Prostate Cancer; Metabolic Risk Factors, Drug Utilisation, Adverse Drug Reactions

BIRGITTA GRUNDMARK
Increased possibilities during the last decades for early detection of prostate cancer have sparked research on preventable or treatable risk factors and on improvements in therapy. Treatments of the disease still entail significant side effects potentially affecting men during the rest of their lives. The studies of the present thesis concern different aspects of prostate cancer from etiological risk factors and factors influencing treatment to an improved methodology for the detection of treatment side effects.

Papers I, II, both based in the population based cohort ULSAM (Uppsala Longitudinal Study of Adult Men), investigate possible risk factors of prostate cancer with options for intervention: selenium levels and the metabolic syndrome. The phenomenon of competing risk of death from other causes than prostate cancer and its impact on and importance for choice of statistical methods is also exemplified and discussed for the first time in prostate cancer research.

- Smokers with low selenium status have an increased future risk of later development of prostate cancer. Influence of genetic variability appears plausible.
- The metabolic syndrome and especially its increased waist circumference component are associated with later development of prostate cancer – taking competing risks of death from other causes into account.

Papers III and IV using pharmacoepidemiological methods investigate aspects of drug utilisation in prostate cancer using nationwide and international databases. In Paper III factors influencing anti-androgen use in prostate cancer are investigated, both from a prescriber- and patient perspective. The age and disease risk group of the patient, unsupported scientifically, influence both the prescribers’ choice of dose and the patients’ adherence to treatment.

- Adherence, not previously investigated in male cancer patients, was considerably higher than reported for adjuvant breast cancer treatment. Subgroups of men suitable for intervention to increase adherence were identified.

Paper IV, investigates the feasibility of improving an established method for screening large adverse drug reactions databases, the proportional reporting ratio (PRR), this by using restricted sub-databases according to treatment area (TA), introducing the concept of PRR-TA.

- The PRR-TA method increases the signal-noise relationship of analyses; a finding highly relevant for possibly conserving manual resources in Pharmacovigilance work in a drug-authority setting.

**Keywords:** Prostate cancer, Epidemiology, Pharmacoepidemiology, Metabolic Syndrome, Selenium, Smoking, hOGG1, MnSOD, Competing Risk, Adherence, Persistence, Medical Possession Ratio, MPR, Signal Detection, PRR, proportional reporting ratio, ULSAM, PcBaSE, EudraVigilance, SPDR, Swedish Prescribe Drug Registry, NPCR, National Prostate Cancer Registry, SDR, Signal, disproportionality analysis, PRR-TA, EudraVigilance

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urn:nbn:se:uu:diva-194297 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-194297)
”Jag vill inte klaga;
jag har faktiskt upptäckt Atlantis.”

Wisława Szymborska ur Lovsång till drömmarna
Department of Surgical Sciences
Uppsala University

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Professor Gunnar Engström
Lund University
List of Papers

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

I  Serum levels of selenium and smoking habits at age 50 influence long term prostate cancer risk, a 34 year ULSAM follow-up.
   BMC Cancer. 2011 Oct 7;11:431

II  The metabolic syndrome and the risk of prostate cancer under competing risks of death from other causes.

III  Anti-androgen prescribing patterns, patient treatment adherence and influencing factors, results from the nationwide PCBaSe Sweden.

IV  Reducing the noise in signal detection of adverse drug reactions by standardizing the background: analyses of Proportional Rate Ratios-by-therapeutic area. Manuscript

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<th>Description</th>
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<tbody>
<tr>
<td>AA</td>
<td>Anti-androgen</td>
</tr>
<tr>
<td>ADR</td>
<td>Adverse Drug Reaction</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CDR</td>
<td>Causes of Death Register</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CR</td>
<td>Cancer Registry</td>
</tr>
<tr>
<td>CT</td>
<td>Clinical Trial</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
</tr>
<tr>
<td>DDD</td>
<td>Defined Daily Dose</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>EGIR</td>
<td>European Group for the study of Insulin Resistance</td>
</tr>
<tr>
<td>EMA</td>
<td>European Medicines Agency</td>
</tr>
<tr>
<td>EV</td>
<td>EudraVigilance database</td>
</tr>
<tr>
<td>GnRH</td>
<td>Gonadotropin Releasing Hormone</td>
</tr>
<tr>
<td>HDL</td>
<td>High Density Lipoprotein</td>
</tr>
<tr>
<td>HDR</td>
<td>Hospital Discharge Registry</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard Ratio</td>
</tr>
<tr>
<td>ICD</td>
<td>International Classification of Diseases</td>
</tr>
<tr>
<td>IDF</td>
<td>International Diabetes Federation</td>
</tr>
<tr>
<td>LUTS</td>
<td>Lower Urinary Tract Symptoms</td>
</tr>
<tr>
<td>MAH</td>
<td>Marketing Authorisation Holder</td>
</tr>
<tr>
<td>MedDRA</td>
<td>Medical Dictionary for Regulatory Activities</td>
</tr>
<tr>
<td>MetS</td>
<td>Metabolic Syndrome</td>
</tr>
<tr>
<td>MnSOD, SOD2</td>
<td>Manganese Superoxide Dismutase</td>
</tr>
<tr>
<td>MPA</td>
<td>Medical Products Agency</td>
</tr>
<tr>
<td>NBHW</td>
<td>National Board of Health and Welfare, Socialstyrelsen</td>
</tr>
<tr>
<td>NCEP-ATP III</td>
<td>National Cholesterol Education Program - Adult Treatment Panel III</td>
</tr>
<tr>
<td>RCC</td>
<td>Regionalt Cancer Centrum</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>NPCR</td>
<td>National Prostate Cancer Register</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>OGG1, hOGG1</td>
<td>human OxoguaninDNA-Glycosylase-1</td>
</tr>
<tr>
<td>PCBaSe</td>
<td>Prostate Cancer Database Sweden</td>
</tr>
<tr>
<td>PDD</td>
<td>Prescribed Daily Dose</td>
</tr>
<tr>
<td>PIN</td>
<td>Prostate Intraepithelial Neoplasia</td>
</tr>
<tr>
<td>PrC, PrCa, PC</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td>PRR</td>
<td>Proportional Reporting Ratio</td>
</tr>
<tr>
<td>PSA</td>
<td>Prostate Specific Antigen</td>
</tr>
<tr>
<td>PT</td>
<td>Preferred (MedDRA-)Term</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised Controlled Trial</td>
</tr>
<tr>
<td>RR</td>
<td>Relative Risk</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>Se, s-Se</td>
<td>Selenium, serum-Selenium</td>
</tr>
<tr>
<td>SDR</td>
<td>Signal of disproportionate reporting</td>
</tr>
<tr>
<td>SDR3</td>
<td>Signal of disproportionate reporting using case count of ≥3</td>
</tr>
<tr>
<td>SDR5</td>
<td>Signal of disproportionate reporting using case count of ≥5</td>
</tr>
<tr>
<td>SES</td>
<td>Socioeconomic Status</td>
</tr>
<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
</tr>
<tr>
<td>SoS</td>
<td>Socialstyrelsen, National Board of Health and Welfare</td>
</tr>
<tr>
<td>SPC</td>
<td>Summary of Product Characteristics</td>
</tr>
<tr>
<td>SPDR</td>
<td>Swedish Prescribed Drug Registry</td>
</tr>
<tr>
<td>TAB</td>
<td>Total Androgen Blockade</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 Diabetes Mellitus</td>
</tr>
<tr>
<td>TNM(-classification)</td>
<td>Tumour, lymph-Nodes, Metastasis (-classification)</td>
</tr>
<tr>
<td>TUR-P</td>
<td>Trans Urethral Resection of Prostate</td>
</tr>
<tr>
<td>ULSAM</td>
<td>Uppsala Longitudinal Study of Adult Men</td>
</tr>
<tr>
<td>UMC</td>
<td>Uppsala Monitoring Centre</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</table>
Introduction

Epidemiological trends in Prostate Cancer

More than one third of all new cancers reported in men in Sweden in the year 2009, 10,404, were prostate cancers (ref SoS). A clear increase in incidence of prostate cancer has been noted since the 1990s (e.g. 1998; n=6120), due to an increase in prostate specific antigen (PSA) testing. In 2009 an average Swedish man ran until the age of 74, the mean age at diagnosis in the 1990s, a 14% risk of being diagnosed with prostate cancer (ref SoS). During 2007 in Sweden, 2500 men died from prostate cancer indicating that a minority of men with prostate cancer die from their disease (ref SoS). Before the screening era the majority of men with prostate cancer were diagnosed due to clinical symptoms in late stages of disease. The mortality rate of the disease in the population remains stable indicating that the increased incidence is mainly in early detected, non-fatal or curable prostate cancer.

The clinical relevance of the screening detected cases is difficult to determine. In autopsy studies of men “without” prostate cancer (PSA and/or biopsy negative) the prevalence of occult disease is a significant 12-22% (Iguchi 2008 A). Many non-symptomatic PSA-detected cases would not cause severe morbidity in the individual if left untreated. There is, compared to other cancers often a long prodromal subclinical disease phase open for effective curative intervention. While active treatment decreases prostate specific mortality (Bill–Axelson 2011) the morbidity from available treatments is not insignificant. There is still a need for further development of more reliable biological markers for distinguishing indolent from fatal disease in order to improve the risk benefit balance of treatment chosen for the individual. Active treatment options for prostate cancer includes surgery, radiation therapy and at later stages hormonal or chemotherapy.

The increased incidence of the disease of 2.7% per year during the last 20 years appears slowing down with an average incidence increase of 0.8% per year during the last 10 years (ref SoS).
In Uppsala County (population base for Papers I and II) the age standardised rate of prostate cancer was in 2008 somewhat lower than in the nation with 194.4 and 233.7 per 100,000 men per year respectively. The papers presented in this thesis reflect the described drift in diagnosing and handling paradigm for prostate cancer during the last decades with Papers I and II being conducted in an environment of almost solely clinically detected prostate cancer and Paper III including a significant proportion of PSA-screening detected early cancers without clinical symptoms. General PSA-screening is currently still not recommended by the National Board of Health and Welfare. Being highly prevalent in the population, prostate cancer, with its often long subclinical stage may be suitable for primary and secondary preventive measures on an individual or population level if such methods are proven non-toxic, affordable and with a high ability to reduce the risk of (clinically relevant) prostate cancer. Research into identifying risk groups and potentially preventable risk factors for prostate cancer is thus highly relevant. Once the disease is diagnosed and treatment chosen, factors with a potential to optimise the treatment and systems to ensure the safety of treatments are of importance to improve. The thesis touches upon all of these issues.

Clinical presentation, diagnosis, grading, staging and risk classification:

Typical symptoms of clinically evident prostate cancer are lower urinary tract symptoms (LUTS) i.e. from local tumour growth, and skeletal (back) pain from metastatic tumour spread. A high PSA value supports the suspicion of a prostate cancer disease which is commonly confirmed by trans-
rectal ultrasound guided needle biopsy (TRUS) with histopathological analysis. Imaging methods, e.g. ultrasound, magnetic resonance imaging, scintigraphic methods may contribute in diagnosing and staging of the disease and choosing optimal treatment. In the very old or ailing a purely clinically determined diagnosis is occasionally used, though in less than 5% of cases at ages above 80 (ref SoS).

The most important histopathological prognostic factor for prostate cancer is the Gleason grade (Gleason 1966) based on architectural growth pattern of biopsies. Primary and secondary patterns are determined; each scored a value between 1 and 5. Together these form the Gleason grade, ranging from the most benign 2, to the most malignant scoring of 10. In prescreening historical data a majority of men presenting with a Gleason grade of 8-10 and treated conservatively died from their disease within 5 years (Albertsen 2005) while with a Gleason grade of 2-6 less than 5% did, emphasising the wide phenotypical span of the disease. Since Gleason’s first description, the method has been modified shifting classifications somewhat upward (Epstein 2005). In this thesis original Gleason grades and cytology grades retrieved from medical records have been used. In Papers 1 and 2 the majority of cases have been being classified using older Gleason criteria while in Paper 3 scoring definitions are mixed. TRUS has replaced the previously common method fine-needle aspiration with cytology grading. Cytology specimens are commonly graded from G1 to G3 according to the WHO (World Health Organisation, 1980) with grade 3 being the most atypical cell appearance. The WHO grading is usually in studies translated into a Gleason score as follows: G1=2-6, G2=7, G3=8-10. The staging of prostate cancer applied is the TNM system (UICC 1992). A further classification on the risk status of the tumours has been made based on a combination of Gleason grade (or WHO status), TNM stage and PSA value, see below.

Risk factors for Prostate Cancer (Papers I, II)

Age
The main and undisputed risk factor for prostate cancer is age. With a mean age at diagnosis for symptomatic disease being more than 70 years, prostate cancer qualifies as a disease of the old man. This makes it especially relevant to take into account competing risks of death and morbidity from other causes in prostate cancer research. The mean age at diagnosis is decreasing with the increased proportion of early screening detected cases.
Hereditary factors, ethnicity, geographic and genetic variation

Prostate cancer is the second most common male cancer in the world with a 25-fold variation in incidence globally (ref IARC 2013). It is the sixth leading cause of death in men in the world (IARC 2013). Europe, Australia and North America have the highest incidence rates (partly explained by the habit of PSA screening) while the lowest rates are found in Asia and Northern Africa (IARC 2013). Globally the highest prevalence is seen in African-American population (Bono 2004). The difference in prevalence may be genetically, life-style or environmentally explained. That it is not entirely genetically determined is apparent when studying large migrant populations which in time approach the prevalence of the population of the new country of residence (Shimizu 1991, Marks 2004) and also when noting that prostate cancer incidence is currently rapidly increasing in Asia (Sim 2005). Still, no other major cancer form shows the same extent of familial influence as prostate cancer. Twin studies suggest that forty percent of prostate cancer risk can be explained by inherited factors (Liechtenstein 2000). There are families with a marked over-representation of early onset (age <55) prostate cancer over the generations in close relatives. Several variable genes are over-represented (Alvarez-Cubero 2012) in hereditary prostate cancer. Hereditary prostate cancer accounts for 40-45% of all cases of early onset prostate cancer cases while in all prostate cancer in they make up a mere 15 % (Klein 2006).

More than 35 genetic polymorphisms have been identified and validated as being associated with prostate cancer. They are common in the population and their individual risk increase contribution modest and hence their potential usefulness as relevant clinical biomarkers still needs to be determined. In Paper I, variations in two of these candidate genes \textit{hOGG1} and \textit{MnSOD}, coding for enzymes active in DNA repair from oxidative stress, are along-
side selenium dependent anti-oxidative mechanisms and smoking explored in relation to prostate cancer.

Oxidative stress, endogenous factors
Naturally occurring free radicals, reactive by-products of oxygen metabolism contribute to chronic disease and ageing processes. The term “oxidative stress” describes a state of an excess of such reactive oxygen species (ROS). ROS creates DNA-base modifications with altered function such as having a role in malignant degeneration, e.g. prostate carcinogenesis (Malins 2001). Both inherited and acquired defects in cellular defence against ROS result in increased oxidative stress. Endogenous (genetic) and exogenous antioxidative factors and mechanisms act interdependently in preventing the development of prostate cancer (Li 2005). Two endogenous factors are explored:

**hOGG1** Oxidative stress (and UV radiation) triggers the formation of the oxidative DNA base mutation, the 7, 8-dihydro-8-oxoguanine (8-oxoG) (Lu 1997, Trzeciak 2004). The enzyme oxoguanine glycosylase (OGG1) is primarily responsible for the repair of 8-oxoG. Several genetic variants of OGG1 have been described with an at least four-fold difference in enzymatic activity (Audebert 2000). Single nucleotide polymorphisms (SNPs) in the hOGG1 gene, are associated with cancer progression in general (Lu 1997, Goode 2002) and with prostate cancer (Xu 2002, Chen 2003, Weiss 2005, Klein 2007) in particular.

**MnSOD** Superoxide dismutases (SODs) are essential enzymes catalyzing the dismutation of superoxide into oxygen and hydrogen peroxide, in all forms of life (Mc Cord 1988). Superoxide, the main ROS, is active in the immune system killing of microorganisms but excessive levels of it risk inactivating important enzymes. SOD serves a key antioxidant defence by maintaining the integrity of enzymes. Three different SODs co-factored with different metallic ions exist in humans. Manganese-SOD (MnSOD) is the primary antioxidant enzyme within mitochondria.

The MnSOD-catalyzed dismutation of superoxide may be written as follows:

\[
\begin{align*}
\text{Mn}^{3+}\text{-SOD} + \text{O}_2^- & \rightarrow \text{Mn}^{2+}\text{-SOD} + \text{O}_2 \\
\text{Mn}^{2+}\text{-SOD} + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{Mn}^{3+}\text{-SOD} + \text{H}_2\text{O}_2
\end{align*}
\]

Over-expression of MnSOD protects against pro-apoptotic stimuli while decline in MnSOD activity is observed in diseases including cancer and also with ageing (Macmillan-Crow 2001). The enzymatic activity of MnSOD varies 50 fold with genotype and has a 15% higher activity in females. It shows a significant inter-ethnic variation which in addition to genetic variation also may be attributable to dietary or other environmental factors (Bastaki 2006). One genetic variant of MnSOD (rs4880) has been associated
with overall increased risk of (primarily) aggressive prostate cancer (Iguchi 2008 B), both in low (Woodson 2003) and high selenium status populations (Li 2005, Chan 2009).

Oxidative stress, exogenous factors
Shamberger and Frost early made the pioneering observation of an inverse association between selenium status of populations and cancer risk (Shamberger 1969). Numerous subsequent studies have investigated this observation further and in prospective studies generally confirmed it for some types of cancer, among them prostate cancer and most often of advanced or aggressive type (Yoshizawa 1998, Vogt 2003, Li 2004, Brinkman 2006, Navarro-Silvera 2007, Pourmand 2008, Rayman 2012). Some have noted that the association predominately is found in smokers (Nomura 2000, Peters 2007).

Selenium is an essential trace element in human metabolism. An adequate selenium level is needed for the function of more than essential 25 selenoproteins/enzymes (Rotruck 1973, Brown 2001, Seo 2002A, Seo 2002B) which are highly expressed in the prostate (Klein 2004). Enzyme functions relevant for prostate cancer include DNA repair from ROS, cell proliferation, cell cycle arrest, apoptosis, androgen receptor signalling and anti-inflammatory effects (Whanger 2004, Rayman 2012). Oxidative stress increases with androgen exposure and thus the antioxidative activity of selenoenzymes is particularly relevant for prostate cancer (Peters 2008B). Polymorphisms in selenoprotein genes (SEPP1, SEP15) have effects on selenoprotein function and of risk of prostate cancer development or prostate cancer mortality (Burk 2009, Reeves 2009).

Selenium has a narrow safety window of intake of 30-900µg/day (Ashton 2009). It is excreted in urine and faeces and homeostasis maintained primarily by the kidneys. Selenium status varies widely in the world, in line with selenium intake (Rayman 2012) of crops and animal products which may vary thousand-fold in selenium content depending on local soil level of selenium. Mean intake of Selenium is 40µg/day in Europe and 90-150µg/day in the USA (Fairweather-Tait 2011). Selenium status is measured in plasma or serum (interchangeably) or from nail clippings (Ashton 2009). 50-60% of selenium is bound in the transport protein selenoprotein P and 10-30% in the anti-oxidant enzyme glutathione peroxidase (Ashton 2009, Brown 2001). In European populations selenium status is generally low with a mean serum concentration of 89.2+/−14.6µg/l, range: 48.2-124 µg/l (Carmona-Fonseca 2010), while in the US the selenium status is often >135 µg/l (Bleys 2008). The recommended minimum daily allowance varies between 30 and 55µg but is disputed (FAO/WHO 2001, NIH 2012).

The association between selenium status and general health effects in a population is U-shaped. Extreme selenium deficiency causes the potentially
fatal Keshan disease, with myocardial necrosis, susceptibility of infections and irreversible central nervous system damage (Burk 2009). Low levels give rise to increased all-cause and cancer mortality, immune system and cognitive deficiency, dementia and thyroid dysfunction (Bleys 2008, Akbaraly 2005). Too low serum selenium levels, <100µg/l inversely relate to overall accumulated DNA damage (Karunasinghe 2004) in prostate cancer patients, i.e. a level common in e.g. Sweden. In large prospective studies serum selenium concentration up to 135µg/l is associated with decreased mortality but above this level mortality again increases (Bleys 2008, Akbaraly 2005). Symptoms of acute or chronic toxicity (selenosis) include disorders of the nervous, muscular, cardiopulmonary and gastrointestinal system along with disorders of hair, nails, skin or teeth. With low selenium supply some selenoproteins in the central nervous system are prioritised at the expense of other tissues (Reeves 2009). Low serum selenium levels measured may hence by this redistribution situation in reality relatively mirror even lower selenium status in some “unprioritized” tissues. Factors known to decrease serum selenium levels are erythrocyte sedimentation rate (ESR), total serum cholesterol and body mass index (BMI) (Ghayour-Mobarhan 2005).

Selenium has been used in cancer prevention trials, both on population level and in high risk groups. The results from such studies have not so far been convincing enough to promote mass interventional measures in a general population. The most well-known example in prostate cancer is the recent SELECT trial (Lippman 2009) – a large interventional study on selenium and/or vitamin E supplementation with the aim of reducing the risk of prostate cancer. The SELECT was prematurely stopped due to lack of effect of selenium and an increased prostate cancer risk in the vitamin E group. Speculatively, the lack of effect of selenium supplementation may have been due to only subgroups of men benefitting from intervention: e.g. men with specific genetic polymorphisms of the MnSOD-gene (Li 2005) or men with low baseline levels of serum selenium (Block 2009, Hatfield 2009, Facompre 2009). Such subgroups were not defined in the SELECT study and could be relevant to study further, particularly in light of later findings of an interaction between selenium and genetic polymorphism in selenoprotein genes (Penney 2010). Paper I contributes to the knowledge of this area by investigating the interaction between selenium level and genetic polymorphism and prostate cancer.

The Metabolic syndrome

The metabolic syndrome (MetS), also known as the insulin resistance syndrome, is a cluster of risk factors associated with cardiovascular disease (CVD). Key features are central obesity, high blood pressure, dyslipidemia and insulin resistance or hyperglycaemia or type 2 diabetes mellitus.
In Paper II where the association between the MetS and prostate cancer is evaluated, we used two clinically oriented definitions of the MetS: the International Diabetes Federation (IDF) definition and the National Cholesterol Education Program (NCEP) definition, for which we had full baseline data at age 50. A harmonised definition has been established to unify the IDF and NCEP definitions (Alberti 2009).

Numerous potential biologic mechanisms for the MetS being associated with prostate cancer have been described (de Nunzio 2012) including increased serum insulin with insulin resistance, elevated levels of IGF-1, increased fasting glucose levels, polymorphisms in the insulin gene, changes in the sex hormone pathways and an increased inflammatory state in general. Previous studies of an association between the MetS and prostate cancer have shown divergent results (Hsing 2007), at least partly due to differences in study size, baseline characteristics, methodology and timing of follow up. Few studies claiming to do so have actually considered the full MetS, i.e. not a MetS truncated or significantly altered due to lack of individual component data. Among the few that have, some have found a positive association in Scandinavians (Lund Håheim 2006, Laukkanen 2004) and in African Americans (Beebe-Dimmer 2007 and 2009) while others found an inverse association in a mixed population (Tande 2006) or no relationship in Scandinavians (Martin 2009) or in whites in the USA (Tande 2006). Most studies have thus only analyzed the association between prostate cancer and selected components of the MetS (Hsing 2000, Baillargeon 2006, Giovannucci 2007, O’Malley 2006). Associations between components of the MetS and aggressive prostate cancer only, have been found e.g. insulin resistance (Stocks 2007) adiposity or high BMI (Hsing 2000, Freedland 2005 and 2009, MacInnis 2003, Gong 2007). Others have found associations between components of the MetS and prognosis or mortality in prostate cancer only, such as for obesity and high plasma C-peptide concentration (Ma 2008), high BMI (Andersson 1997, Rodriguez 2007), hyperinsulinemia and insulin resistance (Hammarsten 2005). Studies have consistently found an inverse association between T2DM and prostate cancer.

Smoking, false protectivity, competing risk

While tobacco smoking is a risk factor for many other types of cancer, its role in relation to prostate cancer is considered unclear (Khan 2010, Adami 2008). As smoking causes oxidative damage (Valavanidis 2009, Thorne 2009) and is associated with lower serum selenium levels (Arnaud 2006, Northrop-Clewes 2007, Ellingsen 2009), an association between smoking and prostate cancer in particular is biologically plausible. Unsurprisingly, there is an increased risk of prostate cancer specific mortality in smokers with prostate cancer (Gong 2008). With smoking being a very strong risk factor for CVD often occurring earlier in life than prostate cancer, it is pos-
sible that an existing over-risk has been overlooked resulting in an apparent “false protectivity” of smoking in relation to prostate cancer. A competing risk of dying from CVD before having reached the common age of prostate cancer diagnosis may overshadow a later-in-life over-risk of getting prostate cancer. With traditionally used statistical methods for survival analysis such effects of competing risk are not taken into account. Similar possibly false protectivity findings of T2DM in relation to prostate cancer may be suspected. An interaction between smoking and selenium levels on the risk of prostate cancer has been noted in some studies (Nomura 2000, van den Brandt 2003, Peters 2008A) where smokers with very high selenium status have a lower prostate cancer risk than smokers with a low selenium status. Others have not seen observed this (Yoshizawa 1998, Allen 2008, Steinbrecher 2010). An association between MnSOD polymorphisms and risk of prostate cancer has also been described to be influenced by smoking status (Kang 2007, Cooper 2008).

Drug utilisation and Pharmacovigilance (Papers III, IV)

Prostate Cancer treatment

Decisions on prostate cancer treatment are made based on the risk classification of the tumour and on individual patient factors e.g. co-morbidity, remaining life expectancy, risk of side-effects of treatment options and patient preferences. National treatment guidelines regularly revised by the professional-scientific community are readily available online (ref SoS D, RCC, MPA). An increasing number of men are being diagnosed in early localized stages of disease (i.e. disease confined within the prostate) which lends the opportunity of curative treatment in the form of radical prostatectomy or combinations of external radiation therapy and interstitial brachytherapy. Both methods show reductions in disease specific mortality but side effects such as urinary incontinence, sexual dysfunction and bowel symptoms are very common. An alternative to active upfront treatment is the option of watchful waiting (active monitoring or symptom based therapy). The choice of treatment in early stages of disease is crucial for both survival and quality of life since significant fraction of men currently diagnosed with prostate cancer could live without treatment for many years without significant disease-specific morbidity.

In advanced stages of disease with overt symptoms of local or metastatic tumour growth, cure is realistically not achievable. Temporarily effective palliative treatment options are available such as general or local radiation therapy, local surgical intervention, chemotherapy but most commonly: hormonal treatment.
Hormonal treatment

Male sex hormones, androgens, have an effect on the progression of prostate cancer and methods to decrease androgen levels or androgen effects are commonly used. Such hormonal therapy or androgen deprivation therapy (ADT) has temporary effect which may last up to several years. Eventually however, the disease becomes hormonally or “castration-“independent and the treatment effect ceases. Periodic treatment to overcome the hormone independence has been demonstrated successful in individual cases. The traditional ADT was orchiectomy (surgical castration), which induces immediate remission giving temporary symptom relief. Another previously used method was female sex hormones, estrogens. These two methods have almost completely been replaced by other drug classes e.g. anti-androgens blocking the effects of androgens and gonadotropine releasing hormone (GnRH) analogues which decrease androgen production. Lately new hormonal treatment approaches have been developed to overcome resistance to existing drugs: the GnRH-antagonist degarelix and the androgen biosynthesis inhibitor abiraterone. The efficacy and effectiveness of hormonally active treatment methods for prostate cancer are undisputed. Notable harmful side effects include loss of libido, impotence, gynecomastia, hot flushes, low mood, and osteoporosis while the suspected risk of cardiovascular side effects is still debated. Men with a high risk of disease progression after intended curative surgery or radiotherapy may have benefit from anti-androgen treatment.

Anti-androgens

Administered as monotherapy or in certain situations combined with GnRH analogues anti-androgens play an important role in the treatment of prostate cancer in the non-curative setting. Bicalutamide is by far the most commonly used anti-androgen. It is a non-steroidal compound without intrinsic endocrine effects. It binds to the androgen receptor thereby inhibiting circulating androgens from exerting their stimulating effect (Furr 1996). In addition, bicalutamide accelerates the degradation of the androgen receptor (Waller 2000).

Prescribing patterns

Treatment guidelines include advice on anti-androgen use. Circumventing a well-founded approved indication by an off-label prescription may be appropriate in the treatment of an individual patient, although it may appear on record as an inaccurate prescribing pattern of the drug. The extent to how treatment guidelines regarding anti-androgens are adhered to by prescribers,
and the determinants of real-life prescription and prescription influencing factors have not been studied previously.

Treatment adherence

Adherence is the extent to which a patient’s behaviour coincides with medical advice (Haynes 1979). There are several methods for estimating it. Adherence to any self-administered long-term drug treatment, often defined as having >80% of days covered by filled prescriptions, is found to be strikingly low at around 50% (Bardel 2007, Williams 2008, Lindberg 2008, d’Inca 2008). Furthermore, contrary to what would be expected, adherence to self-administered, outpatient cancer treatment has been found only marginally higher (Darkow 2007, Nilsson 2006, Atkins 2006, Partridge 2003). With few exceptions, adherence studies in oncology concern adherence to adjuvant breast cancer treatment. Studies in all-male (oncology) populations have not been performed.

Pharmacovigilance

Pharmacovigilance is the pharmacological science relating to the detection, assessment, understanding and prevention of adverse effects of medicines (WHO 2002). Generally pharmacovigilance relates to information from prescribers (primarily physicians) and patients on the adverse effects of medication but it also relates to published scientific literature.

Approval of drugs, benefit/risk evaluation

Any drug a company wishes to market in Europe has first to be authorised by either the European Medicines Agency or a national medicines agency, e.g. the Medical Products Agency (MPA) aka Läkemedelsverket. Marketing authorisation of a drug is granted once the unwavering criterion of it having a positive benefit-risk (B/R) balance in the group of patients for which approval of indication is sought is fulfilled. The B/R balance is established based on preclinical, pharmaceutical and pharmacokinetic studies and usually confirmed in randomised controlled trials (RCT). The benefits and risks of harm of a drug change over time and external validity then transferring results from pre-approval studies to a real life treatment population should not be assumed. The B/R balance of drugs is continuously surveilled during its lifetime by the marketing authorisation holder (MAH) and responsible authorities. A significant part of the risk surveillance is signal detection (see below) in spontaneous reporting. When new serious risks are identified, the B/R of the drug is reassessed. Actions may be taken, from smaller restrictions of the approved indication to the most extreme being the forced withdrawal of the product. The term “risk” in the B/R concept is increasingly
being replaced by the more semantically logical term “harm” (Aronson 2009), which more accurately describes the core assessment of the balance of positive and negative effects of drugs.

Efficacy vs. effectiveness, the risk of harm in trials vs. in real life

Benefits of a drug – the efficacy- for the patients fulfilling often strict inclusion and exclusion criteria in clinical trials are generally well established at the time of approval. The effectiveness of a drug– the benefit in real life clinical use- is rarely as robustly proven. Analogous to the difference between efficacy and effectiveness, the identified and potential harm in the study population does not equal the harm in the real life treatment populations. Patient groups not studied in clinical trials are often the elderly and or populations with concomitant disease and drug treatment. These groups may constitute the majority of the treated population in the real life clinical setting and stand a risk of harm clearly different from RCT populations. Post-marketing information is needed to better define the benefits and risks of harm in real life use of the drug.

Spontaneous Reporting for Signal Detection

The thalidomide disaster in the early 1960s prompted drug authorities to develop systems for detection of unknown side effects and risks of drugs. Spontaneous reporting systems have since been established in more than 100 countries. Signal detection in spontaneous reporting databases has proven to be a simple and cost effective tool for identifying suspected new adverse drug reactions. Some of the better known examples of safety signals detected include apart from phocomelia from thalidomide during pregnancy, vaginal clear cell cancer in girls of mothers using diethylstilbestrol during pregnancy, suicidal ideation and suicide induced by the antiobesity drug rimonabant and the latest example; narcolepsy in relation to the pandemic vaccine Pandemrix.

The spontaneous reporting systems differ by country regarding accepted reporters (e.g. physicians, pharmacists, consumers)and the managing of the systems (national authorities, university based or independent institutions). A few standardised terminologies for coding adverse events and drugs are applied which lend the opportunity to assemble and analyse information from different sources to detect and act on new safety signals.
Pharmacovigilance signal

A signal is: “information that arises from one or multiple sources (including observation and experiments), which suggests a new potentially causal association or a new aspect of a known association, between an intervention and an event or set of related events, either adverse or beneficial, that is judged to be of sufficient likelihood to justify verificatory action” (CIOMS 2010).

Signal detection methods, the PRR:

The original signal detection method of case-by-case assessment of spontaneous reports of adverse drug reactions (ADR) is effective but resource consuming, especially in large ADR databases with high volumes of incoming reports.

This has led to the development and acceptance of semi-automated signal detection methods including primary step(s) of detection by statistical disproportionality analysis, followed by manual clinical validation. Several statistical methods are currently in use (Finney 1974, Bate 1998, Evans 2001, Szarfman 2002) but no gold standard has been established (Puijendebroek 2002, Hochberg 2009). The methods have the ability to detect new safety signals for drugs years earlier than traditional manual methods (Hauben 2004, Alvarez 2010). Strengths, limitations and differences between different pharmacovigilance signal detection methods including their initial disproportionality part have been analysed and described previously (CIOMS 2010).

Within the European Union (EU) signal detection is continuously performed in the common ADR database EudraVigilance (EV, EV2013) using the Proportional Reporting Ratio (PRR) method (Finney 1974, Evans 2001, EVEWG 2008).

The PRR for a drug-ADR combination is defined as:

$$\text{PRR} = \frac{a}{a+c} \div \frac{b}{b+d}$$

With a, b, c and d denoting:

<table>
<thead>
<tr>
<th>Drug of interest</th>
<th>All other drugs in database</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction of interest</td>
<td>a</td>
</tr>
<tr>
<td>All other reactions</td>
<td>c</td>
</tr>
</tbody>
</table>

Briefly: a PRR value > 1 implies that a drug-reaction pair appears more often in the database than would be expected by chance, a PRR value of 2: twice
as often. In Paper IV alterations of the variables “b” and “d” in the table are used for the analysis.

The results from the PRR analysis are delivered as line listings of all PRR values for a drug (exemplified below) which in the next step are clinically evaluated; PRR (- and +) denoting lower and higher 95% confidence interval (CI) of the PRR value:

<table>
<thead>
<tr>
<th>Reaction</th>
<th>PRR(-)</th>
<th>PRR</th>
<th>PRR(+)</th>
<th>Total Case #</th>
<th>Fatal Case #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agranulocytosis</td>
<td>0,05</td>
<td>0,19</td>
<td>0,75</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Anaemia</td>
<td>1,00</td>
<td>1,35</td>
<td>1,81</td>
<td><strong>43</strong></td>
<td>7</td>
</tr>
<tr>
<td>Anaemia haemolytic autoimmune</td>
<td>0,17</td>
<td>1,24</td>
<td>8,82</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Aplastic anaemia</td>
<td>0,48</td>
<td>1,47</td>
<td>4,57</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Bone marrow failure</td>
<td>0,41</td>
<td>0,92</td>
<td>2,04</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Coagulopathy</td>
<td>0,17</td>
<td>0,67</td>
<td>2,67</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Disseminated intra-vascular coagulation</td>
<td>2,15</td>
<td>3,46</td>
<td>5,56</td>
<td><strong>17</strong></td>
<td>7</td>
</tr>
<tr>
<td>Eosinophilia</td>
<td>0,18</td>
<td>0,57</td>
<td>1,76</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Erythropenia</td>
<td>0,65</td>
<td>4,67</td>
<td>33,31</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>0,10</td>
<td>0,41</td>
<td>1,63</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Haemolysis</td>
<td>0,08</td>
<td>0,54</td>
<td>3,82</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Haemolytic anaemia</td>
<td>0,48</td>
<td>1,15</td>
<td>2,76</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Haemorrhagic diathesis</td>
<td>3,73</td>
<td>9,00</td>
<td>21,70</td>
<td><strong>5</strong></td>
<td>1</td>
</tr>
</tbody>
</table>

The PRR method delivers signals of disproportionate reporting (SDRs), reported ADR-drug combinations which
1. have an elevated PRR value (occurs disproportionately often in the database) and
2. reach a case count above an a priori specified threshold.

Isolated reports describing a very rare symptom in the database may give rise to extremely high PRR values, particularly if the total reporting for the drug is limited. The use of the case-count threshold for the SDR is therefore applied to reduce the appearance of irrelevant chance findings.

An SDR again, is a statistical association which may or may not represent a signal of a true causal relationship between a drug and an ADR. Large amounts of SDRs are regularly delivered within the EU system from PRR screening and while the method is sensitive a majority of SDRs delivered represent noise from e.g. statistical chance findings, artefacts, already acknowledged ADRs, SDRs confounded by disease or by “disease spill-over”, i.e. mis-coded disease terms such as diabetes mellitus for a diabetes drug. While a minority of false SDRs are easily dismissible as non-signals, most need profound expert knowledge of the mechanisms of action of the drug and clinical experience of the disease to determine whether or not they are
signals in need of verification or not. This evaluation is very resource intensive. Despite the PRR method’s sensitivity, some SDRs may not be delivered by the PRR despite the existence of a causal relationship. This may appear if an ADR is mistaken for a symptom of the disease under treatment and is not reported or if the ADR is masked by being very commonly reported for other drugs in the database.

A purely statistical approach on signal detection for identifying or refuting suspicions of new adverse drug reactions in databases is thus not sufficient and any refinements of the sensitivity and specificity of the PRR would be welcomed to save manual resources.

Refinements of the PRR can be attempted by adjusting the thresholds defining an SDR, e.g. increasing or decreasing the level of the PRR value and/or adjusting the case count required. Chance findings can thereby partly be prevented to have too great an influence on the output of the analysis while this entails the risk is missing important signals due to decreased sensitivity. Despite adjusting thresholds, with maintained sensitivity the number of delivered SDRs from a database (e.g. the EV) will still be immense. Attempts in the EU to conserve resources of clinical evaluation resources by altering the statistical analysis have recently included re-defining the SDR by increasing the required case count from the conventionally used 3 to 5 at present. This incurs a delay in the delivery of new SDRs and detection of signals.

Improving the performance of disproportionality analysis methods to increase the signal to noise ratio is important for the effectiveness and usefulness of the methods. Experimental measures to achieve this without causing a delay of the signal detection have in literature included restricting analyses to classes of drugs while a restriction by indication which we attempt in Paper IV has not been explored.
Aims of the thesis

Overall aim
To study, during long term follow-up, possible macro- and micro-metabolic risk factors in prostate cancer and the interaction between them; to clarify statistical challenges of competing risks when investigating risk factors for prostate cancer; to explore aspects of palliative drug utilisation in prostate cancer and to investigate possible improvements of a signal detection method for adverse drug reactions.

Specific aims
I To investigate the association between middle age serum selenium levels and clinically relevant prostate cancer, and to assess if smoking modified this association; to explore if polymorphisms in the genes *hOGG1* and *MnSOD* influence any effect of serum selenium on prostate cancer risk.

II To investigate the impact of the metabolic syndrome (MetS) using the NCEP and the IDF definitions, or components of the MetS and life style factors, at baseline on the risk of developing clinically relevant prostate cancer taking competing risks of death into account.

III To describe and analyse aspects of real life anti-androgen utilisation both from a prescriber and a user perspective, specifically the extent of off-label treatment, factors influencing dosage, reasons for and time to discontinuation; factors influencing adherence to treatment.

IV To investigate the feasibility and performance of a hypothesized improvement of the PRR signal detection method, the PRR-TA. To assess in detail the output from the method by studying drugs in prostate cancer and gluco-metabolic disease, in relation to approved product information.
Material and Methods, Papers I, II

The ULSAM cohort:

Papers I and II are based on the population based Uppsala Longitudinal Study of Adult Men (ULSAM) cohort (ref ULSAM). All men born 1920-1924, who were residents of Uppsala, Sweden, were at age 50 (in 1970-73) invited to participate in a health examination aiming at identifying risk factors for diabetes and cardiovascular disease (CVD). Of 2841 men invited, 2322 men (82%) participated in the baseline investigation forming the ULSAM. The men have since been invited to re-examinations at ages 60, 71, 77, 82, 88 and data has been supplemented with annual updates on mortality and in-hospital morbidity. The ULSAM cohort is homogenous as regards the ethnic background and the age (standardized at investigations) of the participants. The participant rate is high and the follow-up up to 34 years almost complete through linkage to national registers with high coverage.

In Papers I and II baseline data except for genotyping data was extracted from investigations at age 50. Data on serum selenium was available for 2045 men who constitute the study base for Paper I (figure 1, Paper 1). The men for whom baseline data was available for determining MetS status (NCEP/IDF) constitute the study base for Paper II. The genotyping for Paper I was performed from blood samples collected at age 71, in 1991-95. Of 1681 men alive and still living in Uppsala at that time, 1221 (73%) participated in the re-investigation. Genotyping was performed and results available for 1005 men. The mean baseline level of serum selenium and two factors negatively correlated to selenium concentration, erythrocyte sedimentation rate (ESR) and total serum cholesterol did not differ significantly between the 1005 genotyped and the full cohort of 2045 at baseline. Therefore, the genotyped men were considered a representative sub-sample of the full cohort.
Investigations at baseline

All ULSAM investigations were carried out under standardised conditions and have been described extensively previously (ref ULSAM). The cohort has been characterized in detail with regard to the MetS (Sundström 2006). In brief:

**Anthropometry:** Body mass index (BMI) was calculated as weight (kg) divided by height (meters) squared. Waist circumference was measured midway between the lowest rib and the iliac crest in a supine position (Zethelius 2002). Waist was only measured in a sub-sample of men and a BMI cut-off point from a linear regression analysis was instead used for the MetS definition as in previous studies (Sattar 2003, Sundström 2006).

**Blood pressure** was measured on the right arm after 10 minutes rest using mercury manometers and read to the nearest 5 mmHg mark.

**Erythrocyte sedimentation rate** was determined by Westergren's method (Westergren 1926).

**Fasting blood-Glucose** was measured by spectrophotometry using the glucose oxidase method and converted to plasma-glucose values used in the definitions of MetS by using the correction factor 1.11.

**Serum cholesterol and triglyceride concentrations** were determined on a Technicon Auto Analyzer type II (Rush 1971) in 1981-82 on serum sam-

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**Figure 1, Paper I. Study base for Paper I.**
amples that had been stored in liquid nitrogen since 1970-73. HDL was assayed in the supernatant after precipitation with a heparin/manganese-chloride solution.

**Serum selenium** was determined using the graphite-furnace atomic absorption spectrometric method (Alfthan 1982). Samples were diluted with a solution containing nickel to reduce the volatility of selenium and nitric oxide, to keep samples free of precipitates, and measured by a standard additions method. The drug clozapine (on the market at baseline) decreases serum selenium levels (Vaddadi 2003), but none of the participants reported clozapine use at the time.

**Smoking status** was based on baseline personal interview reports characterising the men as smokers, non- or ex-smokers (Hedstrand 1975).

**Investigation at age 71**

**Genotyping** was performed on whole blood samples for DNA extraction whereby two selected Single Nucleotide Polymorphisms (SNPs) in candidate genes were typed using the Golden Gate Assay (ref “genotyping”, Fan 2003). Four *hOGG1* SNPs were typed; rs125701, based on the findings by (Figueroa 2007) and another three SNPs randomly distributed over the gene. For the *MnSOD* rs2758331 was for technical reasons typed as proxy for the rs4880, with a correlation coefficient ($r^2$) = 0.92, (Choi 2008, Cooper 2008, Soerensen 2009) and another five SNPs of the distributed over the gene.

**The Metabolic syndrome, definitions:**

Two clinically oriented MetS definitions were used; the National Cholesterol Education Program Adult Treatment panel III (NCEP; ref NCEP 2013) and the International Diabetes Federation (IDF; ref IDF 2013) definitions, both previously applied in relation to CVD in ULSAM (Sundström 2006).

The **National Cholesterol Education Program Adult Treatment Panel III (NCEP)** defines the MetS as established if *three or more* of the following components are present:
- elevated fasting plasma glucose level ($\geq$6.1 mmol/l),
- elevated blood pressure ($\geq$130/85 mm/Hg) or pharmacological treatment for hypertension,
- elevated triglyceride level ($\geq$1.7 mmol/l),
- lowered HDL cholesterol level (<1.03 mmol/l for males)
- central obesity: large waist circumference (>102 cm for males)

The **International Diabetes Federation (IDF)** consensus defines the MetS as established with the presence of
- the absolute criterion central/abdominal obesity with ethnicity-specific values; waist circumference $\geq 94$ cm in Caucasian males. With BMI is $>30$ kg/m², central obesity is assumed and waist circumference does not need to be measured

and any two of the following criteria:
- elevated fasting plasma glucose level ($\geq 5.6$ mmol/l) or pharmacological treatment for and/or previously diagnosed T2DM. Oral glucose tolerance test is recommended but not necessary to define presence of the syndrome.
- hypertension ($\geq 130/85$ mmHg) or pharmacological treatment thereof,
- elevated triglyceride level ($\geq 1.7$ mmol/l) or pharmacological treatment thereof,
- lower than normal levels of HDL cholesterol ($<1.0$ mmol/l for males) or pharmacological treatment thereof.

Follow up and outcome definitions Paper I, II

Follow-up for Papers I and II started at baseline with a censoring date of December 31, 2003 at the age, if still alive, of 79-83 years. Mean follow-up time was 26.5 and 30.3 years in Papers I (full cohort) and II respectively. Diagnosis of invasive prostate cancer (by International Classification of Diseases and Related Health Problems, 10th Revision, ICD-10; C61) was considered an event. Identification of invasive prostate cancer and cause of death was achieved by linking the unique personal identification numbers to the Population Register (ref SCB), the Cancer Register (CR), the Hospital Discharge Register (HDR) and the Causes of Death Register (CDR; ref SOS A, B) all at the National Board of Health and Welfare (NBHW). The CR and the CDR were established in 1958 while the HDR covering all somatic inpatient health care was established in 1987. Reporting to the registers is compulsory and high, with coverage of prostate cancer in the CR of more than 95% (Barlow 2009) and for the other three registers 99% or more (Merlo 2000, Tunstall-Pedoe 1994).

Identified events of prostate cancer were confirmed by systematically reviewing medical records, at the same time collecting clinical tumour characteristics and clinical details of each case. In a few cases (7 and 9 respectively in the Papers I and II), where medical records were not available, the diagnosis, staging and treatment was verified with data from the National Prostate Cancer Registry (NPCR; Varenhorst 2005); the NPCR described more in detail in relation to Paper III. Men without prostate cancer were censored at the time of death from a cause other than prostate cancer or if alive at end of follow-up.
Statistical analysis methods:

All confidence intervals (CI) in the thesis are calculated on the 95% level.

**Paper I:** The full cohort of 2045 men with baseline measurements available was analyzed for the main outcome of prostate cancer in relation to selenium levels and smoking. The 1005 genotyped men were analyzed separately to explore any effect modifications of polymorphisms in OGG1 or MnSOD. The full cohort and the genotyped subcohort were divided into tertiles according to serum selenium concentrations, selenium influencing factors and BMI. Risks of a) prostate cancer and b) death without a diagnosis of prostate cancer were calculated by means of cumulative incidence curves (Kalbfleisch 2002) censoring for end of follow up without events and considering the both types of endpoints a) and b) as competing events.

Smoking status and BMI influence life span expectancy and constitute competing forces of mortality with prostate cancer. Competing risk proportional hazards models were determined for the sub-distribution of prostate cancer through competing risk regression (Fine 1999) considering death without a diagnosis of prostate cancer as a competing risk. Hazard ratios derived from proportional hazards models were used as the estimator of relative risk (RR) of the effect of tertiles of selenium, smoking habits, and SNPs.

**Paper II:** Absolute risks of prostate cancer and of death without a diagnosis of prostate cancer were calculated by means of cumulative incidence proportions (Kalbfleisch 2002), where the events of prostate cancer and of death, whichever came first, were considered as competing events, censoring for end of follow-up. The probability of being diagnosed with prostate cancer was calculated as one minus the Kaplan Meier estimate of prostate cancer free survival, censoring for both death and end of follow up. The conditional probability of prostate cancer given that death did not occur was calculated as the fraction of the cumulative incidence of prostate cancer divided by one minus the cumulative incidence of death without prostate cancer; confidence intervals calculated according to Pepe (Pepe 1993) using R-code (Ihaka 1996). Relative risks in the conditional probability setting were calculated as odds ratios with 95% bootstrap confidence intervals. Relative risks of prostate cancer and death was calculated by means of Cox proportional hazard models (Cox 1972). In the analysis of death, we censored for occurrence of prostate cancer. Similarly, in the analysis of prostate cancer we censored for death.
Material and Methods, Paper III

In Paper III we investigated anti-androgen utilisation patterns: the extent of off-label treatment and factors influencing bicalutamide dosing levels, reasons for and time to anti-androgen discontinuation; adherence to bicalutamide treatment; and factors associated with drug adherence.

We used information from the population-based research database Prostate Cancer Database Sweden (PCBaSe) including data from the Swedish Prescribed Drug Registry (SPDR) in addition to information on marital status and socioeconomic status from Statistics Sweden (ref SCB) and data to calculate the Charlson co-morbidity index (Charlson 1987, Berglund 2011) from the National Patient Register.

PCBaSe Sweden, National Prostate Cancer Register (NPCR)
The PCBaSe is based on linkages between the NPCR and other nationwide health care and socioeconomic registries (Hagel 2009). Between 1996-2006, 80079 cases registered in NPCR were linked to among other data sources the CR, CDR, SPDR, the National Patient Register and the Register of the Total Population. The NPCR is a government funded population based quality data registry for all new cases of prostatic adenocarcinoma in Sweden (Adolfsson 2007). It contains detailed individual data on diagnosing unit, date and cause of diagnosis, tumour grade and stage according to the TNM classification, PSA- levels at diagnosis and data on planned primary treatment for the first 6 months. More than 97% of incident prostate cancer cases in the CR are registered in the NPCR.

Study population
The source population for the study was the 76,624 men registered in the NPCR during the years 1997-2006. Those alive after the initiation of the SPDR; n=58143, on July 1, 2005, and identified as having been dispensed bicalutamide were eligible for analysis of treatment adherence.
Bicalutamide indication, treatment guidelines

Bicalutamide monotherapy improves progression free survival in locally advanced prostate cancer, and overall survival in radiotherapy-treated men (Iversen 2010).

During the main part of the study period, the legally approved indication for monotherapy bicalutamide (150 mg) was: “treatment of locally advanced, non-metastasized prostate cancer when hormonal treatment is indicated and surgical or medical castration is considered unsuitable”. National and regional guidelines recommended during the same period monotherapy anti-androgen in men: “with locally advanced disease or localised symptomatic cancer when curative treatment is not feasible” (MPA, SoS, RCC). The indication term “locally advanced disease” corresponds to the categories “high risk” and “regionally metastatic disease” used in literature including in Paper III (see below). Bicalutamide was also available in a lower dosage (50 mg) approved in combination with GnRH analogues for metastatic prostate cancer, mainly used for flare protection during the initiation of GnRH treatment.

Risk classification

We used a modified version of the National Comprehensive Cancer Network (NCCN) risk group classification (ref NCCN, Berglund 2011):

- **Low risk:** T1–2, Gleason Score (GS) 2–6 and PSA <10 ng/ml
- **Intermediate risk:** T1–2, GS 7 and/or PSA 10 to <20 ng/ml
- **High risk:** T3–4 and/or GS 8–10 and/or PSA 20 to <50 ng/ml
- **Regionally metastatic:** N1 and/or PSA 50 to <100 ng/ml, M0 or MX
- **Distant metastatic:** M1 and/or PSA ≥100 ng/ml

Follow-up and outcome definitions, statistical analysis methods

Reasons for discontinuation was analysed for all anti-androgens (bicalutamide, flutamide and nilutamide), while dosage and adherence was studied for bicalutamide use only since prescription of other anti-androgens was extremely rare (n =88) and approved dosages differ. Adherence was studied in 1406 men with at least 12 months of bicalutamide use recorded and with an on-drug run-in period of 4 months, i.e. with data on 16 months, without evidence of other hormonal treatment (figure 1, Paper III).

Individually prescribed dosages were categorised as “as per approved indication” (150 mg daily) or as “off-label prescription” (<150 mg daily). Fac-
tors possibly influencing the choice of planned dose were explored, and tests of equality within factors were performed with uni- and multivariate logistic regression models.

A time-to-event analysis of drug discontinuation was performed. Reasons for drug discontinuation were categorised into four types:

1. Death
2. Initiation of GnRH analogues
3. Surgical orchiectomy or initiation of estrogens
4. Unexplained drug discontinuation

Unexplained drug discontinuation was defined as occurring 6 months after the last date of drug supply based on the WHO-defined daily dose (DDD; ref WHO) after the last recorded dispensing date and without evidence of 1–3 as a reason. Total androgen blockade (TAB) was defined as remaining on anti-androgen treatment more than 3 months after initiation of GnRH treatment.

Cumulative incidence proportions (Kalbfleisch 2002) of drug discontinuation by prognostic groups were calculated by using a combination of left truncation and right censoring. Right censoring was performed for end of follow-up, whilst left truncation was performed at the end of the run-in period. Patients were followed either until August 1, 2008, date of death/surgical orchiectomy, first dispensing of GnRH/estrogens or drug discontinuation, whichever occurred first.

Figure 1, Paper III; next page: Flow chart of the investigated antiandrogen monotherapy (AA) treatment population. 2,560 men were prescribed anti-androgen monotherapy (A), 779 of them were excluded due to other treatment recorded before the start of the study period or no AA treatment recorded during the study period, leaving 1,812 men for the analysis of reasons for ending AA treatment (B). Further investigations were made for bicalutamide only and in men with at least 16 months of follow-up without other hormonal therapy. These selection criteria resulted in a study base for the analysis of dosage and adherence of 1,406 men (C) prescribed bicalutamide as primary treatment and alive after the initiation of SPDR in July 2005.
Adherence calculations

Adherence to bicalutamide monotherapy treatment was measured by calculating the medical possession ratio using a flexible starting period (MPRf) (Andrade 2006, Vink 2009) defined as number of days of dispensed prescribed supplies/number of days in study period × 100 %. The MPRf calculations were performed using the individual prescribed daily dose (PDD) for each patient instead of the less relevant DDD of 50 mg. In order not to overestimate adherence, a run-in period of 4 months was used for the adherence calculation (figure 2, Paper III) based on the waiting time distribution (Hallass 1997) for anti-androgen dispensing in the SPDR and the rules governing the Swedish reimbursement system.
Figure 2, Paper III: Visualisation of the method of flexible Medical Possession Ratio (MPRf) used for the adherence and persistence calculation for men with a diagnosis before and after July 1, 2005, the date of the start of the Swedish Prescribed Drug Registry. A one year follow-up was used with a run-in period of four months in order not to overestimate adherence. Each horizontal line exemplifies typical patients with boxes representing a dispensed prescription of bicalutamide, the box-size relating to maximum length of treatment in days based on prescribed daily dose (PDD) and amount of bicalutamide dispensed at each occasion.

The men were categorized according to their adherence level as:
- $\geq 90\%$ very good adherence
- $\geq 80\% - < 90\%$ good adherence
- $\geq 50\% - < 80\%$ poor adherence
- $< 50\%$ very poor adherence

The influence on adherence of medical and socioeconomic factor was explored by a univariate and multivariate linear regression analysis using an arcsine transformation of MPRf as dependent variable.
The data used in Paper IV was retrieved from the *EudraVigilance* (EV) ADR database (ref EV 2013). The EV was established in 2001 for signal detection purposes by national competent drug authorities (NCA) within the EU as well as the European Medicines Agency (EMA). All serious ADRs reported worldwide for drugs approved within the EU are mandated to be reported to the EV from all companies, the marketing authorisation holders (MAH) and EU-NCAs. The database is currently accessible online for drugs approved centrally within the EU and access for other drugs is forthcoming. ADRs are coded using the MedDRA hierarchy terminology (MSSO 2013).

Four drugs were used to exemplify the analysis approach, indicated for chronic diseases in the two therapeutic areas of prostate gland disease and T2DM. They belong to different time windows of a drug life cycle; from newly marketed drugs to long term well-established ones:

- **Bicalutamide** (ATC code L02BB03): Approved in the 1990s with current indications in prostate cancer as monotherapy or in combination with GnRN analogues. Side effects include: gynecomastia, liver toxicity, decreased libido, erectile dysfunction, skin rashes and asthenia.

- **Abiraterone** (ATC code L02BX03): Common EU approval in 2011 with indication of second line oral treatment in prostate cancer, combined with per oral steroids. Abiraterone inhibits an enzyme in androgen (testosterone) biosynthesis present in testicular, adrenal, and prostatic tissues. Side effects include adrenal insufficiency, liver toxicity, skeletal and cardiovascular symptoms. Abiraterone is currently the only approved drug in its class.

- **Metformin** (ATC code A10BA02): Approved in the 1950s with current indication of oral use in T2DM as monotherapy, or in combination with other drugs. Metformin, a biguanide, inhibits gluconeogenesis and glycogenolysis, increases insulin sensitivity by enhancing peripheral glucose uptake, delays intestinal absorption of glucose. Insulin levels are not directly affected by metformin. Side effects include: gastrointestinal symptoms and the potentially fatal lactic acidosis.

- **Vildagliptin** (ATC code A10BH02): Common EU approval in 2008 with the indication of oral use in T2DM as monotherapy, or in combination with other drugs. Vildagliptin, a dipeptidyl-peptidase-4-
inhibitor (DPP4I), increases incretin hormone levels raising glucose dependent insulin secretion in diabetes patients (only) thereby decreasing glucose levels. Side effects include: pancreatitis, hypoglycaemia, liver toxicity and peripheral edema.

EV-ADR data until a cut-off date of June 30, 2012, were included in the analysis. In July 2012, new EU-PhV legislation came into effect which included alterations in reporting rules to the EV and definitions of ADRs. All data in the study are on group level with no individual or identifiable patient data used. Hence according to applicable legislation no approval from the ethics review board was needed for the study.

Proportional Reporting Ratios, PRR, thresholds

Signals of disproportionate reporting (SDRs) for the four investigated drugs were identified by calculating the proportional reporting ratio (PRR) for all drug-ADR combinations on a MedDRA “preferred term” (PT) level in the EV. There were at the time 19 294 PTs in the MedDRA terminology, each representing a unique medical condition. The SDRs were identified from the PRR calculations using the a priori defined cut-off thresholds of both a) a case count of ≥3 (SDR3) in EV and b) a lower 95% confidence interval of the PRR of >1.0, as were conventionally recommended in the EU at the time of the study initiation. SDR3 was used in all but one of our analyses. A higher case count cut off level of ≥5 (SDR5) for identifying an SDR, which was recently introduced in the EU, was also analyzed for comparison.

PRR by therapeutic area

For the new method investigated, hereafter called PRR-by-therapeutic area (PRR-TA) we restricted the background drugs for comparison (the terms “b” and “d” in the PRR equation above) to consist of drugs from the following two respective therapeutic areas instead of all drugs in the EV:

Prostate Gland disease In the prostate gland disease examples, the background used for the calculation the PRR-TAs for bicalutamide and abiraterone respectively were all the substances in ATC-codes L02AE, L02BB, L02BX, G04CA and G04CB with indications mainly for benign prostate hyperplasia (BPH) and Prostate cancer (PrC). PRR calculations were performed with a sequentially more restricted background in the therapeutic area of prostate gland disease. The analysis sequence was as follows, models 1-4:

1. PRR: bicalutamide or abiraterone vs. the whole EV database
2. PRR-TA: bicalutamide or abiraterone vs. drugs indicated for PrC or BPH
3. PRR-TA: bicalutamide or abiraterone vs. drugs indicated for PrC
4. Drug class PRR: bicalutamide vs. other anti-androgens
As abiraterone currently is the only approved drug of its class, a class analysis as in model 4 was not applicable. As some BPH drugs have other indications than BPH (e.g. hypertonia) and to decrease the effect of any off-label use, PRR calculations (models 1–4 above) for bicalutamide and abiraterone were performed both including all ADR reports in the analyses and also restricted to include only reports specified as occurring in male patients.

Type 2 Diabetes Mellitus In the T2DM example, the background used for the calculation of the PRR-TAs for metformin and vildagliptin respectively were all the substances in ATC-code A10B. ADR reports on predefined fixed-dose combination products were not included in the PRR-TA analyses as causality assessment in such products is more complicated. PRR calculations were performed with a sequentially more restricted background in the therapeutic area of T2DM. The analysis sequence was as follows, models 5-8:

5. PRR: metformin or vildagliptin vs. the whole EV database
6. PRR-TA: metformin or vildagliptin vs. non-insulin antidiabetic drugs
7. Drug class PRR: metformin vs. biguanides
8. Drug class PRR: vildagliptin vs. DPP4I

Statistical calculations of the PRR-TA were performed using the open access tool “R” (Ihaka 1996), except for the analyses using the full EV database where PRR data was extracted directly from the EV Data WareHouse Tool in use in September 2012.

The approved EU-Summary of Product Characteristics (SPCs) for the four investigated drugs (July 2012) were used as reference of acknowledged (true positive) ADRs. For the two non-centrally approved drugs metformin and bicalutamide, SPCs for originator and generic products were combined into reference lists of acknowledged ADRs.

All SDRs delivered from the calculations using models 1-8 above were clinically classified independently in two steps by experienced clinical experts in the field of oncology, diabetology and pharmacovigilance, as either A true positive SDRs (i.e. acknowledged ADRs in the SPCs for each drug) or B other SDRs representing terms not acknowledged as ADRs in the SPCs. SDRs classified as B were in turn separated into:

C false positive SDRs confounded by indication or by indication spill-over, i.e. irrelevant for further manual evaluation.
D unclassifiable SDRs, relevant for further manual validation.

Results from the classification were compared and differences obtained were resolved by consensus. In the example of bicalutamide, 950 different ADR terms had been reported (table 2, Paper IV), PRR calculation delivered
95 of these as SDR3s and these were in turn classified as “A” or “B” and the SDRs in the “B” group in turn as “C” or “D” according to above.

Possible masking/de-masking (Gould 2003) of SDRs by using restricted backgrounds for the PRR calculations was evaluated by manually comparing true positive SDRs, “A”s detected by the different backgrounds respectively. The concordance between the methods could hereby be evaluated.

A comparative analysis of the ability of models 1-8 to deliver true positive SDRs, “A”s, was performed defining this ability as the percentage detected ADRs of all acknowledged ADR terms in the SPC. A similar analysis using the SDR5 in models 1 and 5 respectively was also performed.

The number of delivered SDRs from the “C” (false positives) and “D” (unclassifiable and therefore relevant) groups using models 1-8 was identified and compared. A similar comparison using the SDR5 in models 1 and 5 respectively was also performed.

Formal calculations of the different PRR methods’ accuracy are not possible to perform; the “usual” two-by-two table (below) to calculate the sensitivity, specificity, positive predictive value is not applicable.

More than one SDR are often representing similar events and may point to one broader reference ADR term acknowledged as a true ADR in the respective SPCs making the “x” in the table below ambiguous. Exemplified: A broad acknowledged term in an SPC, e.g. “rash” may cover several more specific descriptions of rash terms delivered as SDRs such as rash, localized rash, hemorrhagic rash etc.

Furthermore true negative SDRs cannot be firmly established as it is in this group the new not yet established ADRs are waiting to be detected, making the “y” and “w” equally less useful for formal calculations of the mentioned measurements.

<table>
<thead>
<tr>
<th>True positive (=Acknowledged ADR)</th>
<th>True negative (= Not acknowledged ADR)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test + ( = SDR)</strong></td>
<td>x</td>
</tr>
<tr>
<td><strong>Test – ( = non-SDR)</strong></td>
<td>z</td>
</tr>
</tbody>
</table>

Instead we developed a proxy measurement of the positive predictive properties of the methods’, calculated as a ratio between the number of false positive SDRs, “C”, and the unclassifiable and relevant SDRs, “D”, for models 1-8, i.e. “C”/”D”. With presumed ideal noise reduction by a decreased numerator “C” and preserved or increased denominator, “D” this ratio should approach to zero.
During follow-up 10% of men (n= 208) in the full cohort of 2045 and 12.5% (n=126) in the genotyped subcohort (*hOGG1*), were diagnosed with prostate cancer. Notable clinical characteristics in the full cohort in table 1, Paper I show a predominance of advanced disease with: at the mean age at diagnosis of 73 years, more than 80 % of the men having symptomatic disease. PSA measurement at diagnosis was available for >60% and only few men had a PSA value below the threshold for suspecting prostate cancer of 4ng/ml. One in four men had a non-palpable primary tumour (T0-T1c) at diagnosis while similar numbers had a tumours spread beyond the prostate gland (T3 -T4). 48 % had no confirmed metastases or an unknown metastasis status at diagnosis with PSA below or equal to 20ng/ml. Gleason scores - including trans-lated WHO grades- was 6 or below in 41% of the men.

Table 1, Paper I (continued on the next page).
### T-stage‡, n (%)

<table>
<thead>
<tr>
<th>T-stage</th>
<th>n</th>
<th>(%)</th>
</tr>
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<tr>
<td>T0/T1ab</td>
<td>28</td>
<td>(13.5)</td>
</tr>
<tr>
<td>T1c</td>
<td>25</td>
<td>(12.0)</td>
</tr>
<tr>
<td>T2</td>
<td>89</td>
<td>(42.8)</td>
</tr>
<tr>
<td>T3-4</td>
<td>47</td>
<td>(22.6)</td>
</tr>
<tr>
<td>TX/ Information missing</td>
<td>19</td>
<td>(9.1)</td>
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</table>

### N-stage‡, n (%)

<table>
<thead>
<tr>
<th>N-stage</th>
<th>n</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N0</td>
<td>32</td>
<td>(15.4)</td>
</tr>
<tr>
<td>N1</td>
<td>12</td>
<td>(5.8)</td>
</tr>
<tr>
<td>NX</td>
<td>142</td>
<td>(68.3)</td>
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<tr>
<td>Information missing</td>
<td>22</td>
<td>(10.6)</td>
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</table>

### M-stage‡ in combination with PSA, n (%)

<table>
<thead>
<tr>
<th>M-stage</th>
<th>n</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>88</td>
<td>(42.3)</td>
</tr>
<tr>
<td>MX, PSA ≤20</td>
<td>11</td>
<td>(5.3)</td>
</tr>
<tr>
<td>MX, 20&lt;PSA≤100</td>
<td>26</td>
<td>(12.5)</td>
</tr>
<tr>
<td>M1/PSA&gt;100</td>
<td>48</td>
<td>(23.1)</td>
</tr>
<tr>
<td>Information missing</td>
<td>35</td>
<td>(16.8)</td>
</tr>
</tbody>
</table>

### Gleason score*, n (%)

<table>
<thead>
<tr>
<th>Gleason score</th>
<th>n</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-6</td>
<td>50</td>
<td>(41.3)</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>(16.5)</td>
</tr>
<tr>
<td>8-10</td>
<td>31</td>
<td>(25.6)</td>
</tr>
<tr>
<td>Information missing</td>
<td>20</td>
<td>(16.5)</td>
</tr>
</tbody>
</table>

* WHO grade translated into Gleason score G1=2-6, G2=7, G3=8-10 in 42 cases  
** Lower urinary tract symptoms.  
*** Prostate specific antigen  
‡Incidentally discovered under investigation for symptoms not related to the urinary tract  
‡As classified by the International Union against Cancer (UICC)

### Baseline measurements and Prostate Cancer risk

The mean serum selenium level in the genotyped men was 77.4µg/l (range 24-220µg/l) which is low but still not an unusual level for European populations. None of the point estimates for the relative risk for tertiles of investigated factors differed statistically significantly from their references (table 2 Paper I). For the upper two tertiles of serum selenium the estimates were numerically below the reference with the genotyped men in the upper tertile of serum selenium presenting with the lowest risk estimate of developing prostate cancer (RR 0.72; 95% CI, 0.46-1.12). The estimates for the upper two tertiles of ESR and serum total cholesterol were numerically below the
reference with the exception of the highest tertile of ESR for the genotyped men. Trend tests for the estimates were all non-significant. Adjustments by baseline tertiles of ESR and total cholesterol level did not influence the prostate cancer risk estimates for the tertiles of serum selenium (data not shown). The men in the middle tertile of BMI (23.5-26.0) at baseline appeared to have the highest risk of developing prostate cancer (although not to a statistically significant level) and the smokers appeared to have lower risk of developing prostate cancer. These findings were curious and in the case of smoking also clearly biologically implausible. A false protectivity situation caused by competing risks was suspected in the case of smoking which prompted further analysis, as was the suspected pseudo-carcinogenicity in the middle-tertile BMI.

Table 2, Paper I: The unadjusted relative risk (RR) in the full cohort of prostate cancer for baseline tertiles of serum selenium with 95% confidence intervals (CI), the two selenium level modifying factors ESR, total cholesterol, BMI and for smoking status; the lowest tertiles and non-smokers used as reference. Results in the genotyped subcohort available in the published article

<table>
<thead>
<tr>
<th></th>
<th>NonPrCa*</th>
<th>PrCa</th>
<th>PrCa-risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum selenium (µg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤70</td>
<td>675</td>
<td>84</td>
<td>Ref -</td>
</tr>
<tr>
<td>70.1-81.0</td>
<td>588</td>
<td>65</td>
<td>0.89 (0.65 - 1.24)</td>
</tr>
<tr>
<td>81+</td>
<td>574</td>
<td>59</td>
<td>0.83 (0.60 - 1.16)</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate, ESR (mm/h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤4.0</td>
<td>704</td>
<td>84</td>
<td>Ref -</td>
</tr>
<tr>
<td>4.1-8.0</td>
<td>587</td>
<td>67</td>
<td>0.97 (0.70 - 1.33)</td>
</tr>
<tr>
<td>8+</td>
<td>545</td>
<td>57</td>
<td>0.88 (0.63 - 1.23)</td>
</tr>
<tr>
<td>Total serum cholesterol (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤6.24</td>
<td>560</td>
<td>76</td>
<td>Ref -</td>
</tr>
<tr>
<td>6.25-7.27</td>
<td>624</td>
<td>64</td>
<td>0.77 (0.55 - 1.07)</td>
</tr>
<tr>
<td>7.27+</td>
<td>653</td>
<td>68</td>
<td>0.78 (0.56 - 1.08)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤23.4</td>
<td>605</td>
<td>61</td>
<td>Ref -</td>
</tr>
<tr>
<td>23.5-26.0</td>
<td>567</td>
<td>78</td>
<td>1.34 (0.96 - 1.87)</td>
</tr>
<tr>
<td>26+</td>
<td>665</td>
<td>69</td>
<td>1.03 (0.73 - 1.46)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-smoker</td>
<td>449</td>
<td>69</td>
<td>Ref -</td>
</tr>
<tr>
<td>smoker</td>
<td>961</td>
<td>86</td>
<td>0.6 (0.44 - 0.83)</td>
</tr>
<tr>
<td>ex-smoker</td>
<td>427</td>
<td>53</td>
<td>0.82 (0.58 - 1.18)</td>
</tr>
</tbody>
</table>

* prostate cancer, ** relative risk, *** confidence interval

45
Smoking and Selenium: early death competing with Prostate Cancer

We suspected that the point estimates for smoking and middle BMI was influenced by an increased risk of dying early among smokers and among men with extreme BMI values. We therefore estimated for smokers the cumulative incidence of non-prostate cancer death and prostate cancer occurrence respectively by smoking status stratified by serum selenium levels in the genotyped subcohort. BMI was further analysed in Paper II.

The cumulative incidence of death without prostate cancer was, as expected, higher in smokers than in non-smokers/ex-smokers combined, this independently of tertiles of serum selenium (figure 2a, Paper I: low and middle selenium; figure 2b Paper I: high selenium).

Due to their high incidence of early non-prostate cancer death, smokers would be expected to have a reduced cumulative incidence of later prostate cancer, compared to non- and ex-smokers combined. This pattern is confirmed for men in the upper tertile of serum selenium (figure 2d). In contrast to this however, for men in the combined middle and lower serum selenium tertiles (figure 2c, Paper I), the cumulative incidence of prostate cancer in smokers was equal to the cumulative incidence in non- and ex-smokers combined. This would indicate that smoking increases prostate cancer risk in men with low serum selenium levels. The findings for the cumulative incidence are reflected in that smokers with serum selenium in the two lower tertiles (≤80 µg/l) experienced a RR of 2.39 (95% CI, 1.09-5.25) of developing prostate cancer compared to smokers in the high serum selenium tertile. The results were similar when a suggested critical serum selenium threshold of 100µg/l was used for calculation instead of tertile boundaries.
Influence of genotype?

We explored if SNPs in *OGG1* or *MnSOD* genes modified the association between serum selenium levels, smoking status and prostate cancer risk.

None of the investigated SNPs in *OGG1* per se influenced the risk for prostate cancer significantly. Taking serum selenium levels into account, presence of the A- allele of the SNP rs125701 in the *OGG1* gene, was observed to protect from prostate cancer (figure 3, Paper I) in the high tertile of serum selenium compared to lower tertiles (p=0.029). This was almost solely an effect in smokers with a HR of 5.8 (95% CI, 2.13-16.1) with the caveat of this being a small subgroup of the cohort (n=120). The three randomly selected comparison SNPs of the *hOGG1* showed no similar pattern.

Figures 3 and 4, next page: Hazard ratios (HR) for Prostate Cancer by *OGG1* and *MnSOD* genotype respectively with 95% confidence intervals, for prostate cancer diagnosis in tertile 3 vs. 1 (black) and tertile 2 vs. 1(grey) of serum selenium at baseline. Tertile 1: ≤70 µg/l, tertile 2: 70.1-81 µg/l and tertile 3: >81 µg/l.

Figure 3, Paper I (on next page): Results for rs125701 on bottom left.
The investigated MnSOD SNP, rs2758331 (proxy for rs4880) similarly influenced the risk for prostate cancer to a near significant level when taking serum selenium levels into account (figure 4, Paper I). Smoking did not affect the MnSOD-selenium level association. The five randomly selected comparison SNPs of the MnSOD showed no similar pattern. Figure 4, Paper I, results for rs2758331 on top left.
Results Paper II

The overall basic clinical characteristics in Paper II are evidently very similar to Paper 1 since the study populations to a large extent are coinciding. The majority of men had a clinically relevant or advanced stage of disease at time of diagnosis. None of the men presented with a combination of a PSA-value of $<10$, a Gleason sum $<7$ and a T-stage $\leq T2$ which would be typical of screening–detected cancers. There was no clear association between presence of the MetS at age 50 and TNM status, mode of detection or Gleason score; detailed characteristics for subgroups are available in the published article.

2183 and 2287 of participants could be classified according to NCEP-MetS and the IDF-MetS criteria respectively. 237 prostate cancers were identified, 226 of which classified according to NCEP-MetS criteria and 234 according to IDF-MetS criteria.

Baseline measurements and relative risk of Prostate Cancer

Presence of the MetS or any MetS component did not significantly influence the relative risk of prostate cancer, (when analyzed without taking competing risks into consideration) (table 1, Paper 2). Smokers did not have a statistically significantly higher relative risk of developing prostate cancer compared to non-smokers and ex-smokers combined (see table 2, Paper 1 for comparison) but again the point estimate was low. The presence of MetS, any of its components or smoking all respectively conferred a statistically significant higher risk of death without prostate cancer compared to non-presence of these factors. The most common cause of death in ULSAM is CVD (Zethelius 2008). Based on these findings, including the results for smoking status and our findings in Paper I, a cumulative analysis and a competing risk analysis was considered relevant to perform to illustrate the data.

Table 1: Number of men without and with prostate cancer (PC), relative risks (RR) for prostate cancer and RR for death without prostate cancer in men with or without the metabolic syndrome according to the NCEP and IDF definitions and their respective components and smoking at age 50 over 34 years of follow up (next two pages).
<table>
<thead>
<tr>
<th>Plausible Risk factors, n (%)</th>
<th>Not PC</th>
<th>PC</th>
<th>PC RR (95% CI)</th>
<th>Death w/o PC, RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em><em>MetS (NCEP</em>)</em>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1682</td>
<td>195</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td>Yes</td>
<td>275</td>
<td>31</td>
<td>1.3 (0.89, 1.9)</td>
<td><strong>1.86</strong> (1.59, 2.19)</td>
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<td>11</td>
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<td><strong>MetS (IDF†)</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>No</td>
<td>1755</td>
<td>202</td>
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<td>ref</td>
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<tr>
<td>Yes</td>
<td>298</td>
<td>32</td>
<td>1.18 (0.81, 1.71)</td>
<td><strong>1.7</strong> (1.46, 2)</td>
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<td>3</td>
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<td><strong>Abdominal obesity (NCEP)</strong></td>
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<td>No</td>
<td>1373</td>
<td>153</td>
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<tr>
<td>Yes</td>
<td>324</td>
<td>30</td>
<td>0.97 (0.66, 1.44)</td>
<td><strong>1.42</strong> (1.2, 1.67)</td>
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<td>54</td>
<td>(22.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Abdominal obesity (IDF)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>No</td>
<td>1648</td>
<td>190</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td>Yes</td>
<td>436</td>
<td>47</td>
<td>1.11 (0.8, 1.52)</td>
<td><strong>1.5</strong> (1.3, 1.73)</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>(0.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Elevated fasting pl-glucose level (NCEP)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>No</td>
<td>1966</td>
<td>223</td>
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<tr>
<td>Yes</td>
<td>112</td>
<td>12</td>
<td>1.18 (0.66, 2.11)</td>
<td><strong>1.57</strong> (1.23, 2)</td>
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<tr>
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<td>6</td>
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<tr>
<td><strong>Elevated fasting pl-glucose level (IDF)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>No and not DM</td>
<td>1812</td>
<td>206</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td>Yes or DM</td>
<td>266</td>
<td>29</td>
<td>1.04 (0.71, 1.54)</td>
<td><strong>1.23</strong> (1.04, 1.47)</td>
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<tr>
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<td>6</td>
<td>2</td>
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</tr>
<tr>
<td><strong>Hypertension (NCEP, IDF)</strong></td>
<td></td>
<td></td>
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<tr>
<td>No</td>
<td>724</td>
<td>88</td>
<td>ref</td>
<td>ref</td>
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<tr>
<td>Yes</td>
<td>1359</td>
<td>149</td>
<td>1.03 (0.79, 1.35)</td>
<td><strong>1.41</strong> (1.2, 1.61)</td>
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<tr>
<td>Missing data</td>
<td>1</td>
<td>0</td>
<td>(0.0)</td>
<td></td>
</tr>
<tr>
<td>**High triglycerides (NCEP,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Analysing cumulatively

Men with MetS-NCEP at baseline (figure 1A, Paper II, following page) and, slightly less so, men with MetS-IDF (figure 1B, Paper II) appear to have a modestly increased risk of developing prostate cancer than men without MetS. By age 80 the difference is however not statistically significant: 4.4 percent units (CI -1.7%-10.5%; NCEP), 1.7 percent units (CI -3.5%-6.9%; IDF). Among components of the MetS, abdominal obesity (NCEP) at age 50 (figure 1C) appeared to confer a higher probability of developing clinically relevant prostate cancer (n.s. at age 80), whereas plasma glucose levels (IDF; figure 1D) did not appear to have an influence.

The cumulative incidence proportion of death censored for a diagnosis of prostate cancer, was at age 80, 19.2 percent units (CI 13.3%-25.3%) higher in the men with the MetS-NCEP (figure 2A, Paper II, next to following page) and 15.8 percent units (CI 10.5%-21.7%) higher in men with MetS-IDF (figure 2B). In men with abdominal obesity (NCEP; figure 2C) and in men with high fasting plasma glucose (IDF) (figure 2D) cumulative incidence proportion of death was also significantly higher than without these factors present. Presence of any other component of any of MetS definitions i.e. high blood pressure, high triglycerides, and low HDL cholesterol or being a smoker was likewise associated with a significantly higher cumulative incidence proportion of death than non-presence of the factors (not shown).

Figures 1A-D, and 2A-D, Paper II, following two pages:
Presence of the MetS at age 50 in our cohort is thus established as associated with a higher RR of early death without prostate cancer. Death was suspected of acting as a risk competing with a later risk of prostate cancer raising the suspicion of a false protectivity illusion. To overcome this we therefore assessed the risk of prostate cancer in men with the MetS independently of the early risk of death, this by estimating risk of prostate cancer conditioned on survival at a given time (figures 3A-D, Paper II).

Figure 3A-D, Paper II, next page:
By in this way taking the competing risk into account, the conditional probability of becoming diagnosed with prostate cancer by age 80 is statistically significantly higher in the men with baseline MetS-NCEP; with a 7.3 percent unit (95% CI 0.2%-14.5%) higher absolute risk. This translates into an odds ratio (OR) of 1.64 (95% CI 1.03-2.23) (figure 3A, Paper II). The same tendency is seen in figure 3B for men with the baseline MetS-IDF who, at age 80, had a 5.0 percent units (95% CI -1.6%-11.6%) higher conditional probability of becoming diagnosed with prostate cancer than men without the MetS, OR 1.43 (95% CI 0.89-1.90). Abdominal obesity (NCEP; figure 3C) is likewise associated with a non-significantly higher conditional probability of 8.1 percent units (95% CI -1.3-17.5), OR 1.71 (95% CI 0.95-2.78), while high fasting plasma glucose (IDF) did not influence the conditional probability of being diagnosed with prostate cancer in men up to 80 years of age (figure 3D), the difference being 1.5 percent units (95% CI -4.6%-7.6%), OR of 1.12 (95% CI 0.69-1.68). High triglycerides (NCEP, IDF) increased the risk of prostate cancer non-significantly by 3.0 percent units (95% CI -0.8%-6.8%), OR 1.26 (95% CI 0.94-1.55) (not shown in figure) while high blood pressure (NCEP, IDF) and low HDL cholesterol (NCEP, IDF) were only marginally associated with a higher risk of prostate cancer (not shown in figures). Smoking non-significantly increased the conditional probability of later prostate cancer.
Results Paper III

Data on planned primary treatment by age, year of diagnosis and disease stage was available for 58143 men in PCBaSe diagnosed with Prostate Cancer 1997-2006 and alive at 1/11 2005 (see also table 1, Paper III). Notably:

- >60% of all newly diagnosed men were not planned to receive curative treatment.
- >30% of all newly diagnosed men were planned to receive hormonal treatment.
- 2560 (4.4%) were planned to receive monotherapy anti-androgen as first line treatment.
- The highest extent of such treatment (11.3%) was noted in the regionally metastatic risk group.
- Among men with localised, low/intermediate risk disease, 2.1% of men were prescribed first-line anti-androgen monotherapy.

Table 1, Paper III, continued on next page:

<table>
<thead>
<tr>
<th>Study patients, n, %</th>
<th>Localised, low/intermediate risk</th>
<th>Localised, high risk</th>
<th>Regionally metastatic disease</th>
<th>Metastatic disease</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow up Year, mean, sd*</td>
<td>3.6 2.5</td>
<td>3.9 2.6</td>
<td>3.9 2.6</td>
<td>3.3 2.5</td>
<td>3.7 2.6</td>
</tr>
<tr>
<td>Age, n, %</td>
<td>&lt;65</td>
<td>12191 39.6</td>
<td>2916 18.5</td>
<td>888 20.8</td>
<td>995 17.0</td>
</tr>
<tr>
<td></td>
<td>65-74</td>
<td>12698 41.3</td>
<td>5994 38.0</td>
<td>1568 36.8</td>
<td>1907 32.5</td>
</tr>
<tr>
<td></td>
<td>75+</td>
<td>5859 19.1</td>
<td>6883 43.6</td>
<td>1807 42.4</td>
<td>2965 50.5</td>
</tr>
<tr>
<td>Year of diagnosis, n, %</td>
<td>1997 – 2002</td>
<td>12058 39.2</td>
<td>6955 44.0</td>
<td>1859 43.6</td>
<td>2063 35.2</td>
</tr>
<tr>
<td></td>
<td>2003 – 30/6</td>
<td>2005</td>
<td>11595 37.7</td>
<td>5535 35.0</td>
<td>1501 35.2</td>
</tr>
<tr>
<td></td>
<td>1/7 2005 – 2006</td>
<td>7095 23.1</td>
<td>3303 20.9</td>
<td>903 21.2</td>
<td>1618 27.6</td>
</tr>
</tbody>
</table>
Prescribing pattern, dosage influencing factors:

Among the 1 406 men prescribed first line monotherapy bicalutamide treatment and recorded in the SPDR (figure 1, Paper III), 1 109 (79%) were prescribed the approved daily dose of 150 mg during the full treatment period (table 2, Paper III). No patient was prescribed a daily dose higher than 150 mg (range 45-150 mg). A lower, off-label, dose was prescribed to 297 men (21%) with one third of them being prescribed 50 mg daily.

In univariate analysis of the whole treated group, men older than 75 years, with low risk disease and who were married or widowers were significantly more likely to receive an off-label dose. Year of diagnosis and time of follow-up were also associated with prescription pattern, possibly due to that men diagnosed during the first years of the existence of the register constitute a selected group of long-term survivors. Neither the patients’ socio-economic status, nor their co-morbidity, nor the medical specialty of the treatment-initiating prescribing physician influenced the dose of bicalutamide in univariate analyses.

In multivariate analysis only age and risk class remained as factors significantly influencing the choice of dose in the whole group. The effect of marital status in the univariate analysis disappeared in the multivariate analysis. However, age group (<65, 65-74 and ≥75 years and above) was closely associated with marital status (chi-squared 91.26; p<0.001), and the statistical power for individual variables was weaker in the multivariate models.

Table 2, Paper III, next page:
<table>
<thead>
<tr>
<th>Time of follow up, mean (sd)</th>
<th>As per approved Posology (n=1109)</th>
<th>Off-label &lt;150 mg (n=297)</th>
<th>P-value Univariable model</th>
<th>P-value Multivariable model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>140 (12.6)</td>
<td>23 (7.7)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>65-74</td>
<td>573 (51.7)</td>
<td>124 (41.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75+</td>
<td>396 (35.7)</td>
<td>150 (50.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year of diagnosis, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1997 - 2002</td>
<td>268 (24.2)</td>
<td>90 (30.3)</td>
<td>0.02</td>
<td>0.74</td>
</tr>
<tr>
<td>2003 – 30/6 2005</td>
<td>431 (38.9)</td>
<td>120 (40.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/7 2005 - 2006</td>
<td>410 (37.0)</td>
<td>87 (29.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk class at diagnosis, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localised, low risk</td>
<td>87 (7.8)</td>
<td>34 (11.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localised, intermediate risk</td>
<td>211 (19.0)</td>
<td>89 (30.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localised, high risk</td>
<td>491 (44.3)</td>
<td>115 (38.7)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Regionally metastatic disease</td>
<td>199 (17.9)</td>
<td>36 (12.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastatic disease</td>
<td>115 (10.4)</td>
<td>15 (5.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missing data</td>
<td>6 (0.5)</td>
<td>8 (2.7)</td>
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</tr>
<tr>
<td>Marital status, n (%)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>770 (69.4)</td>
<td>221 (74.4)</td>
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<td></td>
</tr>
<tr>
<td>Unmarried</td>
<td>78 (7.0)</td>
<td>13 (4.4)</td>
<td>0.03</td>
<td>0.23</td>
</tr>
<tr>
<td>Divorced</td>
<td>153 (13.8)</td>
<td>27 (9.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Widower</td>
<td>108 (9.7)</td>
<td>36 (12.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SES*, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>571 (51.5)</td>
<td>159 (53.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>524 (47.2)</td>
<td>132 (44.4)</td>
<td>0.47</td>
<td>0.7</td>
</tr>
<tr>
<td>Not gainfully employed/Missing</td>
<td>14 (1.3)</td>
<td>6 (2.0)</td>
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<td></td>
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<tr>
<td>Charlson co-morbidity index, n (%)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>803 (72.4)</td>
<td>208 (70.0)</td>
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</tr>
<tr>
<td>1</td>
<td>183 (16.5)</td>
<td>51 (17.2)</td>
<td>0.75</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>87 (7.8)</td>
<td>25 (8.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3+</td>
<td>36 (3.2)</td>
<td>13 (4.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prescriber#, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical unit</td>
<td>224 (54.6)</td>
<td>43 (49.4)</td>
<td>0.33</td>
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<tr>
<td>Urology unit</td>
<td>150 (36.6)</td>
<td>32 (36.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* SES - Socioeconomic Status  # for men diagnosed after 1/7 2005

Age was the only factor with a significant influence on dosage in multivariate analysis both in an off-label treatment indication subgroup of men with
The more severe the disease, the lower the treatment persistence

Median treatment persistence of anti-androgen monotherapy varied from almost six years among the men with localised low/intermediate risk group (figure 3, Paper III, medians marked by vertical lines) down to two years in men with metastatic disease. After 10 years the proportion of men remaining on anti-androgen monotherapy was less than 20% in the localised low risk/intermediate group and the localised high risk groups while in the two higher risk groups no men remained on therapy after ten years.

Reasons for treatment discontinuation vary with disease severity

In men with localised low/intermediate risk, the first occurring reason for monotherapy anti-androgen discontinuation was equally divided between death, a switch to GnRH (+/- anti-androgen) treatment or remained unexplained, whereas in men with more severe disease the most common reason for discontinuation was switching to treatment including a GnRH analogue (Fig 3, Paper III).

Figure 3, Paper III, next page:
Adherence to bicalutamide treatment

-Sixty percent of the men adhered well or very well to monotherapy bicalutamide treatment (>80%).
-Median adherence was 84 %.
-Men >75 years old had a lower treatment adherence than <65 year-olds.
-Men in the localised high risk and metastatic group adhered to their prescribed bicalutamide to a significantly higher extent than men in the localised, low risk group (table 5, Paper III).
-Neither marital status, nor socioeconomic status, nor co-morbidity according to the Charlson co-morbidity index nor the medical speciality of physician initiating the treatment had a significant impact on the adherence.

Restricting the analysis to men diagnosed after the start of the SPDR, July 1, 2005, i.e. analysing adherence only in the initial phase of anti-androgen treatment, yielded a similar overall pattern, but only age above 75 years remained as a factor significantly influencing adherence. Factors influencing initial versus later treatment adherence may thus differ (table 5, Paper III)
Table 5, Paper III: Adherence and adherence-influencing factors in men with Prostate Cancer prescribed Bicalutamide

<table>
<thead>
<tr>
<th></th>
<th>Very good adherence ≥ 90% (n=430)</th>
<th>Good adherence ≥80%&lt;90% (n=419)</th>
<th>Poor adherence ≥50%&lt;80% (n=425)</th>
<th>Very poor adherence &lt;50% (n=132)</th>
<th>All (n=1406)</th>
<th>P-value univariable/multivariable</th>
<th>P-value for restricted analysis univariable/multivariable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of follow up, mean (SD)</td>
<td>2.4 (1.9)</td>
<td>2.6 (2.0)</td>
<td>3 (2.1)</td>
<td>3.3 (2.3)</td>
<td>2.7 (2.1)</td>
<td>&lt;0.001 / 0.004</td>
<td>1.7 / 0.2</td>
</tr>
<tr>
<td>Age, n (%)</td>
<td>56 (13.0)</td>
<td>58 (13.8)</td>
<td>37 (8.7)</td>
<td>12 (9.1)</td>
<td>163 (11.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>232 (54.0)</td>
<td>201 (48.0)</td>
<td>197 (46.4)</td>
<td>67 (50.8)</td>
<td>697 (49.6)</td>
<td>0.009 / 0.005</td>
<td>0.002 / 0.007</td>
</tr>
<tr>
<td>65-74</td>
<td>142 (33.0)</td>
<td>160 (38.2)</td>
<td>191 (44.9)</td>
<td>53 (40.2)</td>
<td>546 (38.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year of diagnosis, n (%)</td>
<td>1997 - 2002</td>
<td>84 (19.5)</td>
<td>90 (22.9)</td>
<td>31 (30.8)</td>
<td>47 (35.6)</td>
<td>358 (25.5)</td>
<td></td>
</tr>
<tr>
<td>2003 – 30/6 2005</td>
<td>172 (40.0)</td>
<td>167 (39.9)</td>
<td>190 (46.4)</td>
<td>60 (45.6)</td>
<td>300 (21.3)</td>
<td>&lt;0.001 / 0.85</td>
<td>NA</td>
</tr>
<tr>
<td>1/7 2005 - 2006</td>
<td>174 (40.5)</td>
<td>156 (37.2)</td>
<td>198 (44.9)</td>
<td>65 (40.2)</td>
<td>497 (35.3)</td>
<td>&lt;0.001 / 0.002</td>
<td>0.06 / 0.33</td>
</tr>
<tr>
<td>Risk class at diagnosis, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localised, low risk</td>
<td>31 (7.2)</td>
<td>31 (7.4)</td>
<td>40 (9.4)</td>
<td>19 (14.4)</td>
<td>121 (8.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localised, intermediate risk</td>
<td>84 (19.5)</td>
<td>77 (18.4)</td>
<td>103 (24.2)</td>
<td>36 (27.3)</td>
<td>300 (21.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localised, high risk</td>
<td>182 (42.3)</td>
<td>185 (44.2)</td>
<td>179 (42.1)</td>
<td>60 (45.5)</td>
<td>606 (43.1)</td>
<td>&lt;0.001 / 0.002</td>
<td>0.06 / 0.33</td>
</tr>
<tr>
<td>Regionally metastatic disease</td>
<td>82 (19.1)</td>
<td>80 (19.1)</td>
<td>62 (14.6)</td>
<td>11 (8.3)</td>
<td>235 (16.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastatic disease</td>
<td>47 (10.9)</td>
<td>43 (10.3)</td>
<td>36 (8.5)</td>
<td>4 (3.0)</td>
<td>130 (9.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missing data</td>
<td>4 (0.9)</td>
<td>3 (0.7)</td>
<td>5 (1.2)</td>
<td>2 (1.5)</td>
<td>14 (1.0)</td>
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<tr>
<td>Marital status, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>310 (72.1)</td>
<td>292 (69.7)</td>
<td>308 (72.5)</td>
<td>81 (61.4)</td>
<td>991 (70.5)</td>
<td>0.4 / 0.21</td>
<td>0.86 / 0.36</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>(%)</td>
<td>n</td>
<td>(%)</td>
<td>n</td>
<td>(%)</td>
<td>n</td>
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<td>----------------------</td>
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<td>-----</td>
<td>------</td>
<td>-----</td>
<td>------</td>
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<tr>
<td>Unmarried</td>
<td>29</td>
<td>(6.7)</td>
<td>24</td>
<td>(5.7)</td>
<td>29</td>
<td>(6.8)</td>
<td>9</td>
</tr>
<tr>
<td>Divorced</td>
<td>53</td>
<td>(12.3)</td>
<td>58</td>
<td>(13.8)</td>
<td>45</td>
<td>(10.6)</td>
<td>24</td>
</tr>
<tr>
<td>Widower</td>
<td>38</td>
<td>(8.8)</td>
<td>45</td>
<td>(10.7)</td>
<td>43</td>
<td>(10.1)</td>
<td>18</td>
</tr>
<tr>
<td>SES*, n (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>High</td>
<td>237</td>
<td>(55.1)</td>
<td>213</td>
<td>(50.8)</td>
<td>216</td>
<td>(50.8)</td>
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<tr>
<td>Low</td>
<td>188</td>
<td>(43.7)</td>
<td>200</td>
<td>(47.7)</td>
<td>203</td>
<td>(47.8)</td>
<td>65</td>
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<td>Not gainfully employed/Missing</td>
<td>5</td>
<td>(1.2)</td>
<td>6</td>
<td>(1.4)</td>
<td>6</td>
<td>(1.4)</td>
<td>3</td>
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<tr>
<td>Charlson co-morbidity index, n (%)</td>
<td></td>
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<tr>
<td>0</td>
<td>304</td>
<td>(70.7)</td>
<td>313</td>
<td>(74.7)</td>
<td>302</td>
<td>(71.1)</td>
<td>92</td>
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<tr>
<td>1</td>
<td>71</td>
<td>(16.5)</td>
<td>64</td>
<td>(15.3)</td>
<td>72</td>
<td>(16.9)</td>
<td>27</td>
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<tr>
<td>2</td>
<td>41</td>
<td>(9.5)</td>
<td>26</td>
<td>(6.2)</td>
<td>36</td>
<td>(8.5)</td>
<td>9</td>
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<tr>
<td>3+</td>
<td>14</td>
<td>(3.3)</td>
<td>16</td>
<td>(3.8)</td>
<td>15</td>
<td>(3.5)</td>
<td>4</td>
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<td>First prescriber#, n (%)</td>
<td></td>
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<tr>
<td>Surgical unit</td>
<td>96</td>
<td>(55.2)</td>
<td>80</td>
<td>(51.3)</td>
<td>75</td>
<td>(56.8)</td>
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<td>Urology unit</td>
<td>62</td>
<td>(35.6)</td>
<td>69</td>
<td>(44.2)</td>
<td>37</td>
<td>(28.0)</td>
<td>14</td>
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<tr>
<td>Other/missing</td>
<td>16</td>
<td>(9.2)</td>
<td>7</td>
<td>(4.5)</td>
<td>20</td>
<td>(15.2)</td>
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</table>

*SES-Socioeconomic status

#For men diagnosed after 1/7 2005

$Restricted to men diagnosed after 1/7 2005
Results Paper IV

The total number of reports of ADRs in EV database for the four investigated drugs ranged from 2400 for abiraterone to close to 50 000 for metformin (table 2, Paper IV). To compare: the total number of ADR reports for all drugs in the EV database was roughly 3.5 millions. The average number of reports per ADR term was for metformin 18 and for bicalutamide, abiraterone and vildagliptin around 5 mirroring the increased patient year exposure and market time of metformin compared to the other investigated drugs.

Conventional PRR calculations using the SDR3 and SDR5 thresholds

The relative frequencies of delivered SDRs using 3 as a case count threshold (SDR3) among all reported ADR terms ranged from 10 % (i.e. 95/950) for bicalutamide to 17.9% for vildagliptin (table 2, Paper IV). The rest of the reported ADR terms (82-90%), were thereby eliminated from clinical evaluation. Increasing the SDR-defining case count from ≥3 (SDR3) to ≥5 (SDR5) reduced the number of SDRs for further validation and verification by between 14% in the abiraterone (men only) analysis and 36% for vildagliptin. This also removed between 33-70% of the unclassified SDRs relevant for manual evaluation, potentially delaying detection and validation of important safety signals.

Table 2, Paper IV: Number of adverse drug reaction reports, reported ADR terms and signals of disproportionate reporting detected using the conventional PRR method, i.e. with the EV database as background for the four investigated drugs; number of SDRs not delivered when increasing the threshold of the SDR-defining case count number from 3 to 5; number and fraction of the above of non-disease related SDRs relevant for manual validation. Continued on next page.

<table>
<thead>
<tr>
<th>Gender</th>
<th>bicalutamide</th>
<th>abiraterone</th>
<th>metformin</th>
<th>vildagliptin</th>
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<tr>
<td>Unspecified</td>
<td>5 161</td>
<td>5 055</td>
<td>2 405</td>
<td>49 719</td>
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<tr>
<td>Men only</td>
<td>2 335</td>
<td>2 335</td>
<td>49 719</td>
<td>5 551</td>
</tr>
<tr>
<td>ADR reports in EV; n</td>
<td>950</td>
<td>939</td>
<td>492</td>
<td>2 667</td>
</tr>
<tr>
<td>ADR terms in EV; n</td>
<td>950</td>
<td>939</td>
<td>492</td>
<td>2 667</td>
</tr>
</tbody>
</table>

64
<table>
<thead>
<tr>
<th>SDR3s; n, (% of all ADR terms)</th>
<th>95(10.0)</th>
<th>79(8.4)</th>
<th>70(14.2)</th>
<th>63(12.9)</th>
<th>371(13.9)</th>
<th>190(17.9)</th>
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<tbody>
<tr>
<td>SDR5s; n</td>
<td>66</td>
<td>55</td>
<td>58</td>
<td>54</td>
<td>304</td>
<td>120</td>
</tr>
<tr>
<td>SDRs undetected when moving from SDR3 to SDR5; n</td>
<td>29</td>
<td>24</td>
<td>12</td>
<td>9</td>
<td>67</td>
<td>70</td>
</tr>
<tr>
<td>SDRs relevant for manual evaluation but undetected when moving from SDR3 to SDR5; n, (%)</td>
<td>13 (44.8)</td>
<td>13 (54.1)</td>
<td>5 (41.7)</td>
<td>3 (33.3)</td>
<td>44 (70.0)</td>
<td>46 (65.7)</td>
</tr>
</tbody>
</table>

PRR calculations by restricting the background of comparison, detection of acknowledged ADRs in SPCs = true positive SDRs

Figures 1 a-d, Paper IV presents the PRR-TA method’s ability to detect and deliver true positive SDRs-compared to the conventional PRR method using SDR3 or SDR5 thresholds. In the case of bicalutamide, abiraterone and vildagliptin this ability was increased or unchanged (figs 1a, b, d). In the case of metformin the PRR-TA failed to deliver as an SDR one of the twelve ADR terms delivered by the conventional PRR method (figure 1c).

Using the more strict SDR5 threshold (far right bar in figures 1a-d, models 1 and 5), led to a failure of the PRR to identify between 5-20 % of acknowledged ADRs as compared to using the SDR3 threshold. Compared to the PRR-TA (SDR3) method, the PRR applying the SDR5 threshold failed to identify between 8-31% of acknowledged ADRs.

Reducing the background further down to drug class resulted in marked loss of ability to detect true positive SDRs in the bicalutamide/anti-androgen (model 4) and vildagliptin/ DPP4I (model 8) analyses and an absence of ability to detect any true positive SDRs in the metformin/biguanides analysis (model 7) indicating that class-models 4, 7, 8 were not useful. For abiraterone no drug class analysis corresponding to model 4 was applicable.

Figures 1a-d, next page:
The proportion of detected acknowledged ADRs i.e. true positive SDRs for bicalutamide (a), abiraterone (b), metformin (c), vildagliptin (d) using from left to right for (a, b): the conventional PRR defining the SDR by a case count of \( \geq 3 \) (model 1, SDR3); PRR-TA, prostate gland disease drugs (model 2, SDR3); PRR-TA prostate cancer drugs (model 3, SDR3); PRR-class (model 4, SDR3, not for abiraterone); and the conventional PRR defining the SDR by a case count of \( \geq 5 \) (model 1, SDR5) and for (c, d) from left to right the conventional PRR defining the SDR by a case count of \( \geq 3 \) (SDR3); the PRR-TA(SDR3); PRR-class(SDR3); and the conventional PRR defining the SDR by a case count of \( \geq 5 \) (SDR5).

The analysis restricted to male gender for bicalutamide and abiraterone did not differ markedly compared to the analysis not restricted to male gender; however they appeared to perform less well (not shown).

The ability to detect true positive SDRs by the PRR methods using SDR 3 and SDR5 thresholds and the inter-method concordance using different backgrounds in models 1 through 8 were high for each drug investigated. A few true positive SDRs were de-masked using the PRR-TA method as compared to the conventional PRR. For all drugs the ability to detect true positive SDRs using the PRR-SDR5 was generally lower than for the PRR-SDR3 and the PRR-TAs.

Detection of SDRs not acknowledged as ADRs in the SPCs

The number of false positive SDRs confounded by disease or disease spill-over and thus less relevant for further evaluation decreased when moving from the conventional PRR analysis to the PRR-TA (figure 2a-d; grey bars, from left to right for each drug in figures); the number of SDRs for metformin (figure 2b) decreasing by 101, or 63%. The number of unclassified SDRs relevant for further manual validation, i.e. the group of SDRs where new not yet acknowledged ADRs signals may be present, increased (black bars, figures 2a-b)), when moving from the conventional PRR analysis to the PRR-TA for all drugs except for metformin. In the case of bicalutamide, the wider therapeutic area background (model 2) performed better than the narrower therapeutic area background (model 3) while for abiraterone the opposite was found (model 3 better than model 2).

Figures 2a-b, next page:
Figures 2 a-b, Paper IV represent the PRR-TAs and the PRR class methods’ ability to detect and deliver SDRs not acknowledged as ADRs in the SPCs for each drug, either false positive SDRs confounded by disease or disease spill-over (grey bars) or unclassified SDRs relevant for further manual validation (black bars); figure 2a: bicalutamide and abiraterone, figure 2b: metformin and vildagliptin analyses.

Reducing the background further down to drug class, delivered for metformin and bicalutamide (models 4, 7) few or no unclassified SDRs relevant for manual validation, while for vildagliptin (model 8) the numbers were the same as with the larger backgrounds (models 5, 6). Drug class level PRR calculations hence appeared less useful.

An analysis restricted to male gender for bicalutamide and abiraterone regarding ability to deliver SDRs not acknowledged as ADRs in the SPCs did not differ markedly compared to the analysis not restricted to male gender.

The ratio of false positive SDRs and unclassified SDR, as a measure of the signal to noise ratio, for each of the drugs is consistently improved when decreasing the comparator background from the conventional PRR (SDR3)
output to the PRR-TA (from left to right, figure 3, Paper IV). For bicalutamide and abiraterone this was again done in two steps (models 2 and 3) while for metformin and vildagliptin in one step. Analyses using the most restricted background of drug class (models 4, 7, 8) were not considered relevant to include based on their poor performance regarding the ability to detect true positive SDRs and remove false positive SDRs.

Figure 3, Paper IV The ratio of false positive SDRs confounded by indication or disease spill-over and unclassified SDRs relevant for further manual validation; the ratio should ideally be as close to zero as possible with as few confounded SDRs as possible (numerator) delivered by the method in relation to the relevant SDRs (denominator). From left to right for each drug analysis the ratios when analyzing by the conventional PRR, PRR-TA (model 2 and 6) and for bicalutamide and abiraterone also the PRR-TA (model 3)
Discussion and Conclusions, Papers I, II

Discussion and Conclusions on Papers I, II and III reflect and current knowledge on the topics of the Papers including new information since their publication.

Papers I and II are both based in the population based cohort ULSAM with a high participation rate. The ULSAM cohort is homogenous in age and ethnicity. Regarding prostate cancer, the results from the ULSAM cohort pertain mainly to the pre-screening era of symptomatically detected advanced prostate cancer. Only few screening detected prostate cancer cases are present in ULSAM and the absolute majority of the men diagnosed with the disease died or suffered significant morbidity from it during the remainder of their lives. For cancer studies, ULSAM is comparatively small and hence modest or weak associations may go undetected but its annotation is unprecedented in details and precision.

"Clinically relevant" prostate cancer
We chose in Papers I and II the term “clinically relevant” for the type of pre-PSA-screening era prostate cancer for lack of consensus wording. Screening detected prostate cancer undoubtedly also is “clinically relevant” as it may give rise to extensive monitoring and/or advanced treatment.

Screening detected vs. non-screening detected prostate cancer
The relative and absolute incidence of early screening detected subclinical cancer is growing, at the “expense” of more advanced disease. Studies on prostate cancer in general are becoming increasingly dominated by screening detected disease. Are the findings from Papers I and II still relevant for prostate cancer disease panorama of today and the future?

The results from Papers I and II pertain in this respect in essence to the same subgroup that others identify as aggressive or advanced prostate cancers; so yes, results from our studies are still relevant. In the future advanced prostate cancer may become a rare disease in parts of the world, but we are far from there yet. The associations identified between the exposures under study in the ULSAM may hence be compared to results in subgroups or strata of “advanced” disease in more recent study cohorts. The study population of Paper III represents in this regard an intermediate position with an
increased proportion of screening detected prostate cancer. The results in Papers I and II would presumably have been different had occult cases of prostate cancer been identified by invasive methods and/or screening. 12-22% of “prostate cancer free” men in studies have in reality harboured occult prostate cancer “contaminating” control groups (Iguchi 2008A). Occult cases in our study may have reduced the power of the analysis if results were in reality relevant for all prostate cancer, or the estimates of risk could have been different.

Main results and conclusions, Papers I and II:

- Taking competing risks into account, middle age selenium status and smoking habits give long term information regarding the risk of prostate cancer presenting in a setting with a low prevalence of screening and a low selenium status of the population.
- Smoking at age 50 may be a risk factor for prostate cancer in men with low serum selenium levels (≤80µg/l) during long term follow up. Possible preventive effects of selenium appear modified by level of oxidative stress induced by smoking.
- When analysing possible risk factors for diseases of an elderly population such as clinically relevant prostate cancer, competing risks of developing the outcome, other than the ones primarily of interest for the study, should always be considered and taken into account when appropriate.
- We identify a need for further development and validation of statistical methods to take competing risk into account in prospective studies of risk factors for diseases with an incidence and prevalence peak late in life.
- The association between serum selenium levels and prostate cancer risk was modified by genetic polymorphisms, in hOGG1 and MnSOD.
- Smoking- an oxidant stressor -in combination with unfavourable genetic profile as regards enzymes for DNA repair of oxidative damage hOGG1 and MnSOD increases the risk of prostate cancer in a population with suboptimal selenium levels.
- Both exogenous (e.g selenium) and endogenous antioxidants (e.g. MnSOD, OGG1) may play interdependent roles in preventing clinically significant prostate cancer. Patterns of exposure to selenium and smoking combined with data on genetic variation can be valuable to explore further.
- The presence of the metabolic syndrome (NCEP) at age 50 is a risk factor for clinically relevant prostate cancer.
- Components of the MetS predominately attributing to the risk increase appear to be abdominal obesity and high serum triglycerides.
The role of selenium

Our data indicate an overall modest inverse association of serum selenium level and prostate cancer risk, fully in line with what others have found also after the publication of our study. A recent meta-analysis indicates a 10% decrease in the risk of advanced/aggressive prostate cancer for every 10µg/l increase in plasma/serum selenium in the range of 60-170 µg/l (Hurst 2012) and a Cochrane analysis on selenium for cancer (i.e. all cancer) prevention notes reduced cancer incidence and mortality in men with high vs. low selenium exposure with a composite risk estimate of 0.74 (95% CI, 0.61-0.88) (Zwahlen 2012). In a Swedish population the range of selenium exposure is limited, with very few high serum selenium levels. Baseline serum selenium levels and co-variates were for Paper I measured at a median of 23 years before diagnosis, a disadvantage, which however is shared with other studies in the field (Willett 1983, Yoshizawa 1998, Brinkman 2006)- however in our study at a standardized age of all participants.

The role of smoking- competing risk

Our finding of an increased risk for prostate cancer in smokers with low serum selenium is surprisingly a new finding. The role of smoking as a risk factor for prostate cancer has been unclear (Furuya 1998, Khan 2010). As smoking increases oxidative damage, an association is biologically reasonable. The smoking exposure was in ULSAM recorded crudely at age 50; further stratification of smokers would not have been feasible given the limited size of the cohort.

The lack of association between smoking and prostate cancer in other studies have been due to masking by the strong competing risk of concomitant early death from other causes, predominately CVD, in smokers. If smoking is associated with both early death from other causes and risk of prostate cancer later in life, conventional time to event analyses may give a false impression that smoking protects from prostate cancer since methods traditionally employed assumes that censoring is non-informative which is not the case. The use of such less appropriate statistical methods may give rise to false results.

In the “EPIC” cohort of 145000 men whereof 4600 within 17 years were diagnosed with prostate cancers, long time or heavy smokers had an increased prostate cancer mortality while prostate cancer incidence was lower compared to non-smokers (Rohrmann 2012). Similar results have been reported by others (Huncharek 2010). These biologically implausible results of smoking protecting from prostate cancer may be the result of not addressing the concept of competing risk in these studies. The mentioned analyses would have impressed more had an analysis of the competing risk of death
analysis been performed. Smoking has been confirmed by others to increase the mortality in prostate cancer (Kenfield 2011).

We had already noted and analysed a similar— not biologically plausible—false protectivity pattern for obesity in relation to prostate cancer in the UL-SAM cohort (Grundmark 2010, Paper II). The issue of competing risk and false protectivity findings may be particularly evident in this study representing a pre-screening era where the median age at diagnosis of prostate cancer was high. With screening programs for prostate cancer introducing long lead times the pattern may be altered.

The question addressed and answered in studies which do vs. do not take competing risk into account are both relevant but different: Without taking competing risk into account the question addressed is “how many cases of prostate cancer will you diagnose in men classified at baseline as smokers vs. non smokers” whereas when including competing risk into the equation the question addressed comes closer to “does smoking contribute to causing prostate cancer”. Both questions are in their own respect relevant and give complimentary results but failing to observe the difference must now be considered inadequate. Another way of putting it is that the first question/answer is relevant for health care budget planning purposes whereas the second is more relevant for those interested in the aetiology and preventability of the disease. The interests of these two groups may not always be coinciding.

*Competing risk simply put:*

> If you smoke you don’t live long enough to die from prostate cancer.

* A foolish argument would be to recommend smoking to protect from prostate cancer.

**The role of genetic variation, OGG1, MnSOD**

Our hypothesis generating results that serum selenium levels and the polymorphic genes *hOGG1* and *MnSOD*, involved in the protection from oxidative stress, act concurrently in the defence of prostate cancer development are in accord with previous knowledge (Rayman 1997, Yoshizawa 1998, Vogt 2003, Li 2005, Brinkman 2006, Pourmand 2008). Large studies on genetic polymorphisms of other central selenoproteins find associations with a decreased risk of prostate cancer incidence, mortality, or modifications in the association between serum selenium and prostate cancer survival, similarly to our study for some only in a subset of subjects with low selenium status (Penney 2010, Steinbrecher 2010, Meplan 2012). The Papers support our findings of an interaction between selenium levels and genetic variation as risk factors for prostate cancer, again emphasizing the complex interplay between genetic and environmental factors as risk factors for prostate cancer.

If, as these reports suggests and our study may be interpreted, genetically different subgroups of men would benefit differently from high serum sele-
nium levels, then beneficial effects of supplementation in interventional studies may pertain only to subgroups and therefore be difficult to detect in mass intervention studies. The interplay between selenium levels and genetic factors has not yet been fully clarified.

Few studies on the relation between \textit{hOGG1} polymorphism and cancer have been conducted since Paper I was published. The ones published have been inconclusive in part due to small size and lack of statistical power (Zhang 2011, Karahalil 2012). The enzyme coded for by the OGG gene, the DNA oxoguanine glycosylase, has a specialized function in human DNA repair mechanisms compared to e.g. MnSOD. As such it is still unclear what impact decreased function of the OGG has on cancer risk. Polymorphisms in the \textit{MnSOD} and their association to cancer have in contrast to \textit{hOGG1} gained more interest with more than one hundred articles published since 2011. Associations between MnSOD polymorphisms and aggressive prostate cancer and modification of the association between selenium levels and aggressive prostate cancer (Abe 2011) have been found.

The size of the studied population in Paper I implies that our analyses of the interaction of gene polymorphism and serum selenium levels are of an exploratory character. The impact of genetic variation in relation to smoking being a risk factor for prostate cancer was not possible to analyse in depth. A disadvantage was also that a proxy SNP was used for the analysis of the intended \textit{MnSOD} polymorphism which made a detailed analysis on a molecular level unattainable. The genotyping of Paper I was performed during the “candidate gene era” whereas today, studies on genetic polymorphisms and their possible associations with disease are usually performed using genome-wide screening technique analyses. Analysing one gene at the time, as was done in Paper I, is now more common for more detailed analyses after a screening procedure has identified an association between a gene and an outcome.

\textbf{Attempting prevention of prostate cancer by selenium supplementation}

The importance of research on the association between selenium levels and prostate cancer obviously lies in the hope of using selenium supplementation for cancer prevention. The Nutritional Prevention of Cancer Trial (NPCT) in a low selenium status area of the USA, studied selenium supplementation as a means to prevent secondary non-melanoma skin cancers with negative results. For the secondary endpoints, all cancer mortality and prostate cancer incidence, a risk reduction was observed (Clark1996) most pronounced in the bottom tertile of plasma selenium (<106 µg/l; Duffield-Lillico 2003).

The NPCT finding, in addition to a large number of supporting epidemiological studies, led to the initiation of the large US-Canadian Selenium and
Vitamin E Cancer Prevention study (SELECT; Lippman 2009). As already mentioned, SELECT failed to achieve evidence of benefits from selenium supplementation on prostate cancer incidence. The study was terminated prematurely due to an increased incidence of prostate cancer in a parallel Vitamin E supplementation arm. The lack of positive effect from Selenium in SELECT led to the general conclusion by the consortium that selenium supplementation can not be used for prostate cancer prevention. This conclusion may however be too hasty. Other explanations for a negative results could be the difference in baseline selenium status in SELECT (135-138µg/l) compared to the NPCT (113-114µg/l) where SELECT included almost no participants with low selenium status (<106µg/l). The lack of benefit may thus reflect an already selenium-saturated study population in which surplus selenium made no difference.

Remember that our population in Paper I had a lower boundary for the upper tertile range of only 81µg/l. Smokers, the group for whom the strongest protective effect of high selenium levels has been detected (Peters 2008A), were greatly underrepresented in SELECT and relevant genetic variability was not analysed. Only 1% of prostate cancers in SELECT were non-localised, and only one participant died from prostate cancer during the study. This study which has had a huge impact is hence in fact not generalizable to other populations and certainly not to populations outside the USA and Canada. The results instead emphasize the remaining possibility for success in selenium supplementation in populations with poor selenium status, in smokers and possibly with certain genetic selenoprotein profiles as Paper I also suggests.

Risk of harm vs. chance of benefit of supplementation- more is not always better

Essential nutrients can cause harm when ingested in excess. Significant side effects in the NPCT and SELECT studies (Clark 1996, Lippman 2009, Klein 2011) were: gastrointestinal side effects, alopecia and dermatitis. Both studies found a possible association with T2DM although not statistically significant. Theoretically, excessive levels of antioxidants could decrease free radicals to harmful levels; ROS in moderate concentrations are essential mediators of precancerous cell death (Bjelakovic 2007). Selenium supplementation in populations of already high selenium status may thus theoretically be negative and baseline levels should always be considered when discussing benefits and risks of any supplementation.

This is often disregarded when it comes to vitamins, trace elements/antioxidants etc which up to 1/3 of adults use for health promoting reasons (Lawson 2007) while in reality excessive use may have harmful health effects (Melhus 1998, Lippman 2009) the general public may not be
aware of. Promotion and marketing of nutritional products such as selenium is not strictly regulated. It has been argued that these products be controlled in a way similar to what is required for pharmaceutical products (Lawson 2007). This would include defining indications, performing clinical trials investigating benefits and risk of harm in large populations before recommended, advertised and marketed. This is an interesting view but perhaps not realistic for political and commercial reasons.

Prevention of Prostate Cancer, is it really relevant?

No disease-preventive measure should be attempted in the context of only one single disease. In the case of prostate cancer a more holistic view would be to at least include an analysis on effects on CVD, the number one cause of morbidity and mortality in the population. Weak positive preventive effects on prostate cancer in a population could easily be counteracted by an even smaller increase in cardiovascular risk on account of its higher incidence and prevalence. Selenium supplementation for the prevention of CVD has been investigated in RCTs, both in high and low selenium status areas, without definite evidence of positive effects (Navas-Acien 2008). Somewhat disturbing are instead findings in high selenium status populations of increased incidence of T2DM, hypercholesterolemia (Navas-Acien 2008) and increased all-cause mortality (Bleys 2008).

The holistic approach also applies to our findings on smoking and prostate cancer in Papers I and II. While when taking competing risk into account it appears that smoking is a risk factor for prostate cancer, this is naturally clinically overshadowed by it being a very strong risk factor for CVD. In general what is positive for cardiovascular health is usually most important in a population. As long as effects of different factors on different diseases point in the same direction, as in the case of smoking, results are not controversial. When results point in different directions, care must be exercised to make the right decisions. Taking this into account, selenium levels in Europe are generally very low and health benefits from increasing the selenium status of the population by supplementation are probable but not proven. Chances are that the question of if there is a benefit of selenium supplementation in Europe on prostate cancer and or other disease will never be scientifically answered.

Better chance of success in high risk populations?

At least two groups have attempted prevention of prostate cancer with selenium in high risk populations without much success. Men with a negative biopsy but with suspected prostate cancer and with a mean baseline selenium level of 126µg/l did not benefit from further selenium (Algotar 2012) while in a high grade PIN study a non-significant positive result in the bottom
baseline selenium quartile (<106µg/l; Marshall 2011) was reached. In these studies the baseline selenium status of the populations studied was high and a substantial proportion of the studied populations likely had a semi-occult but established prostate cancer disease. Expecting to be able to treat manifest cancer with selenium is optimistic. Further attempts in this direction should identify high risk men in other ways.

Other Prostate Cancer prevention attempts

Other examples of large scale interventional mass-prevention studies are studies using 5-α-reductase inhibitors, vitamins A and E and toremifene with mixed results.

The role of the Metabolic Syndrome or its components

We show in Paper II that the presence of the MetS according to the NCEP definition at age 50 is a risk factor for clinically relevant prostate cancer during long term follow-up. Results for the MetS according to the IDF definition are of similar magnitude, however not formally statistically significant. The findings are more marked when taking account of the competing risk of early death without prostate cancer. The components of the MetS predominately attributable to the findings appear to be abdominal obesity and a high serum triglyceride level. High fasting plasma glucose, high blood pressure and a low HDL cholesterol level are only marginally associated with the conditional probability of developing prostate cancer.

The measurements used in Paper II were made on average 25 years before diagnosis, but these long-term lifestyle based measurements will not likely vary much over time, once established in middle aged men. Any misclassification of men who later developed the MetS would most probably have lead to an underestimation of any risk observed rather than overestimating it. Our study is similar in size to other published studies on the relationship between a fully characterized MetS and prostate cancer and the follow-up is considerably longer. Previous studies have come to divergent conclusions regarding the MetS and prostate cancer risk, probably due to the different definitions of the MetS used and the lack of consideration given in other studies to effects of competing risk.

There were four generally accepted definitions of the MetS at the time of planning Paper II: the World Health Organization (WHO), the NCEP, the European Group for the Study of Insulin Resistance (EGIR) and the IDF definitions. None of these had a gold standard status and they emphasize different aspects of the MetS. Furthermore, several investigators used modified versions of these definitions (Lund Håheim 2006, Beebe-Dimmer 2009, Martin 2009) or only selected parts of them (Beebe-Dimmer 2007).
The metabolic syndrome and competing risk

An important reason for the equivocal results in the field is the lack of consideration given to the effect of competing risk. Many men with the MetS will, as already has been mentioned, not live to an age when prostate cancer incidence is highest but will die early from other causes leaving a possible excess risk of prostate cancer undetected. The commonly used statistical analyses method assumes that censored individuals have the same risk of the event under study as those observed until the endpoint (Cox 1972). When the exposure is associated both with risk of early death and the disease under study, especially when the outcome is increasing in frequency with age, this criterion is not fulfilled. We used the conditional probability method (Pepe 1993) to try to circumvent possible violation of the assumptions underlying the standard techniques. This revealed that the full MetS and key elements of it are linked to prostate cancer risk. Paper II is the first study using this methodology when analyzing the longitudinal relationship between the metabolic syndrome and prostate cancer.

Other explanations for apparent diverging results of previous studies may be differences in age at baseline measurement and length of follow-up, e.g. measurements vary from those done early in life (Martin 2009) to the elderly, closer to a mean age of diagnosis of prostate cancer (Beebe-Dimmer 2007). Several studies end their follow-up before the mean age of diagnosis of non-screening detected prostate cancer (Lund Håheim 2006, Laukkanen 2004, Martin 2009), thereby studying only a younger part of the prostate cancer spectrum. Prostate cancer in the young is in 30-40% of cases associated with dominantly inherited genetic traits with high penetrance (Bratt 2002) and could hence be anticipated to be less influenced by external factors such as the MetS.

Our findings are in line with two other studies. The first one (Laukkanen 2004) working with the WHO definition in young cohort, found a 1.9 fold higher relative risk (95% CI, 1.1-3.5) of prostate cancer. This study excluded diabetics due to an expected high morbidity and mortality of this disease thereby indirectly taking competing risk of death into account. The second large study (Lund Håheim 2006) working with a modified NCEP definition of the MetS in a young cohort, found with the MetS present a slightly increased relative risk of 1.56 (95% CI, 1.21-2.00) of prostate cancer.

Contrary to this, in a cohort study on comparatively young and overweight American men with a low participation rate, a decreased relative risk of developing prostate cancer of 0.77 (95% CI, 0.60-0.98) working with MetS (NCEP) was observed (Tande 2006). The cases were identified by a combination of questionnaire and registry findings and the prostate cancers were to an unknown extent screening detected. The concept of competing risk of death in their obese cohort was neither discussed in this study and nor in another study working with a revised NCEP definition of the MetS where
little evidence was found of a relationship between the MetS or most of the components thereof and later clinically relevant prostate cancer, RR= 0.91 (95% CI, 0.77-1.09) (Martin 2009). The average age of the men at baseline was here very low at 50 years with a follow-up of a mere 9.3 years, i.e. an age of low prostate cancer risk.

The findings in Paper II are thus well in accordance with other research in the field once analyzing it closely. The findings indicate that life style factors giving rise to the MetS according to either the NCEP/IDF definition, in particular abdominal obesity, increase the risk of clinically relevant prostate cancer once the competing risk of dying early from other outcomes of the MetS is taken into account. Further research into the underlying mechanisms and into finding out whether a reversal of the MetS components, e.g. obesity, will attenuate the risk is now needed.

Reverse the Metabolic Syndrome and prevent Prostate Cancer?
The metabolic syndrome is defined in relation to CVD. Interventions to attempt to slow down the development of or reverse an established MetS or components thereof to lower the risk of later CVD are a part of clinical everyday treatment routine. They include attempting lifestyle changes such as increasing physical activity, switching to a healthy, reduced calorie diet which due to lack of compliance however is successful in only a minority of individuals. MetS intervention studies with cancer as an endpoint have not been made per se. Such studies would need longer follow-up and be larger to provide reliable results and as emphasized above the general health effects on CVD would overshadow any effects on cancer.

Drug treatment of the individual components of metabolic syndrome is common and effects of such treatment on cancer development have indeed been investigated. Metformin for the treatment of T2DM appears to reduce the risk of several cancers, prostate cancers not included (Zhang 2013).

The lack of effect in prostate cancer may be age and competing risk related. Data on statins for dyslipidemia and their relation to cancer are conflicting. In a recent study statin use was associated with substantial protection against prostate cancer death, adding to some epidemiologic evidence for an inhibitory effect on prostate cancer. Whether such findings are effects mediated via the metabolic syndrome or via direct effects on cancer development is unknown.

The Metabolic Syndrome, its Raison d’être
Already at the time of planning Paper I a debate questioning the concept of the MetS had started (Kahn 2005 and 2008, Grundy 2007). The cluster of risk factors for CVD included in the definitions of the MetS was questioned as to its indicating the existence of a medical condition or a true syndrome in
itself. The MetS has not gained WHO disease status. The debate at the time also concerned the (lack of) precision of the definitions, the lack of concordance between populations identified using different existing definitions (Nilsson 2007), the lack of quality evidence of its pathogenesis and its value as a risk marker for CVD compared to its ingoing factors. The latest consensus opinion is that the metabolic syndrome in relation to CVD still is useful as concept e.g. for clinically educating patients (Simmons 2010). Still, with several of the ingoing defining factors of the metabolic syndrome being discussed as risk factors for cancers, the value of investigating the concept of metabolic syndrome in relation to prostate cancer was at the time Paper II was planned not questioned.

What happened since Paper II

In a European multicentre cohort of nearly 300 000 men with a mean recruitment age of 44 years and up to 12 years of follow-up, no association between “metabolic factors” including a metabolic composite and incident prostate cancer (n = 6673) was found (Häggström 2012). BMI, hypertension and the composite variable were associated with an increased risk of progression and death from prostate cancer. There are some concerns in the unusual handling of laboratory data, the use of complex statistical methods to correct for lack of data and the inaccurate referencing of previous knowledge in the field in the paper published. The age of the cohort was very low and the results may as such have strongly affected by a high proportion of early onset-hereditary prostate cancers. The strongest associations were seen in those included during the first years of establishing the cohort, i.e. with less screening detected prostate cancer. As in most studies the possible influence of competing risk of death from other causes was never discussed. The conclusion that some metabolic factors are not related to the development of prostate cancer but to its progression pertains here to a very young population with a very short follow-up and is difficult to compare to the results from Paper II.

A recent case-control study on absence/presence of MetS (harmonised definition) at diagnosis of incident prostate cancer (n=1294) vs. hospital controls (Pelucchi 2011) found an increased odds ratio of 1.66 (95% CI, 1.22-2.28) in men with the MetS compared to men without it. With four MetS components present the OR was 3.99 (95% CI, 1.03-15.4). The study results represent the situation at diagnosis of prostate cancer and give as such a snapshot in time independent of the competing risk situation affecting longitudinal studies. As the MetS is a chronic condition the results likely reflect the impact of long term influence of the MetS on the risk of developing prostate cancer and thereby support the conclusions of Paper II.
Type 2 Diabetes Mellitus - does it really protect from Prostate Cancer?

The gross overlap between Type 2 Diabetes Mellitus (T2DM) and the MetS populations (~80%), which both are associated with CVD and premature death, makes some insight into the association between T2DM and prostate cancer risk relevant. Two recent reviews (Bansal 2012, Xu 2013) confirm some previous findings of an inverse relationship between T2DM and prostate cancer incidence, strongest in localized stages of disease. T2DM is a strong risk factor for several cancers including endometrial, kidney, colon, pancreas, and thyroid cancers with a hazard ratio in a large meta-analysis for persons with T2DM vs. without of 1.25 for death of any cancer and 2.32 for death from CVD (Emerging Risk Factors Collaboration 2011). The apparent protective effect on prostate cancer disease is therefore puzzling. Suggested explanations include alterations of lifestyle to a healthier one after T2DM diagnosis, use of metformin, decreased insulin levels in later stages of T2DM, decreased levels of testosterone in T2DM, leptin, IGF-1or genetic links but there is a lack of causal evidence to support any of the theories. Some authors (Frayling 2008) observe that “the depletion of diabetic cohorts by early mortality will lead to an apparent lower rate of prostate cancer in survivors” thereby describing the competing risk situation.

There are however some evidence of genetic links between T2DM and prostate cancer (Gudmundsson 2007, Meyer 2010, Pierce 2010, Machiela 2013) which could explain the inverse relationship between T2DM and prostate cancer, which appears strongest in Asians.

While the possible explanatory factors of the apparent inverse association between T2DM and prostate cancer exist, analyses of any effects of competing risks are still lacking. However, in patients with a first hospitalization for T2DM a decreased risk of being diagnosed with prostate cancer was seen already after one and five years, which was not found for any other forms of cancer (Hemminki 2010). This was confirmed in the oldest age groups when stratifying for age at hospitalization thereby in part controlling for competing risks and hence gives support to the general finding of an inverse relationship between T2DM and prostate cancer.

Competing risk - does it matter in the screening age of Prostate Cancer?

One may argue that with the decreasing age at diagnosis of prostate cancer due to PSA screening, the effect of competing risk both in real life and in studies would decrease. In the future, larger proportions of these studies will concern younger men with low risk disease but still many prostate cancer studies presented today still reflect cohorts of men not undergone screening.
That accurate estimate of long term risk for disease may require adjustments for competing risk of mortality, remains true.
Discussion and Conclusions, Paper III

Paper III provides important new information on prescription patterns and adherence to oral pharmacological treatment as estimated in a large older male oncology population. One in 20 men diagnosed with prostate cancer in Sweden between the years 1997-2006 (n=58143) was prescribed a mono-therapy anti-androgen as first line treatment for the disease.

Main results and conclusions, Paper III:

• The large majority of men prescribed monotherapy anti-androgens for prostate cancer was treated according to national guidelines and approved indication.

• We noted a possible over-treatment with anti-androgens in men with low risk cancer.

• Among the bicalutamide treated men 21% were prescribed an off-label low dosage, predominately in ages >75 years and in low risk disease.

• Adherence to prescribed bicalutamide was high (median 84%),

• Men older than 75 years had lower adherence and men in the localised high risk and metastatic group had a higher adherence to their prescribed bicalutamide than other groups.

• Neither the choice of dosage nor the patient adherence was influenced by the socioeconomic status of the patient, his marital status, other significant morbidity or the medical speciality of physician initiating the treatment

• intervention attempts to increase adherence could be aimed at men above the age of 75 years and in men with less severe disease once the adequacy of the indication for treatment has been consolidated.

Treatment in relation to guidelines

Current guidelines appear to be fairly synchronised with real life clinical prescription practice and vice versa. Still 2.1 % of men in the localised low/intermediate risk group, where the benefits of anti-androgen treatment have not been proven and where approved indication is lacking, were also prescribed anti-androgen. No subgroup of men with prostate cancer could be identified in which under-treatment or excessive treatment with monotherapy anti-androgen was apparent as per investigated other background factors.
Persistence

In the 94 % of men who did initiate a planned monotherapy anti-androgen treatment, persistence in use and reasons for discontinuation followed expected patterns with a median treatment time twice as long for the low risk patients compared to the high risk patients who in turn more quickly proceeded to second line treatment usually including GnRH analogues.

Off-label use

The 21 % of men who were prescribed an off-label, low dose of bicalutamide more commonly had a low risk prostate cancer, and belonged to the oldest age group, >75 years.

Married men with disease classified as low risk may be more likely to request from their physician an active treatment for their disease or the physician may prescribe a “light hormonal treatment” with the intent of reducing side effects, e.g. loss of libido, impotence, gynecomastia, and asthenia. In the multivariate analysis the apparent effect of marital status was weak, but age and risk class at diagnosis remained clearly statistically significant. Due to a high correlation between age and marital status and a loss of statistical precision in the multivariate models, our data cannot exclude that marital status also is a determinant for prescribing patterns.

Analysing the men according to their risk status at diagnosis, i.e. separating men of the combined high risk class treated as per approved indication from men with low risk prostate cancer and off-label indication, revealed that age influenced the choice of dose irrespective of risk class.

In all, the physicians appeared to be more likely to prescribe a lower than recommended dose to older men with less severe disease, perhaps to avoid side effects. The risk-benefit balance of the treatment in this situation is uncertain and critical reflection on the scientific and clinical basis for the chosen dosage should be emphasised.

On adherence to prescribed treatment

Adherence is defined as the extent to which a patient’s behaviour coincides with medical advice (Haynes 1979). It is an important aspect of the effectiveness of self-administered cancer treatments as the responsibility shifts from the health care professionals to the patient. Few studies of adherence have been carried out in oncology since most oncology drugs are delivered in a hospital setting with few adherence problems. Non-adherence may result in less effective therapy, increase in physician visits and activities (Partridge 2002), and may lead to a compromised patient-physician relationship. A substantial proportion (60 %) of men in our study had a very good or good adherence (>80%, MPR); better than what has been reported from several
similar studies of adjuvant breast cancer treatment (Partridge 2002, Nilsson 2006, Atkins 2006, Ziller 2009, Hershman 2010) for the prevention of recurrence of disease. This is in contrast to the situation we studied, where the men would have been informed that they were suffering from an “active” cancer disease requiring treatment. Endocrine treatment of prostate cancer results in a rapid decrease in serum PSA and alleviation of symptoms, providing individual patient feedback likely to increase the adherence.

Men with more advanced disease adhered to a higher degree, indicating that perceived personal benefit of the treatment was important for adherence, in line with other studies (Partridge 2002). At the beginning of treatment, however, in bicalutamide-naïve patients, disease severity did not clearly influence adherence.

Adherence to oral medication among older male oncology patients has not been previously studied but is now more relevant than before, given the emergence of several new oral treatment options for genitourinary cancer, e.g. abiraterone for advanced prostate cancer, sunitinib and sorafenib for, among other indications, renal cell cancer. To retain the effectiveness of these drugs, knowledge of adherence-influencing factors is crucial. We found that younger age at diagnosis was associated with higher adherence. Interventions to increase bicalutamide adherence in the oldest age group and for men with a low risk disease could be considered, once the relevance of the indication for treatment is fully established for the individual patient, especially in the low risk group.

In contrast to our findings, in studies in female populations, young age (Bardel 2007, Güth 2011) or age below 50 or over 65 years (Hershman 2010) had a negative influence on adherence. In reviewing mixed gender population studies (Balkrishnan 1998) age as a predictor of adherence was not consistent across studies. These studies were mostly based on study cohorts outside the oncology field, often with study subjects considerably younger than ours of Paper III. Neither socioeconomic factors, nor marital status, nor co-morbidity, nor the speciality of the prescribing physician influenced the adherence or the prescribing pattern in Paper III. Studies on the multi-factorial determinants of adherence show conflicting results, and there is currently no unified theory or picture of non-adherence (Partridge 2002, Becker 1975).

On measuring adherence

This study is based on nationwide registries with high coverage. Compared to adherence studies based on self reporting, which tend to overestimate adherence, prescription registry data more accurately estimate real life medication use and adherence (Avorn 1998, Ziller 2009,) in large populations. Our registry data show the distribution of bicalutamide use in a population to whom all prescribed drugs are readily available through a national reim-
bursement system regardless of the financial situation of the patient. A limitation of the study is the relatively short follow-up time in PCBaSe, which prohibits a study of those on treatment for very long times. However, the follow-up is sufficient to study a length of treatment that is realistic for a large proportion of the patient population.

Adhering to guidelines and approved indication

The extent to which prescribing oncologists adhere to guidelines and or approved indications for drug treatments has not been not much studied. In lung and colon cancer studies performed in the USA predictors of chemotherapy under-use and prescription were socioeconomic factors in patients such as old age, income, lack of insurance, transportation barriers, or transferring between facilities of care (Salloum 2012, Boland 2012). Some of these predictors are less relevant in a Scandinavian setting with more equal access to health care. Higher adherence to local guidelines in the studies conferred a survival benefit for patients. Studies into the area would be of interest to determine the relevance of the guidelines and for identifying and possibly addressing issues with a possible negative patient impact.

Awareness of adherence to improve effectiveness of oral cancer treatment

The steady increase in availability of patient-administered oral anti-cancer treatments is putting the spotlight on the importance of addressing adherence when planning treatment and assessing outcome. Adherence has hitherto largely been a non-issue in oncology with almost exclusively healthcare professional administered drugs. Self-administration has so for been restricted mostly to hormonal adjuvance or treatment in breast and prostate cancer. The increased awareness with oncologists of the adherence as factor for treatment success is noticeable: Adherence in imatinib leukaemia treatment and similar drugs has been studied (Abraham 2012, Yood 2012) and even monitored by electronic equipment (Abraham 2012). Factors predicting good adherence were here: absence of side-effects, faith in health care team and drug, good understanding of illness and treatment. The authors state for the field of oncology that we “no longer have any excuse not to evaluate medication behaviour, not to prevent or correct non-adherence and not to promote adherence as a part of routine clinical practice” (Abraham 2012). In the best interest of the patients a room for non-adherence should not be assumed.
Adherence affects effectiveness

Lack of adherence may differ substantially between anti cancer drugs of the same class with the same indication and similar dosing schemes (Yood 2012). If equal efficacy is assumed the difference in adherence between drugs could mean a difference in effectiveness again emphasizing the importance of evaluating adherence and preventable reasons for non-adherence. This also emphasises that the risk-benefit analysis of a drug at the time of approval is relevant mainly for the populations in the pre-approval studies in which it was determined. This may and will not reflect the real life risk benefit of the drug in clinical use. Adherence to long-term medication therapy for a chronic disease such as prostate cancer is pivotal for optimal clinical outcome.

Recent comments on methods for measuring adherence

Low concordance between different methods for assessing adherence has repeatedly been confirmed (Oberguggenberger 2012, Font 2012) when comparing self-reporting vs. physician reporting or registry data such as ours. Comparisons between different studies should be done cautiously.

Future

There is a need to further investigate possible modifiable adherence factors, similar to what has been investigated for other disease populations (Abraham 2012, Murphy 2012) especially with the recent introduction of novel oral medications for prostate cancer e.g. abirateron. Identification of factors influencing adherence to oral cancer treatment in order to intervene to possibly improve them will in the future be a cornerstone in effective cancer treatment - as important as improvements in the efficacy of drugs. The cost effectiveness of new oral anti-cancer drugs will be highly dependent on adherence.

Simply put:

“Drugs don’t work in patients who don’t take them”

C. Everett Koop
Discussion and Conclusions Paper IV

Paper IV evaluates a novel approach for the PRR method of disproportionality analysis; the PRR-by-therapeutic area (PRR-TA) using a background restriction; this in a drug authority pharmacovigilance setting. The evaluation of the PRR-TA is exemplified by drugs from areas of chronic disease, Prostate gland disease and Type 2 Diabetes Mellitus, with the investigated drugs bicalutamide, abiraterone, metformin and vildagliptin. We could not choose any MetS drugs per se as no such drugs are approved for that indication. Main findings:

- The novel approach PRR-TA performed better or equally well regarding its ability to detect true positive SDRs compared to the conventional full database PRR approach.
- The novel approach PRR-TA performed better in reducing the noise from false positive SDRs compared to the conventional full database PRR approach.
- The PRR-TA decreased the ratio between false positive SDRs and unclassified SDRs relevant for further evaluation i.e. it increased the signal-to-noise relationship compared to the PRR.

The good results when including drugs for the treatment of both BPH and PrC into one therapeutic area suggest that the indication for treatment does not have to be identical in all included drugs in a restricted comparative database for the PRR-TA method to apply. It appears to suffice that the symptoms of the treated disease areas are largely coinciding. The PRR-TA furthermore performed better for background noise reduction than the recently suggested method of restricting the number of SDRs delivered by the conventional PRR method by increasing the threshold case count to \( \geq 5 \) instead of the conventional \( \geq 3 \).

Analyses restricted by gender for the prostate gland disease analyses did not markedly improve the outcome implying that gender restrictions may be less useful than restriction to disease area even in gender specific drugs. Eight unclassified new potential signals were detected and forwarded for clinical expert validation within the regulatory system.
Comparison with literature

A recent general review on practical aspects of pharmacovigilance methodology in different setting briefly touches on the possible impact on disproportionate analyses from restricting the background or by stratifying analyses. However, this is predominately suggested for the analyses of vaccines and paediatric drugs due to their particular use and target population (CIOMS 2010).

Among seven possible sources of improvements in signal detection suggested by one author (Egberts 2007) is “selection of appropriate control groups and restriction to subsets of people/reports” which has not resulted in further published research. Another author suggests that subgroups of a database could be used as a background for disproportionate analyses, theoretically exemplifying this with removing per-oral products when analysing injectables, chemotherapeutics when evaluating emesis for other drugs or all ADR terms in the background which do not appear for the drug under investigation (Gogolak 2003). Differences in output from such manipulated PRR calculations are exemplified by single drug-ADR pairs but no general analysis is presented. A few studies presenting results of systematic analysis of alterations of background for different disproportionality analyses methods have been published; almost exclusively concerning analyses for the specific situation of analysing paediatric- vaccines (Woo 2008, Zeinoun 2009, de Bie 2012). Substantial statistical differences are found in different subgroups of analysis (de Bie 2012); increased numbers of false negatives are noted in vaccine subgroup analyses with only small samples (5-10%) of SDRs analysed in detail. Other exploratory vaccine studies stratifying for age, gender etc and/or alteration of background database also note statistical differences in the output from disproportionality analyses (Woo 2008, Zeinoun 2009) some with low concordance between methods (Woo 2008). The authors recommend combined analyses (Zeinoun 2009) and conclude that “stratification likely increased efficiency” (Woo 2008) without supporting the statements with data. Clinical details and the impact of the mentioned statistical differences are not analysed in more depth.

The masking phenomenon (Gould 2003) has mostly been discussed in relation to narrow commercial databases with a single drug constituting a large proportion (Almenoff 2005, Zeinoun 2009, Wang 2010). It may have a large impact on the vaccine analyses. We noted sporadic cases of de-masking of acknowledged ADRs as SDRs in the PRR-TA compared to the PRR. The published studies hitherto generally represent a “drug-statistics” perspective on explorations of variants of restricted (vaccine) backgrounds or stratification in disproportionality analyses, with for one, small samples of the output analysed in detail (de Bie 2012). Our study instead concentrates on the “drug-patient” perspective. We focus on therapeutic area classifying each SDR in detail to determine its relevance. Practical and generalizable conclu-
isions drawn from stratified analyses and background alterations in vaccine signal detection are not applicable for other drugs as vaccines are only used in few occasions in a healthy young population not representative of long time drug users in general. Our study populations are in this respect representative of patients in general regarding variation in age, variation in background morbidity, and different administration forms. The vaccine analyses are similar to our drug-class restricted PRR analyses which performed too poorly to be of substantial value in the long term drug use setting.

No other researchers have investigated systematically and in clinical detail the role of reduction of background to a therapeutic area when evaluating the results from calculating the PRR-TA in continuous medication for chronic disease.

Strengths and weaknesses of the PRR-TA

The PRR-TA in our study performs better for background noise reduction than restricting the number of signals by redefining the SDR thresholds of the PRR from SDR3 to SDR5. A further drawback of the increased threshold method is the inevitable delay of the possibility to discover a new signal until cases number 4 and number 5 are reported.

Since our aim of performing the PRR-TA was to remove the SDRs confounded by disease or disease spill-over, a weakness of the PRR-TA is the presumed inability to detect ADRs mimicking symptoms of the treated disease. This is however a drawback shared with any disproportionate analysis method; as such SDRs likely would be discarded early at the time of manual expert validation. Pre- or post-marketing safety studies stand a better chance of succeeding in detecting such signals. Validation of a signal subsequent to the disproportionality analysis normally includes ascertaining reliable information on the background incidence of the suspected new ADR in the general population and if possible in the population at risk, i.e. in patients with the same disease without treatment with the drug in question. To some degree our method incorporates this task by using the treatment area patient population.

The PRR-TA presents a way of introducing clinical knowledge into the statistical disproportionality analysis to optimize it and to provide the manual evaluators to a greater degree focus on relevant SDRs with the noise of irrelevant SDRs being reduced.

The PRR-TA could replace the possible gains of stratification since it effectively mimics a one-stratum-stratified analysis streamlining background factors to the investigated therapeutic area.
Clinical implications

Study IV represents a successful exploration of new methods for signal detection with the intent to decrease background noise while maintaining the ability to detect true signals. Our method provides an opportunity to standardise data in a relatively simple way to improve the output from the PRR in a large general database. A mere mechanical reduction of the PRR background into ATC groups would not be as sufficient as the reduction of confounding of disease and disease spill-over requires the therapeutic use to be the factor for clustering drugs rather than the ATC code (although these may sometimes partly coincide).

The PRR-TA would, if generalizable to other drugs/therapeutic areas, provide opportunity for more cost efficient use of manual expert resources in the signal validation step. Other authors have also emphasized this “importance of minimizing the amount of false positive signals (SDRs) which if excessive could detract from optimal pharmacovigilance activities” (Zeinoun 2009).

Over-stratification or using advanced, complex statistical methods which are used may give an exaggerated confidence in or “seduction bias” (Hauben 2005) as to what disproportionality methods may perform. Even if a signal to noise ratio or other features of a disproportionality analysis method is improved, these methods should indeed only be used for screening purposes. The ensuing manual expert evaluation is still indispensable in determining whether a delivered SDR should be considered a signal or not. More complex analysis methods do not necessarily yield better output, especially if they are used at the expense of clinical expertise. We believe that our method balances well the method complexity in relation to clinical knowledge in areas exemplified. If proven generalizable the PRR-TA both improves the screening method output and leaves room for better use of clinical expert resources.

Unanswered questions and future research specified

The PRR-TA was in Paper IV performed for drugs with one single approved indication in a very large general ADR database. This does not exclude the method from being useful in other types of drugs or drug areas, database settings or with other disproportionality methods, but this would have to be validated before applying the PRR-TA in general signal detection routine.

Conclusions

The PRR-TA method opens a possibility to increase the performance in signal detection by decreasing the number of false SDRs confounded by indication and indication spill-over, while maintaining the ability to detect true
positive SDRs in drugs for chronic diseases. Exploring and validating the methods applicability also in other treatment areas is suggested. If found generalizable into other therapeutic areas, one could expect a possible gain in a more effective use of valuable manual validation resources from the use of the PRR-TA.
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