-1.6); 1.22 (1.22-1.42); 1.32 (1.01-1.71), respectively).

		Univariate		Multivariate		p-value
	Biomarker	HR	95% CI	HR	95% CI	
According to set clinical Cut-offs	GLU	1.11	0.84-1.46	0.87	0.64-1.19	0.42
	TGC	**1.37**	**1.18-1.58**	**1.37**	**1.17-1.6**	**<0.001**
	TC	**1.33**	**1.15-1.54**	**1.22**	**1.04-1.42**	**0.01**
	GGT	**1.37**	**1.07-1.76**	**1.32**	**1.02-1.71**	**0.03**
	FAMN	1.27	0.88-1.82	0.91	0.59-1.39	0.64

Table 1. Univariate and multivariate Cox proportional regression analyses for the association between biomarkers and risk of SPTs. Adjusted for age, the remaining biomarkers, fasting status, PCa treatment, body mass index , comorbidities at PCa diagnosis, diabetes at PCa diagnosis, time between blood test and PCa diagnosis, and education .



Panel A. Multivariate Cox proportional regression analysis for the association between biomarkers and specific SPTs

CONCLUSION

Biomarkers of lipid metabolism and GGT measured before PCa diagnosis were associated with a higher risk of developing specific types SPTs. Our results point towards the need for large prospective cohort studies with repeated measurements to disentangle common aetiological mechanisms for primary and secondary tumours.

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Appendix II

Radiotherapy and the risk of thromboembolic disease in men with prostate cancer.

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Introduction

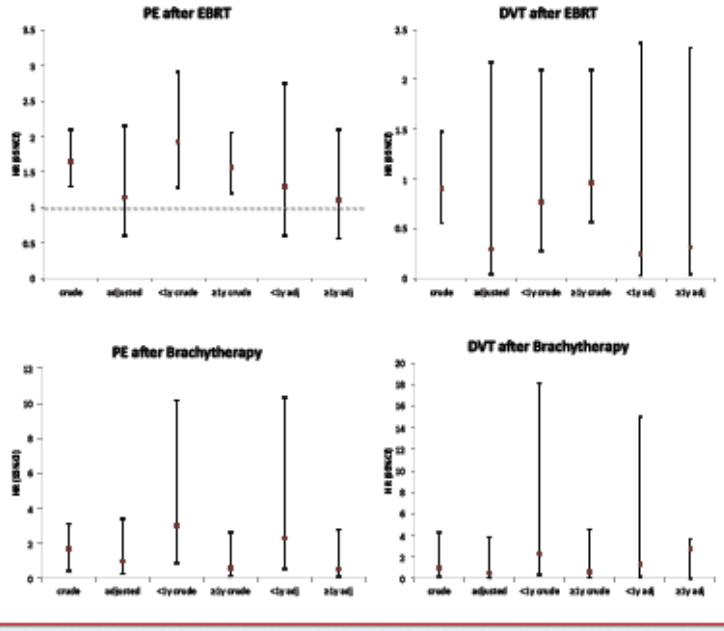
- Using data from PCBaSe Sweden, we have previously shown that the risk of deep venous thrombosis (DVT) and pulmonary embolism (PE) is increased among men with prostate cancer (PCa), especially among those who undergo surgery or are treated with androgen deprivation therapy.
- Several case reports and experimental studies have now also suggested an association between radiotherapy (RT) and risk of TED.
- The current study therefore investigates in detail risk of TED following RT in men with PCa.

Study population and methods

- Apart from data on tumour characteristics and primary treatment for men with PCa, PCBaSe Sweden also contains a Retrospective collection of Radiotherapy data (RetroRad).
- We identified all men who received RT as curative treatment (n=71,352) and grouped them into external beam RT (EBRT) or brachytherapy.
- By comparing with an age and county-matched cohort of PCa-free men (n=300,679), we investigated the risk of TED following RT using Cox proportional hazard regression.
- The model was adjusted for tumour characteristics, demographics, comorbidities, PCa treatments, and known risk factors of TED such as recent surgery and disease progression.

Results

- Between 2006 and 2013, 67398 men with PCa received EBRT and 3954 underwent brachytherapy.
- A statistically significant association was found between EBRT and risk of PE in the crude analysis HR: 1.65 (95%CI: 1.30-2.10). However, upon adjusting for potential confounders this association remained positive but non-statistically significant.
- A more detailed analysis investigating risk of PE by time since EBRT showed a decreasing trend over time for PE , HR: 1.93 (95%CI: 1.28-2.91) and HR:1.57 (95%CI: 1.20-2.06) <1y and ≥1y, respectively. Upon adjusting for potential confounders the trend remained however it became non-statistically significant.



CONCLUSION

In this large representative cohort of men with PCa, we did not find an increased risk of TED following curative intent RT. However the crude analysis trends remained after adjusting for potential confounders. Further analysis will be performed according to radiation delivery methods subtypes and units of radiation dose.

PCBaSe Prostate Cancer data Base Sweden

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NCRI National Cancer Research Institute

Appendix III

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Clinical Investigation

Prostate Cancer Radiation Therapy and Risk of Thromboembolic Events

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Summary

We investigated the risk of thromboembolic disease (TED) after radiation therapy (RT) with curative intent for prostate cancer in a cohort including 6232 men who received external beam RT (EBRT) and 3178 who underwent brachytherapy (BT). No significant associations

Purpose: To investigate the risk of thromboembolic disease (TED) after radiation therapy (RT) with curative intent for prostate cancer (PCa).

Patients and Methods: We identified all men who received RT as curative treatment (n=9410) and grouped according to external beam RT (EBRT) or brachytherapy (BT). By comparing with an age- and county-matched comparison cohort of PCa-free men (n=46,826), we investigated risk of TED after RT using Cox proportional hazard regression models. The model was adjusted for tumor characteristics, demographics, comorbidities, PCa treatments, and known risk factors of TED, such as recent surgery and disease progression.

Results: Between 2006 and 2013, 6232 men with PCa received EBRT, and 3178 underwent BT. A statistically significant association was found between EBRT and BT

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Conflict of interest: none.

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were found between EBRT or BT and risk of deep venous thromboembolism or pulmonary embolism. Curative RT for prostate cancer using contemporary methodologies was thus not associated with an increased risk of TED.

and risk of pulmonary embolism in the crude analysis. However, upon adjusting for known TED risk factors these associations disappeared. No significant associations were found between BT or EBRT and deep venous thrombosis.

Conclusion: Curative RT for prostate cancer using contemporary methodologies was not associated with an increased risk of TED.

Introduction

Cancer increases the risk of embolic or thromboembolic diseases (TED) because tumor cells can activate the coagulation system (1). Previously we have shown that men with prostate cancer (PCa) are at higher risk of TED (2), and this risk was especially high for those who had undergone PCa-related surgeries while receiving androgen deprivation therapy (ADT) (3).

No large epidemiologic study has yet investigated the association between radiation therapy (RT) and risk of TED. It has been suggested that veins are less susceptible to radiation effects; however, there are several case reports of arterial thrombosis for patients who received RT for breast, lung, or uterine cancer (4-6). There is also a considerable body of experimental and epidemiologic evidence showing that RT causes damage to endothelial cells in the arteries via different mechanisms (7). For instance, the association between RT for breast cancer and higher risk of myocardial infarction and coronary heart disease is well established (8, 9). On the basis of this evidence, endothelial damage to veins is possible, and therefore quantifying the risk of TED after RT is of relevance.

In this study we investigated the association between curative RT given with contemporary standards for prostate cancer and risk of TED in a nationwide population-based cohort in Sweden.

Patients and Methods

Study population

We selected all men with PCa who received curative RT for prostate cancer between 1996 and 2013, as registered in Prostate Cancer data Base Sweden (PCBaSe) (n = 9410), which is described in detail elsewhere (Fig. 1) (10, 11). Briefly, PCBaSe Sweden was created by linking the National Prostate Cancer Register (NPCR) of Sweden with a number of other population-based registers via the use of the Swedish personal identity number. It also contains a control series of men free of PCa at the time of sampling. These men were matched by county of residence and birth year with an index case. For the present study we selected 46,826 men free of PCa. This comparison with a non-PCa cohort has been successfully applied previously in Prostate Cancer data Base Sweden when investigating the risk of TED, cardiovascular disease, or diabetes after ADT or surgery (2, 3, 12-14). Radiation therapy data were obtained from the NPCR, as well as from RetroRadioTherapy, a separate retrospective data collection at radiation units in Sweden. For this register data on treatment type, timing, total dose, and fractionation were retrieved directly from the verification/oncology information systems and local databases of the RT departments in Sweden. Men were followed up starting on the day of RT until the end of the study, death, immigration, or loss to follow-up. Prostate cancer–free men inherited an RT date according to their matched PCa men. The Research Ethics Board at Umea University approved this study (11).

The main outcomes were deep venous thrombosis (DVT) (International Classification of Diseases, 10th revision code: I80-82) and pulmonary embolism (PE) (International Classification of Diseases, 10th revision code: I26) as primary diagnoses in the National Inpatient Register and National Outpatient Register or Cause of Death Register. All 3 registers were used to avoid underestimation of severe cases of PE that may have only been captured as fatal in the Cause of Death Register (2).

The following information on potential confounders was also obtained. On the basis of information from the National Patient Register, comorbidities were measured using the Charlson comorbidity index (CCI), which assigns weights to a number of medical conditions. Each condition is assigned a score of 1, 2, 3, or 6, and the final CCI is given as the sum of these scores (15). Individuals were grouped into CCI categories for final scores of 0, 1, 2, or 3+. Information on age, serum prostate-specific antigen level, treatment at time of diagnosis, tumor grade, and stage, educational level, and history of TED was also used. Prostate cancer risk category was defined according to a modification of the National Comprehensive Cancer Network guideline (16): low risk: T1-2, Gleason score 2 to 6, and PSA <10 ng/mL; intermediate risk: T1-2, Gleason score 7, and/or PSA 10 to 20 ng/mL; high risk: T3 and/or Gleason score 8 to 10 and/or PSA 20 to 50 ng/mL; regionally metastatic/locally advanced: T4 and/or N1 and/or PSA 50 to 100 ng/mL in the absence of distant metastases (M0 or MX); and distant metastases: M1 and/or PSA >100 ng/mL. Information on surgeries was taken from the National Patient Register and included transurethral resection of the prostate (TURP), open or laparoscopic

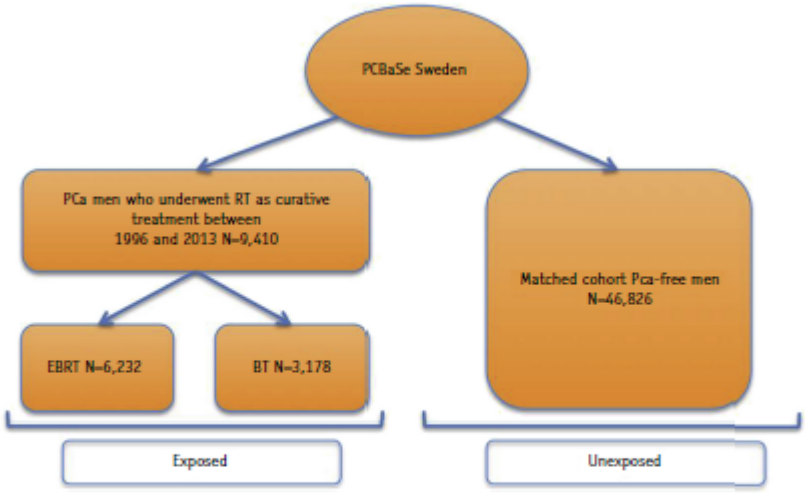

Fig. 1. Selection of study population from Prostate Cancer Database Sweden.

radical prostatectomy, pelvic lymph node dissection, and orchiectomy (3). Information on filled prescriptions of anti-androgens and gonadotropin-releasing hormone agonists was obtained from the National Prescribed Drug Register, in which all filled prescriptions have been registered since July 1, 2005. This allowed us to create a time-updated covariate for adjuvant and neoadjuvant ADT. Disease progression was defined by using the following proxy variables as time-dependent covariates: transurethral resection of the prostate indicating infravesical obstruction; palliative RT indicating a rise in serum PSA level or skeletal pain; and use of nephrostomy indicating overgrowth on the ureter. This is consistent with previously published work on the association between ADT and TED (13).

Statistical analysis

First we conducted univariate Cox proportional hazards models to evaluate the association between known clinical risk factors (ie, lymph node dissection, palliative RT, ADT due to disease progression, hydronephrostomy, non–prostate cancer related surgeries) and TED. This then confirmed the need to take these factors into account as time-updated covariates in our multivariate models. To further justify our choice for time-updated covariates related to PCa only, we performed a sensitivity analysis in which we censored for these events (eg, ADT for disease progression) or used delayed entry (eg, 1 year after lymph node dissection). The results were virtually the same as for the adjusted models (results not shown). Univariate and multivariate Cox proportional hazards models with age as a time-scale were then conducted to determine the hazard ratios (HRs) and 95% confidence intervals (CIs) for risk of DVT and PE by types of RT (brachytherapy [BT] and external beam RT [EBRT]). The assumption of proportionality of the Cox model covariates was tested by plotting Schoenfeld residuals (17). The multivariate analyses were conducted stepwise, allowing us to identify the effect of each confounder: CCI, education, PCa risk categories, PCa-related surgeries, history of TED, disease progression markers, other surgeries, adjuvant and neoadjuvant ADT. Exposure to surgeries, neoadjuvant and adjuvant ADT, and markers of disease progression were incorporated as time-updated covariates. Because of the rather small sample size for BT, we only performed an additional stratified analysis by time since RT for EBRT: 0 to 6 months, 6 to 12 months, 1 to 2 years, and >2 years.

Data management was performed using SAS version 9.3 (SAS Institute, Cary, NC), and data analysis was conducted with R version 2.13.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Between 1996 and 2013, 9410 men received curative RT as registered in PCBaSe Sweden, out of which 6232 underwent EBRT and 3178 BT. The latter group consisted of patients receiving either high-dose-rate BT to the prostate (n=2452), combined with EBRT in the majority of the patients, or low-dose-rate BT via implanted radioactive seeds (n=726). There were a total of 144 TED events in the exposed groups (43 in the BT group and 101 in the EBRT group) and 483 in the comparison cohort. Baseline characteristics of the study cohort are presented in Table 1.

Univariate analyses for the association between known TED risk factors and PE and DVT are presented in Table 2,

Table 1 Baseline characteristics of PCBaSe

Characteristic	BT		EBRT		PCa-free men	
	n	%	n	%	n	%
Total no. of men	3178	100	6232	100	46,826	100
Age (y)						
<60	490	15.4	566	9.1	5299	11.3
60-64	772	24.3	1179	18.9	9678	20.7
65-74	1747	55.0	3827	61.4	27,706	59.2
75+	169	5.3	660	10.6	4143	8.8
CCI						
0	2574	81.0	4632	74.3	35,975	76.8
1	382	12.0	935	15.0	5751	12.3
2	158	5.0	436	7.0	2944	6.3
3+	64	2.0	229	3.7	2156	4.6
Stage group						
No PCa	0	0.0	0	0.0	46,826	100.0
Low risk	864	27.2	900	14.4	0	
Intermediate risk	1059	33.3	2387	38.3	0	
High risk	1106	34.8	2503	40.2	0	
Regionally metastatic	126	4.0	391	6.3	0	
Missing data	23	0.7	51	0.8	0	
Prior DVT						
0	3171	99.8	6190	99.3	46,529	99.4
1	7	0.2	38	0.6	140	0.3
2+	0	0.0	4	0.1	157	0.3
Prior PE				0.0		
0	3151	99.2	6157	98.8	46,497	99.3
1	26	0.8	65	1.0	146	0.3
2+	1	0.0	10	0.2	183	0.4
Neoadjuvant ADT						
No ADT	1029	32.4	2463	39.5	46,826	100.0
AA	200	6.3	309	5.0	0	
GnRH	1949	61.3	3460	55.5	0	
Educational level						
Low	869	27.3	2279	36.6	16,861	36.0
Middle	1333	41.9	2525	40.5	18,684	39.9
High	959	30.2	1388	22.3	10,652	22.7
Missing	17	0.5	40	0.6	629	1.3
Follow-up time (y), mean (SD)	5.1 (2.1)		4.6 (2.1)		4.7 (2.2)	

Abbreviations: AA = anti-androgens; ADT = androgen deprivation therapy; BT = brachytherapy; CCI = Charlson comorbidity index; DVT = deep venous thrombosis; GnRH = gonadotropin-releasing hormone agonist; PCa = prostate cancer; PCBaSe = Prostate Cancer data Base Sweden; PE = pulmonary embolism.
Adjuvant ADT: BR group (AA = 222, GnRH = 134); EBRT group (AA = 484, GnRH = 678).

confirming the need for time-updated covariates in the multivariate analyses.

There was a positive association between EBRT and BT and the risk of PE, although after adjusting for CCI, PCa risk category, PCa-related surgeries, previous TED, disease progression markers, other surgeries, education, adjuvant ADT, and neoadjuvant ADT it was no longer statistically significant (HR 1.05, 95% CI 0.61-1.79; and HR 0.97, 95% CI 0.29-1.44, respectively) (Table 3). In the stratified analysis, the highest HR was observed for the first period (0-6 months); however, after adjustment for the named covariates it remained not statistically significant (data not shown). No associations between EBRT or BT and the risk of DVT were found. Residual plots for all covariates versus time at risk showed the residuals centered around zero, indicating no violation of the hazards proportionality assumption.

Discussion

The present study shows that in a cohort of Swedish men with PCa, curative RT for prostate cancer was not associated with an increased risk of TED. Our analyses compare men with PCa receiving RT with matched men from the general population, so that our results cannot entirely disentangle the effects of RT and the tumor itself on development of TED. The observed lack of an association

Table 2 Univariate hazard ratios (HRs) and 95% confidence intervals (CIs) for risk of DVT and PE according to known clinical risk factors for TED

	No. of events		Univariate			
			PE		DVT	
TED known risk factors	BT	EBRT	HR	95% CI	HR	95% CI
PCa men						
Lymph node dissection (LND within last 12 mo vs no LND within last 12 mo)	759	1166	2.03	0.82-4.99	3.44	0.80-14.76
Palliative RT	25	90	1.68	0.23-12.06	17.72	4.16-75.47
AA due to disease progression vs no AA	181	665	1.09	0.50-2.58	2.64	0.92-7.56
GnRH due to disease progression	183	537	2.46	1.30-4.65	9.41	3.83-23.06
Hydronephrostomy	4	24	7.56	1.03-55.44	NA*	NA
Non-PCa related surgeries†	427	863	7.83†	4.88-12.56	5.04†	1.86-13.62

Abbreviations: EBRT = external beam radiation therapy; LND = lymph node dissection; NA = nonapplicable; TED = thromboembolic disease. Other abbreviations as in Table 1.

* No events.

† PCa-free men included for this variable (no. of events = 5106).

between RT and TED when comparing with the general population can be explained by one of the following reasons: (*1*) RT is truly not associated with risk of TED; or (*2*) men receiving RT are heavily selected according to their TED risk factors so that a potential increased risk of TED from RT is at most as big as the risk reduction due to the selection. However, because cancer itself is a risk factor for TED, this indicates that the second explanation is unlikely.

To the best of our knowledge, no large study to date has investigated the association between RT for prostate cancer and TED. Experimental data show that RT can induce changes in artery walls, sinusoids, and capillaries (7). The different layers of the wall vessels can suffer several alterations after radiation exposure, such as endothelial cell damage, neointima lipid deposit, necrosis, fibrosis rupture, and thrombosis (7, 18). Moreover, EBRT to the pelvis has been found to increase the risk of bleeding in men who were on an anticoagulant scheme before receiving RT (19). Less evidence has been found for large veins (20), except for hepatic and large intestine veins, which RT frequently affects. Little is known regarding the biological mechanisms for this lesser impact of RT in large veins, although it has been suggested that large veins that do get affected by RT were probably invaded by the neoplasm before RT (20). Our results suggest that large veins from the pelvic area of patients who received RT for PCa do not seem to suffer enough alterations that can lead to a short-term thromboembolic event. However most of the reported RT changes in the arteries and heart seem to happen several years after receiving RT, and our mean follow-up time was 5 years, so that the present study may not be sensitive for long-term events.

Men who undergo radical prostatectomy are at a slightly increased risk of TEDs (2). Moreover, results from a recent observational showed that ADT also increases the risk of TED (13). In our analysis we included adjuvant and neoadjuvant ADT as potential confounders; however, this adjustment did not alter the final point estimates for the association.

A major strength of our study is the use of comprehensive data in PCBaSe Sweden, a large nationwide population-based register from which information on complete follow-up, PCa treatment, PCa stage, surgeries, disease progression, ADT, comorbidities, and socioeconomic status can be retrieved, which allowed us to adjust for known TED risk factors. Additionally, the use of a PCa-free, age- and residence-matched comparison cohort allowed for accurate risk estimation. The availability of data regarding delivered RT doses for this large cohort is another strength of this study. It allowed us to confirm that the selected patients had received radiation doses with curative potential to the prostate.

Detailed information on irradiated volumes was lacking, which excluded the possibility of examining dose–volume effects on TED. Even though we had data on type and dosage of EBRT, it was not possible to divide this further into subtypes owing to the low number of TED events. However, it is unlikely that we have missed strong

Table 3 Multivariate analysis HRs and 95% CIs for risk of DVT and PE

	DVT		PE	
Analysis	HR	95% CI	HR	95% CI
No RT	1.00	Reference	1.00	Reference
Unadjusted				
BT	0.60	0.26-1.36	1.47	1.05-2.07
EBRT	1.09	0.68-1.74	1.73	1.35-2.2
Adjusted*				
BT	0.34	0.08-1.11	0.97	0.29-1.44
EBRT	0.44	0.14-1.4	1.05	0.61-1.79

Abbreviations as in Tables 1 and 2.

* Charlson comorbidity index, PCa risk category, PCa-related surgeries, previous thromboembolic events, TED known risk factors as determined in Table 2, education, adjuvant ADT, and neoadjuvant ADT.

associations because none of our findings suggested any indication of a positive trend. Additional limitations include lack of information on lifestyle factors and residual confounding, which could not be accounted for (21, 22). However, adjustment for CCI and history of TED served as proxies for lifestyle and health status at initiation of RT.

Conclusion

Our data indicate that curative RT for PCa is not associated with the risk of developing PE or DVT.

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Appendix IV

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Drugs for metabolic conditions and prostate cancer death in men on GnRH agonists

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Objective

To evaluate whether drugs for metabolic conditions influence prostate cancer-specific mortality in men starting gonadotrophin-releasing hormone (GnRH) agonists, as it is unclear whether metabolic syndrome and its related drugs is affecting treatment response in men with prostate cancer on GnRH agonists.

Patients and Methods

We selected all men receiving GnRH agonists as primary treatment in the Prostate Cancer data Base Sweden (PCBaSe) ($n = 9267$). Use of drugs for metabolic conditions (i.e. anti-diabetes, anti-dyslipidaemia, and antihypertension) in relation to all-cause, cardiovascular disease (CVD), and prostate cancer-specific death were studied using multivariate Cox proportional hazard and Fine and Gray competing regression models.

Results

In all, 6322 (68%) men used at least one drug for a metabolic condition at GnRH agonist initiation: 46% on antihypertensive drugs only, 32% on drugs for dyslipidaemia and hypertension, and ~10% on drugs for more than two metabolic conditions. Cox models indicated a weak increased risk of prostate cancer death in men who were on drugs for hypertension only (hazard ratio [HR] 1.12, 95% confidence interval [CI] 1.03–1.23) or drugs for hyperglycaemia (HR 1.19, 95% CI 1.06–1.35) at GnRH agonist initiation. However, upon taking into account competing risk from CVD death, none of the drugs for metabolic conditions were associated with an increased risk of prostate cancer death.

Conclusion

We did not find evidence for a better or worse response to GnRH agonists in men with prostate cancer who were also on drugs for hypertension, dyslipidaemia, or hyperglycaemia.

Keywords

dyslipidaemia, GnRH agonist, hyperglycaemia, hypertension, metabolic syndrome, prostate cancer death, #PCSM, #ProstateCancer

Introduction

A recent meta-analysis estimated that the risk of prostate cancer is 1.54-times (95% CI 1.23–1.94) higher for those with metabolic syndrome (MetS), as compared to those without MetS [1]. Recent studies also suggest that the presence of MetS or some of its features is associated with higher grade disease in men with prostate cancer and can lead to more rapid progression [2,3]. In contrast, drugs that treat components of MetS (e.g. metformin for diabetes or statins for dyslipidaemia) have also been associated with a reduced risk and progression of prostate cancer [4–7]. However, the underlying biological mechanisms for these observations have not been fully elucidated [8].

GnRH agonists are associated with an increased risk of type 2 diabetes, as well as other components of the MetS in men with prostate cancer who are treated with androgen-deprivation therapy (ADT) [9]. Moreover, one recent study found that having MetS may shorten time to castrate-resistant prostate cancer and overall survival [3], whereas another study did not find any statistically significant associations between baseline MetS and prostate cancer death [10].

Given this complex interaction between MetS, its related drugs, and prostate cancer progression, the present study aimed to evaluate how the use of drugs for metabolic conditions (below referred to as 'metabolic drugs') at the time

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of GnRH agonist initiation may affect response to treatment by studying time to prostate cancer death.

Patients and Methods

We selected all men with prostate cancer who received primary GnRH agonists between 2007 and 2013 (n = 9267), as registered in the **P**rostate **C**ancer data **Ba**se **Se**weden (PCBaSe) Traject, which is described in detail elsewhere [11,12]. Briefly, the PCBaSe was created by linking the Swedish National Prostate Cancer Register (NPCR) with a number of other population-based registers using the Swedish personal identity number for record linkage. The Research Ethics Board at Umeå University approved this study [12].

Based on the underlying causes of death registered in the Cause of Death Register, the following main outcomes were defined for this study: death from prostate cancer (International Classification of Diseases-10 [ICD-10] code: C61), death from cardiovascular disease (CVD; ICD-10: I10–I99), as well as other deaths (remaining ICD-10 codes), and overall mortality [13].

The main exposure variables for this study were newly filled prescriptions, prescribed before GnRH agonist initiation, for treatment of diabetes (metformin, sulphonylurea, insulin), dyslipidaemia (statins), hypertension (angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, β-blockers, calcium channel blockers, or thiazide and thiazide-like diuretics), or anti-obesity agents in the Prescribed Drug Register. As only 20 men received anti-obesity agents, exposure to these drugs was not considered as part of the analysis a priori. As many men often take drugs for more than one of the metabolic conditions listed above, we looked at each metabolic drug group individually, as well as the most common combinations: dyslipidaemia only (n = 241), hyperglycaemia only (n = 67; 38 on insulin and 29 on metformin or sulphonylurea), hypertension only (n = 2933), dyslipidaemia and hyperglycaemia (n = 41), dyslipidaemia and hypertension (n = 1996), hyperglycaemia and hypertension (n = 333), and treated for more than two metabolic conditions (n = 651). The analyses focused on the four most common groups of drugs: hypertension only, hypertension and dyslipidaemia, any hyperglycaemia, dyslipidaemia only (Fig. 1).

The following information on potential covariates was also obtained: age, tumour grade and stage and educational level. Prostate cancer risk category was defined according to a modification of the National Comprehensive Cancer Network Guideline [14]: low risk: T1–2, Gleason score of 2–6 and PSA level of <10 ng/mL; intermediate risk: T1–2, Gleason score of 7 and/or PSA level of 10–20 ng/mL; high risk: T3 and/or Gleason score of 8–10 and/or PSA level of 20–50 ng/mL; regionally metastatic/locally advanced: T4 and/or N1 and/or PSA level of 50–100 ng/mL in the absence of distant metastases (M0 or MX); and distant metastases: M1 and/or PSA level of >100 ng/mL. In addition, we collected information on history of CVD, defined by any CVD hospital admission (ICD-10 codes: I20–I25, I50, I60–I69, I70–I79) as primary diagnoses in the National Patient Register. Comorbidities were also measured using the Charlson Comorbidity Index (CCI), which assigns weights to a number of medical conditions. Each condition was assigned a score of 1, 2, 3 or 6, and the final CCI was the sum of these scores [15]. Individuals were grouped into CCI categories for final scores of 0, 1, 2 or ≥3. History of CVD and CCI were included for descriptive purposes, as careful assessment of the causal pathway did not indicate that these covariates need to be included in multivariate models (Fig. 2) [16]. The use of a directed acyclic graph (DAG) helps represent causal relations among variables to determine which ones need to be controlled for in the estimation of causal effects [17].

Statistical analysis

We conducted univariate and multivariate Cox proportional hazard regression models to assess the association between individual metabolic drugs, as well as common combinations

Fig. 1 Combinations of metabolic drugs studied.

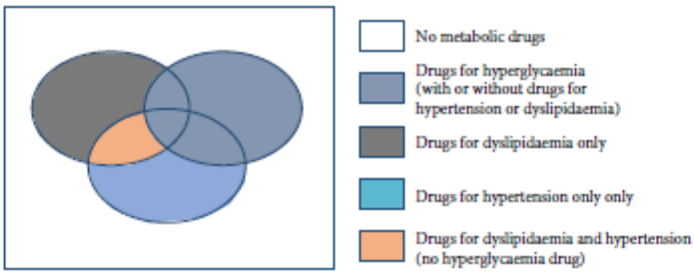
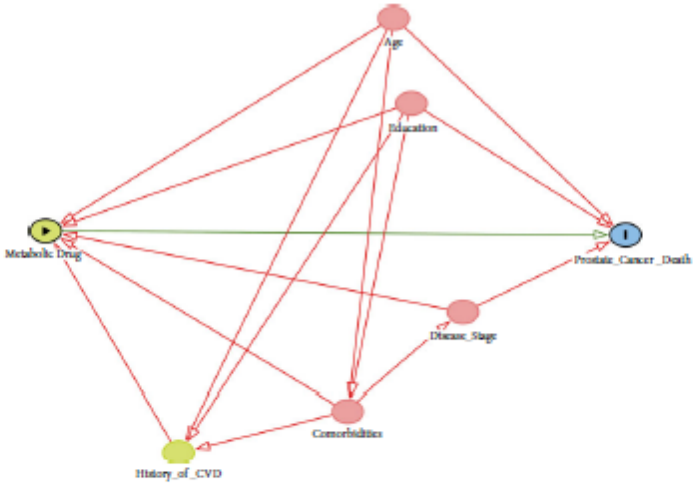


Fig. 2 Directed acyclic graph for the association between metabolic drugs and prostate cancer death in men who start on GnRH agonists.



(Fig. 2) and death from prostate cancer, CVD, and overall mortality. Multivariate models were adjusted for age, education level, disease stage, and civil status. Adjustments for age were done using natural cubic splines with four degrees of freedom. To consider competing risks, we repeated the analyses using Fine and Gray competing risk regression [18]. As all the men were on primary GnRH agonists and disease stage was taken into account, all analyses are based on the intention-to-treat assumption, i.e., all men stayed on ADT, which was the standard drug for advanced prostate cancer in Sweden at the time of data collection.

To further illustrate the associations between metabolic drugs and causes of death, stacked cumulative incidence proportion functions for all-cause, CVD, and prostate cancer-specific death were displayed by categories of metabolic drug use.

Data management was performed using the Statistical Analysis System (SAS), version 9.3 (SAS Institute, Cary, NC, USA) and data analysis was conducted with R version 2.13.2 (R Foundation for Statistical Computing, Wien, Austria).

Results

In all, 6322 (68%) men used at least one drug for a metabolic condition at the time they started GnRH agonists. Most of these men were on antihypertensive drugs only (46%), followed by men on drugs for dyslipidaemia and hypertension (32%). About 10% of men were on drugs for more than two metabolic conditions. Table 1 shows the baseline characteristics of all men included in the study based on the type of metabolic drugs they were taking at time of GnRH agonist initiation.

Multivariate Cox proportional hazards regression adjusted for age, education, and prostate cancer risk category showed that use of most metabolic drugs were associated with an increased risk of CVD death and hence also overall death (Table 2). For instance, those men on antihypertensive drugs only were 1.87-times more likely to die from CVD than men not taking any metabolic drugs (95% CI 1.56–2.24) and this increased to 2.46-times if these men were also taking drugs for dyslipidaemia (95% CI 2.03–2.98). For prostate cancer death, there was a small increased risk for those on drugs for hypertension only or any hyperglycaemia (hazard ratio [HR] 1.12, 95% CI 1.03–1.23); and HR 1.19, 95% CI 1.06–1.35, respectively).

Given the strong association with death from CVD, competing risk regression showed little impact on the association between metabolic drugs and death from CVD (Table 2). However, the associations seen for prostate cancer death disappeared and even became inverse for those on drugs for hypertension and dyslipidaemia, although not statistically significant (HR 0.92, 95% CI 0.83–1.02).

Figure 3 shows these findings using stacked cumulative incidence proportions. The largest proportion of CVD-deaths (red) can be seen amongst those on metabolic drugs, with the biggest proportion for those who are on drugs for both hypertension and statins. The proportion of prostate cancer deaths (blue) was fairly similar across all the groups studied.

Table 1 Baseline characteristics according to use of drugs for metabolic conditions at time of GnRH agonist initiation.

Variable, *n* (%)	All men (*n* = 92657)	Dyslipidaemia only (*n* = 241)	Hyperglycaemia only (*n* = 67)	Hyperglycaemia with insulin only (*n* = 38)	Hyperglycaemia with metformin/SU only (*n* = 29)	Hypertension only (*n* = 2933)	Dyslipidaemia and hyperglycaemia (*n* = 41)	Dyslipidaemia and hypertension (*n* = 1996)	Hyperglycaemia and hypertension (*n* = 333)	≥3 Metabolic conditions (*n* = 681)
Age, years										
<65	896 (97)	15 (6.2)	7 (10.4)	2 (5.3)	5 (17.2)	175 (6.0)	5 (12.2)	112 (5.6)	19 (5.7)	49 (6.1)
65–74	2399 (260)	76 (31.5)	28 (41.8)	18 (47.4)	10 (34.5)	651 (22.2)	12 (29.3)	548 (27.5)	76 (22.8)	217 (33.3)
75–84	4309 (464)	133 (55.2)	29 (43.3)	17 (44.7)	12 (41.4)	1968 (66.6)	21 (51.2)	1068 (53.5)	173 (52.0)	319 (46.8)
≥85	1963 (169)	17 (7.1)	3 (4.5)	1 (2.6)	2 (6.9)	739 (25.2)	3 (7.3)	268 (13.4)	65 (19.5)	75 (11.5)
CCI										
0	5755 (621)	199 (82.2)	33 (49.3)	24 (63.2)	9 (31.0)	1857 (63.3)	17 (41.5)	827 (41.4)	124 (37.2)	179 (27.5)
1	1683 (182)	35 (22.8)	18 (26.9)	8 (21.1)	10 (34.5)	545 (18.6)	12 (29.3)	607 (30.4)	77 (23.1)	151 (23.2)
2	983 (106)	25 (10.4)	10 (14.9)	5 (13.2)	5 (17.2)	313 (10.7)	6 (14.6)	278 (13.9)	57 (17.1)	133 (20.4)
≥3	846 (91)	11 (4.6)	6 (9.0)	1 (2.6)	5 (17.2)	218 (7.4)	6 (14.6)	284 (14.2)	75 (22.5)	188 (28.9)
Educational level										
High	1411 (152)	52 (21.6)	9 (13.4)	4 (10.5)	5 (17.2)	425 (14.5)	9 (22.0)	283 (14.2)	30 (9.0)	78 (12.0)
Low	4551 (491)	104 (43.2)	34 (50.7)	22 (57.9)	12 (41.4)	1507 (51.4)	21 (51.2)	999 (50.1)	194 (58.3)	333 (51.2)
Middle	3172 (342)	81 (33.6)	24 (35.8)	12 (31.6)	12 (41.4)	960 (32.7)	9 (22.0)	699 (35.0)	106 (31.8)	229 (35.2)
Missing	133 (1.4)	4 (1.7)	0	0	0	41 (1.4)	2 (4.9)	15 (0.8)	5 (1.5)	11 (1.7)
Civil status										
Married	5664 (611)	157 (65.1)	33 (49.3)	17 (44.7)	16 (55.2)	1776 (60.6)	28 (68.3)	1284 (64.3)	183 (55.0)	411 (63.1)
Not married	3683 (389)	84 (34.9)	34 (50.7)	21 (55.3)	13 (44.8)	1157 (39.4)	13 (31.7)	712 (35.7)	150 (45.0)	240 (36.9)
Risk category										
Low risk	98 (1.1)	6 (2.5)	1 (1.5)	1 (2.6)	0	33 (1.1)	2 (4.9)	26 (1.3)	2 (0.6)	9 (1.4)
Intermediate risk	778 (8.4)	33 (13.7)	5 (7.5)	4 (10.5)	1 (3.4)	263 (9.0)	1 (2.4)	290 (14.6)	29 (8.7)	66 (10.4)
High risk	2976 (32.1)	80 (33.2)	22 (32.8)	14 (36.8)	8 (27.6)	1018 (34.7)	17 (41.5)	768 (38.5)	122 (36.6)	223 (34.3)
Regional metastatic	1458 (15.5)	32 (13.3)	14 (20.9)	6 (15.8)	8 (27.6)	462 (15.1)	4 (9.8)	309 (15.5)	63 (18.9)	91 (14.0)
Distant metastases	3977 (42.9)	90 (37.3)	25 (37.3)	13 (34.2)	12 (41.4)	1177 (40.1)	17 (41.5)	753 (37.7)	117 (35.1)	292 (39.9)

SU, sulphonylurea.

Table 2 Cox proportional HRs and competing risks regression ratios with 95% CIs for the associations between drugs for metabolic conditions and prostate cancer death, other deaths, CVD deaths, and overall death.

	No. of events	Univariate Cox HR (95% CI)	Multivariate Cox* HR (95% CI)	Competing risk regression* HR (95% CI)
Prostate cancer death				
No metabolic drugs	1117	1.00 Ref	1.00 Ref	1.00 Ref
Only hypertension	986	0.97 (0.89–1.06)	1.12 (1.03–1.23)	1.03 (0.94–1.13)
Hypertension + dyslipidaemia	578	0.81 (0.74–0.90)	1.02 (0.93–1.14)	0.92 (0.83–1.02)
Hyperglycaemia	336	0.93 (0.82–1.05)	1.19 (1.06–1.35)	1.00 (0.89–1.14)
Only statins	73	0.78 (0.62–0.99)	1.06 (0.83–1.34)	1.01 (0.80–1.29)
Other death				
No metabolic drugs	294	1.00 Ref	1.00 Ref	1.00 Ref
Only hypertension	394	1.48 (1.27–1.72)	1.23 (1.05–1.43)	1.12 (0.96–1.31)
Hypertension + dyslipidaemia	238	1.29 (1.09–1.53)	1.20 (1.01–1.43)	1.07 (0.90–1.27)
Hyperglycaemia	185	1.97 (1.64–2.37)	1.85 (1.53–2.22)	1.59 (0.32–1.91)1
Only statins	31	1.26 (0.87–1.82)	1.23 (0.85–1.79)	1.19 (0.83–1.72)
CVD death				
No metabolic drugs	174	1.00 Ref	1.00 Ref	1.00 Ref
Only hypertension	385	2.45 (2.05–2.93)	1.87 (1.56–2.24)	1.71 (1.43–2.05)
Hypertension + dyslipidaemia	283	2.59 (2.14–3.13)	2.46 (2.03–2.98)	2.26 (1.87–2.74)
Hyperglycaemia	149	2.69 (2.16–3.35)	2.53 (2.03–3.16)	2.15 (1.72–2.69)
Only statins	15	1.03 (0.61–1.74)	1.06 (0.63–1.80)	1.01 (0.59–1.71)
Overall Death				
No metabolic drugs	1585	1.00 Ref	1.00 Ref	1.00 Ref
Only hypertension	1765	1.23 (1.15–1.31)	1.22 (1.14–1.31)	N/A
Hypertension + dyslipidaemia	1099	1.10 (1.02–1.18)	1.23 (1.14–1.33)	N/A
Hyperglycaemia	670	1.32 (1.20–1.44)	1.49 (1.36–1.63)	N/A
Only statins	119	0.90 (0.74–1.08)	1.08 (0.89–1.30)	N/A

**Adjusted for allocation, prostate cancer risk category, civil status, and age.*

Fig. 3 Stacked cumulative incidence of prostate cancer-specific, CVD, and other deaths based on exposure to metabolic drugs. DM, diabetes mellitus.

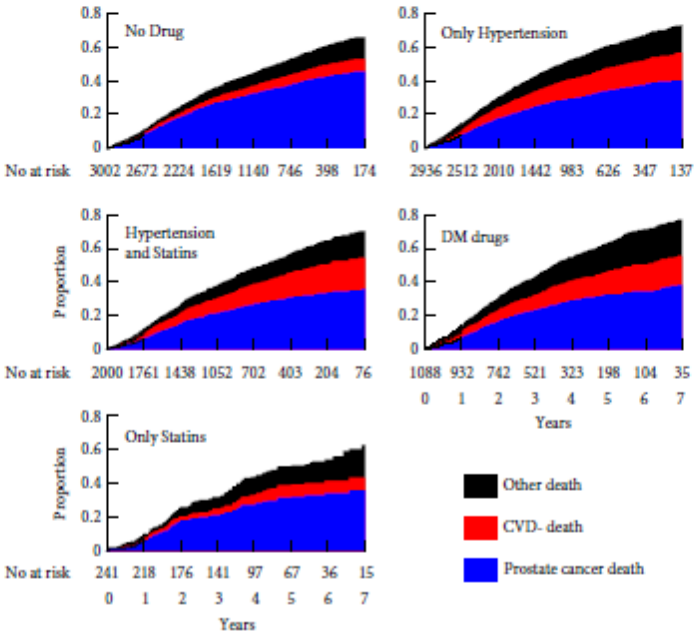


Table 3 Cox proportional HRs and competing risks regression ratios with 95% CIs for the associations between drugs for metabolic conditions and prostate cancer death, other deaths, CVD deaths, and overall death, stratified by metastatic status.

Metastatic disease only	No. of events	Univariate Cox HR (95% CI)	Multivariate Cox* HR (95% CI)	Competing risk regression* HR (95% CI)
Prostate cancer death				
No metabolic drugs	820	1.00 Ref	1.00 Ref	1.00 Ref
Only hypertension	614	1.12 (1.01–1.24)	1.07 (0.96–1.2)	0.99 (0.88–1.1)
Hypertension + dyslipidaemia	361	0.98 (0.87–1.11)	1.01 (0.89–1.14)	0.91 (0.81–1.04)
Hyperglycaemia	204	1.13 (0.97–1.32)	1.16 (0.99–1.36)	0.97 (0.83–1.14)
Only statins	48	1.07 (0.8–1.43)	1.11 (0.83–1.49)	1.05 (0.78–1.42)
Other death				
No metabolic drugs	105	1.00 Ref	1.00 Ref	1.00 Ref
Only hypertension	124	1.78 (1.37–2.30)	1.41 (1.08–1.84)	1.30 (0.99–1.70)
Hypertension + dyslipidaemia	69	1.48 (1.09–2.00)	1.34 (0.98–1.82)	1.22 (0.90–1.67)
Hyperglycaemia	50	2.20 (1.57–3.09)	1.95 (1.39–2.75)	1.63 (1.16–2.30)
Only statins	0	1.40 (0.68–2.86)	1.28 (0.62–2.63)	1.18 (0.58–2.43)
CVD Death				
No metabolic drugs	59	1.00 Ref	1.00 Ref	1.00 Ref
Only hypertension	118	2.99 (2.19–4.09)	2.06 (1.50–2.83)	1.90 (1.38–2.61)
Hypertension + dyslipidaemia	78	2.97 (2.12–4.16)	2.58 (1.83–3.64)	2.38 (1.69–3.35)
Hyperglycaemia	50	3.90 (2.67–5.68)	3.31 (2.26–4.85)	2.77 (1.90–4.06)
Only statins	6	1.86 (0.80–4.32)	1.64 (0.71–3.81)	1.57 (0.69–3.54)
Overall death				
No metabolic drugs	984	1.00 Ref	1.00 Ref	1.00 Ref
Only hypertension	856	1.30 (1.19–1.43)	1.18 (1.07–1.29)	N/A
Hypertension + dyslipidaemia	508	1.15 (1.04–1.28)	1.14 (1.03–1.28)	N/A
Hyperglycaemia	304	1.41 (1.24–1.61)	1.40 (1.23–1.59)	N/A
Only statins	62	1.15 (0.89–1.49)	1.16 (0.90–1.50)	N/A
Non-metastatic disease only				
Prostate cancer death				
No metabolic drugs	297	1.00 Ref	1.00 Ref	1.00 Ref
Only hypertension	372	1.16 (1.00–1.36)	1.22 (1.05–1.43)	1.14 (0.97–1.32)
Hypertension + dyslipidaemia	217	0.96 (0.81–1.15)	1.08 (0.9–1.29)	0.96 (0.81–1.14)
Hyperglycaemia	132	1.14 (0.93–1.40)	1.26 (1.03–1.55)	1.09 (0.89–1.34)
Only statins	25	0.79 (0.53–1.20)	0.96 (0.64–1.44)	0.95 (0.64–1.42)
Other death				
No metabolic drugs	189	1.00 Ref	1.00 Ref	1.00 Ref
Only hypertension	270	1.32 (1.10–1.59)	1.15 (0.95–1.38)	1.04 (0.86–1.26)
Hypertension + dyslipidaemia	169	1.170.95–1.44)	1.14 (0.92–1.40)	1.00 (0.81–1.23)
Hyperglycaemia	135	1.81 (1.45–2.26)	1.78 (1.43–2.22)	1.55(1.25–1.94)
Only statins	23	1.15 (0.74–1.77)	1.20 (0.77–1.85)	1.17 (0.76–1.79)
CVD death				
No metabolic drugs	115	1.00 Ref	1.00 Ref	1.00 Ref
Only hypertension	267	2.15 (1.73–2.68)	1.77 (1.42–2.21)	1.63 (1.30–2.03)
Hypertension + dyslipidaemia	205	2.33 (1.86–2.93)	2.37 (1.88–2.98)	2.19 (1.73–2.76)
Hyperglycaemia	99	2.19 (1.67–2.86)	2.22 (1.69–2.91)	1.90 (1.45–2.50)
Only statins	9	0.74 (0.38–1.46)	0.84 (0.43–1.67)	0.79 (0.40–1.57)
Overall death				
No metabolic drugs	601	1.00 Ref	1.00 Ref	1.00 Ref
Only hypertension	909	1.40 (1.26–1.55)	1.30 (1.17–1.44)	N/A
Hypertension + dyslipidaemia	591	1.29 (1.15–1.45)	1.35 (1.20–1.51)	N/A
Hyperglycaemia	366	1.55 (1.36–1.77)	1.62 (1.42–1.84)	N/A
Only statins	57	0.90 (0.68–1.18)	1.02 (0.78–1.34)	N/A

Adjusted for education, prostate cancer risk category, civil status, and age.

Discussion

Traditional Cox proportional hazards models indicated a weak increased risk of prostate cancer death in men who are on drugs for hypertension or hyperglycaemia at the time they start GnRH agonists. However, upon taking into account competing risk from CVD death none of the drugs for metabolic conditions were associated with an increased risk of prostate cancer death and a trend towards an inverse association was seen for those who were on drugs for both hypertension and dyslipidaemia.

To our knowledge, few studies have investigated the effect of drugs for metabolic conditions in relation to response to treatment for men on GnRH agonists [19]. Most studies to date have explored the effect of single drugs, predominantly metformin, statins, or β-blockers, in relation to prostate cancer death [5,20–27]. Moreover, these existing observational

studies have found contradicting results and they did not specifically study those men on GnRH agonists, a drug that in itself is also associated with an increased risk of metabolic conditions [9]. Some reasons for these contrasting findings have been summarised previously [7], but by investigating several drugs for metabolic conditions in a specifically defined group of men with prostate cancer, the present study aimed to improve our understanding of possible metabolic drug effects in the context of prostate cancer progression.

Thus, to our knowledge the present study is the first to specifically investigate the overall use of drugs for metabolic conditions and prostate cancer death in men on GnRH agonists. Our present results are consistent with a small study (n = 273) investigating the effect of MetS (without looking at the related drugs) on prostate cancer death in men on ADT using data from the Health Professionals Follow-up Study and the Veteran's Administration [10]. The authors concluded that there was no association of prostate cancer-specific death and MetS, but the latter was associated with an increased risk of death from all causes. However, there was a weak positive association between hypertension only and prostate cancer-specific death (HR 1.59, 95% CI 0.89–2.84). Although the exposure assessment in that study was different from what we have done here, none of the studies support a strong association between metabolic aberrations and prostate cancer-specific death in men on GnRH agonists. The latter is relevant from a clinical point of view and could inform future studies looking into how development and treatment of MetS after initiation of GnRH agonists (as an adverse event) may affect response to hormonal treatment or prostate cancer progression.

A major strength of our present study is the use of comprehensive data in the PCBaSe Traject, a large nationwide population-based register from which information on complete follow-up, prostate cancer treatment, prostate cancer risk category, comorbidities, and socio-economic status can be retrieved. As with other currently published studies, our follow-up was rather short and a follow-up study might provide more power to investigate risk of prostate cancer death in a competing risk setting (e.g. death from CVD [28]). Another limitation of the present study is the lack of direct measures of MetS (e.g. serum glucose and cholesterol levels [7]) and information on ethnicity or lifestyle factors. However, the latter could be approximated by the CCI, which was not needed as a covariate in the statistical models (Fig. 2).

Conclusion

Despite the suggested complex interaction between MetS, metabolic drugs, and prostate cancer progression, the present study did not find any evidence for a better or worse response to GnRH agonists in men who were also on drugs for hypertension, dyslipidaemia, or hyperglycaemia. These findings suggest that treatment of MetS is important in men on GnRH agonists, but not a contraindication for their prostate cancer treatment.

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Conflict of interest

None to be declared.

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Abbreviations: ADT, androgen-deprivation therapy; CCI, Charlson Comorbidity Index; CVD, cardiovascular disease; HR, hazard ratio; ICD-10, International Classification of Diseases-10; MetS, metabolic syndrome; NPCR, National Prostate Cancer Register of Sweden; PCBaSe, Prostate Cancer data Base Sweden.