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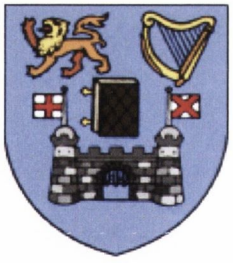
PROSTATE CANCER PHARMACOEPIDEMIOLOGY:

DIGOXIN, ASPIRIN

AND PATIENT OUTCOMES

A thesis submitted to the University of Dublin,

for the degree of Doctor of Philosophy



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2013

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DECLARATION

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Eva Flahavan

Date: December 2013

THESIS SUMMARY

Pharmacoepidemiology is the study of the effects of medicines in a real-world population; combining pharmacology, the study of medicines, with epidemiology the study of diseases. Prostate cancer is the most commonly diagnosed non-cutaneous malignancy in Irish men and the second most common cause of cancer death. This thesis contains the first pharmacoepidemiology studies to be carried out in a cohort of Irish prostate cancer patients. These studies were carried out using linked patient records from the National Cancer Registry of Ireland (NCRI) and prescription claims data from the Primary Care Reimbursement Services (PCRS) General Medical Services (GMS) scheme. Exposure to two medicines, digoxin and aspirin, commonly used for the treatment and prevention of cardiovascular disease were examined in relation to prostate cancer patient outcomes.

Digoxin is a member of the cardiac glycoside family, and is prescribed as second line therapy in the treatment of atrial fibrillation and heart-failure. Digoxin and other cardiac glycosides have been shown to impede cancer cell growth and tumour progression in a variety of cancer types and in mouse tumour models. These anti-cancer activities have been attributed to the pharmacological activity of digoxin on the sodium/potassium ATPase pump, and the more recently documented effects of digoxin on gene transcription; demonstrated through inhibition of Hypoxia Inducible Factor-1α (HIF-1α) expression. Digoxin exposure has also been associated with reduced risk of prostate cancer.

In this thesis, two studies were carried out investigating digoxin exposure in men with prostate cancer. The first study examined the association between digoxin exposure prior to cancer diagnosis and tumour characteristics (stage or grade) at diagnosis; digoxin exposure was not found to be associated with tumour stage or grade at diagnosis. The second study investigated the association between digoxin exposure at diagnosis and prostate cancer-specific mortality. In this study no association was observed between digoxin exposure and prostate cancer-specific mortality in the main analysis or in a propensity score matched cohort. There are a number of possible reasons why improved outcomes were not observed in men with prostate cancer exposed to digoxin; the most critical of these is that the therapeutic plasma concentrations of digoxin in humans are much lower than those used in pre-clinical studies. However clinical research is on-going, investigating digoxin in patients with breast cancer and in the treatment of recurrent prostate cancer.

Aspirin is the most commonly prescribed drug on community drugs schemes in Ireland. It was originally used for its anti-inflammatory and anti-pyretic properties, mediated through the

inhibition of cyclooxygenase enzyme-2 (COX-2). Currently aspirin is most commonly prescribed at low doses for its anti-thrombotic effects, as it reduces the risk of stroke and myocardial infarction. This effect is mediated through the inhibition of COX-1 in platelets. Inhibition of COX-1 and/or COX-2 by aspirin has been proposed to impede the development, growth and dissemination of a number of cancers, including prostate cancer.

The findings of observational studies investigating aspirin exposure and prostate cancer incidence have been equivocal; meta-analyses of these studies have reported aspirin to be associated with an approximately 10% reduction in risk of prostate cancer. Recent studies have also reported aspirin exposure to be associated with reduced prostate cancer mortality. The studies carried out in this thesis examined the association between aspirin exposure prior to diagnosis and prostate cancer-specific mortality in two cohorts; firstly in men diagnosed with stage I-III prostate cancer and secondly in men with prostate cancer of Gleason score >7.

In the first study, no association was observed between any aspirin use and prostate cancer-specific mortality; however men with higher intensity of aspirin use had a non-significant reduced risk of prostate cancer specific mortality, similar to other studies which examined this association in men with daily aspirin use. A statistically significant reduction in risk of prostate cancer-specific mortality was observed in men who received higher doses (>75mg) of aspirin. In the second study, carried out in men with prostate cancer of Gleason score >7, no association was observed between any aspirin use and prostate cancer-specific mortality. However, there was the suggestion that aspirin exposure may be associated with a non-significant increased risk of prostate cancer-specific mortality in men with stage IV disease. Future research of aspirin in prostate cancer should be directed towards identifying patient and tumour molecular characteristics which are predictive of therapeutic response to aspirin; as have been recently investigated in colorectal cancer patient cohorts.

In summary, no benefit was observed between digoxin use and prostate cancer outcomes and there was the suggestion of a possible benefit in men with localised disease who used aspirin at high intensity or high dose. The benefits of cancer pharmacoepidemiology are many-fold. Many pharmacoepidemiological studies are based on biological and pharmacological rationale from pre-clinical studies, proposing anti-cancer effects of existing drugs. The testing of hypotheses at the population level, using existing data sources, is an efficient means of verifying whether these medicines are associated with disease risk or outcomes in humans. The identification of clinically relevant molecular or pharmacological pathways as targets for new cancer therapies will further advance progress in improving patient outcomes.

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ACKNOWLEDGEMENTS

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My supervisors Dr Kathleen Bennett, Dr T. Ian Barron and Dr Linda Sharp; I truly appreciate your work on establishing the linked database, and its success as a research resource is testament to the excellent collaboration you have, long may it continue. From January 2010, when we put the grant proposal together for this project, I have always had your support. You have been generous with your time, your patience and your advice; thank you most sincerely.

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The Irish Cancer Society has supported my PhD research through their Research Scholarship Programme. I really appreciated their interest, support and investment in me as a researcher.

I couldn't have asked for better colleagues in the Department of Pharmacology and Therapeutics, you are among the most eclectic groups of people I have met. We've shared many laughs and stories over morning coffee, which was always entertaining. Furthermore you have always been on hand for advice and most encouraging of my academic work (and my baking!). Teresa Mulroy you have been a great friend, and my office "Mum" I really appreciate the many chats and your constant good humour. Susan Spillane, you've been a great ally, we've had each other's backs in the post-graduate office. Your friendship and reassurance have been invaluable through the past three years.

To my friends, some in Dublin, others scattered around the world. Thank you for being there for me in person and in spirit as this work evolved. These three years would have seemed much longer without the many outings, parties and enjoyable occasions we've shared. Your support in big ways and small has been fantastic and kept my spirits up, I truly appreciate it all.

My little brother Ed, it has been great to share the Trinity experience with you these few years. Congratulations on your degree this year as well as your other achievements in college. I really appreciate your words of reassurance, your perspective and friendship not just through the PhD but always. Finally my proud parents, who I have to admit, have not had an easy task! Since I could talk, you have channelled my abundant energy in a constructive way. You have guided but never pushed me and always encouraged and supported me in pursuing and developing both my academic skills and my varied hobbies. Mum and Dad, thanks for everything.

Dedication

For my family, remembering especially my two grandmothers who would have been ever so proud.

PUBLICATIONS ASSOCIATED WITH THIS THESIS

Flahavan EM, Sharp L, Bennett K, Barron TI. *A cohort study of digoxin exposure and mortality in men with prostate cancer.* BJU Int 2013. PMID:23937513 doi: 10.1111/bju.12287 (Appendix 1)

Flahavan EM, Sharp L, Bennett K, Barron TI. *Aspirin use and prostate cancer mortality in men with high-grade prostate cancer: a cohort study.* Ann Oncol 2014; doi: 10.1093/annonc/MDT428 (Appendix 2)

Flahavan EM,* Drummond FJ,* Barron TI, Bennett K, Sharp L. *Prostate specific antigen testing is associated with men's physical, and psychological health and healthcare utilisation, in a nationally representative sample.* Under review (Appendix 3)

Cardwell CR, Flahavan EM, Hughes CM, Coleman HG, O'Sullivan JM, Powe DG, Murray LM. *Low dose aspirin and survival in men with prostate cancer: A study using the UK Clinical Practice Research Datalink*. Cancer Causes Control. In press (Appendix 4)

Barron TI, Flahavan EM, Sharp L, Sharp L, Bennett K, Visvanathan K. *Aspirin use, lymph node metastasis and mortality in women with stage I-III breast cancer: a prospective cohort study.* Under review. (Appendix 5)

List of Abbreviations

ADT	Androgen Deprivation Therapy
AF	Atrial Fibrillation
AJCC	American Joint Committee on Cancer
ASR	Age Standardised Rate
AR	Androgen Receptor
ATC	Anatomical Therapeutic Classification
BPH	Benign Prostate Hyperplasia
CAB	Combined Androgen Blockade
CaPSURE	Cancer of the Prostate Strategic Urologic Research Endeavour
CHF	Chronic Heart Failure
CI	Confidence Interval
COX	Cyclooxygenase
CPRD	Clinical Practice Research Datalink
Cdk5	Cyclin-dependent kinase 5
CYP	Cytochrome-P
DDD	Defined Daily Dose
DHT	Di-hydro-testosterone
DRE	Digital Rectal Examination
EAU	European Association of Urology
EGF	Epidermal Growth Factor
EMA	European Medicines Agency
ENCePP	European Network of Centres for Pharmacoepidemiology and Pharmacovigilance
ER	Oestrogen Receptor
FDA	Food and Drug Administration
FGF-2	Fibroblast Growth Factor-2
FSH	Follicle Stimulating Hormone
GLUT	Glucose Transporter
GMS	General Medical Services
GnRH	Gonadotropin Releasing Hormone
HIF	Hypoxia-Inducible Factor
HK	Hexokinase
HR	Hazard Ratio

HSE	Health Services Executive
ICD-O	International Classification of Diseases - Oncology
IGF-1	Insulin-like Growth Factor-1
ISPE	International Society for Pharmacoepidemiology
LOX	Lipoxygenase
LH	Luteinising Hormone
MAPK	Mitogen Activated Protein Kinase
Na^+/K^+ ATPase	sodium/potassium Adenosine Tri-Phosphate-ase
NCRI	National Cancer Registry Ireland
NF-κB	Nuclear Factor Kappa-B
NSAID	Non-Steroidal Anti-Inflammatory Drug
NSCLC	Non-Small Cell Lung Cancer
OR	Odds Ratio
PCRS	Primary Care Reimbursement Services
PDEF	Prostate Derived ETS Factor
PDGF	Platelet Derived Growth Factor
PG	Prostaglandin
PGP	P-Glycoprotein
PIA	Proliferative Inflammatory Atrophy
PIN	Prostatic Intraepithelial Neoplasia
PSA	Prostate Specific Antigen
RR	Relative Risk
ROS	Reactive Oxygen Species
SD	Standard Deviation
STROBE	Strengthening The Reporting of Observational studies in Epidemiology
TGF-β	Transforming Growth Factor-β
TRAIL	Tumour necrosis factor- Related Apoptosis-Inducing Ligand
TRUS	Trans-Rectal Ultrasound
TURP	Trans-Urethral Resection of the Prostate
TX-A_2	Thromboxane A_2
uPA	urokinase Plasminogen Activator
VEGF	Vascular Endothelial Growth Factor

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Chapter 1 INTRODUCTION

This chapter outlines the field of pharmacoepidemiology; its evolution and its contribution to evidence based medicine, particularly in the area of cancer. Prostate cancer is the focus of the thesis; thus the prostate gland, prostatic disease, and the development, classification, and epidemiology of prostate cancer are described. Digoxin and aspirin, the two medicines examined in this thesis are introduced; and finally the overall objectives of the studies in thesis are outlined.

1.1 Pharmacoepidemiology and Its Application in Cancer

Pharmacoepidemiology is the study of the use and effects of drugs in large numbers of people.[1] It combines clinical pharmacology, the study of the effects of drugs in man, with the methods of epidemiology, the study of disease and determinants of disease in a population. The disciplines of epidemiology and pharmacology are described below to illustrate the benefits of combining these scientific approaches. This type of study enables a better understanding of both disease incidence and outcomes and the association between these and medicines usage.

1.1.1 Background to Pharmacoepidemiology

1.1.1.1 Epidemiology

Epidemiology is 'the study of what is upon the people', which comes from the Greek; *"epi"* upon, *"demos"* people, *"logos"* the study.[2] The ancient Greek physician Hippocrates (460-370BC) is the first person known to have examined logical relationships between disease and environmental factors.[3] Epidemiology includes the study of disease occurrence, prevalence, and outcomes as well as the study of the causal associations between environmental or lifestyle exposures and disease. The discipline of epidemiology has evolved in the past 200 years. In the nineteenth century, early epidemiologists such as William Farr, John Snow and Florence Nightingale identified poor sanitation as the cause of many preventable deaths. They gathered and presented meticulous data to the responsible authorities, illustrating what changes were required, and where, in order to stimulate the public health reform for which they are now celebrated.[3] The application of statistics in the field of medicine was driven by these public health objectives.

In cancer epidemiology many aspects of disease development and progression such as racial, genetic, environmental, behavioural and dietary causes are studied.[4,5] The relationship between smoking and lung cancer, first described by Doll and Hill in 1950,[6] was one of the most important causal associations identified in cancer epidemiology. Also in the middle of the twentieth century, another causal association in medicine was emerging, that of birth defects in babies of mothers who had taken the anti-emetic drug thalidomide.[7] This was the trigger for more rigorous regulation of quality, safety and efficacy of medicinal products in Europe.[8] Prior to this medicines could be prescribed and administered without proving their therapeutic efficacy or safety. This thesis is focused on cancer outcomes and specifically prostate cancer which is the most commonly diagnosed non-cutaneous cancer in Irish men.[5]

1.1.1.2 PHARMACOLOGY

Pharmacology is the study of the effects of drugs. Many of these effects are determined through laboratory studies in tissues and animal models. Typically, drugs which have clinical and therapeutic benefits in laboratory studies proceed through the development cycle to clinical trials in humans; this is in order to improve the understanding of their therapeutic benefits, pharmacokinetics and pharmacodynamics (Figure 1-1). Clinical trials, usually double-blind randomised controlled trials, are required for regulatory approval, to prove the safety and efficacy of a medicine for a particular indication.

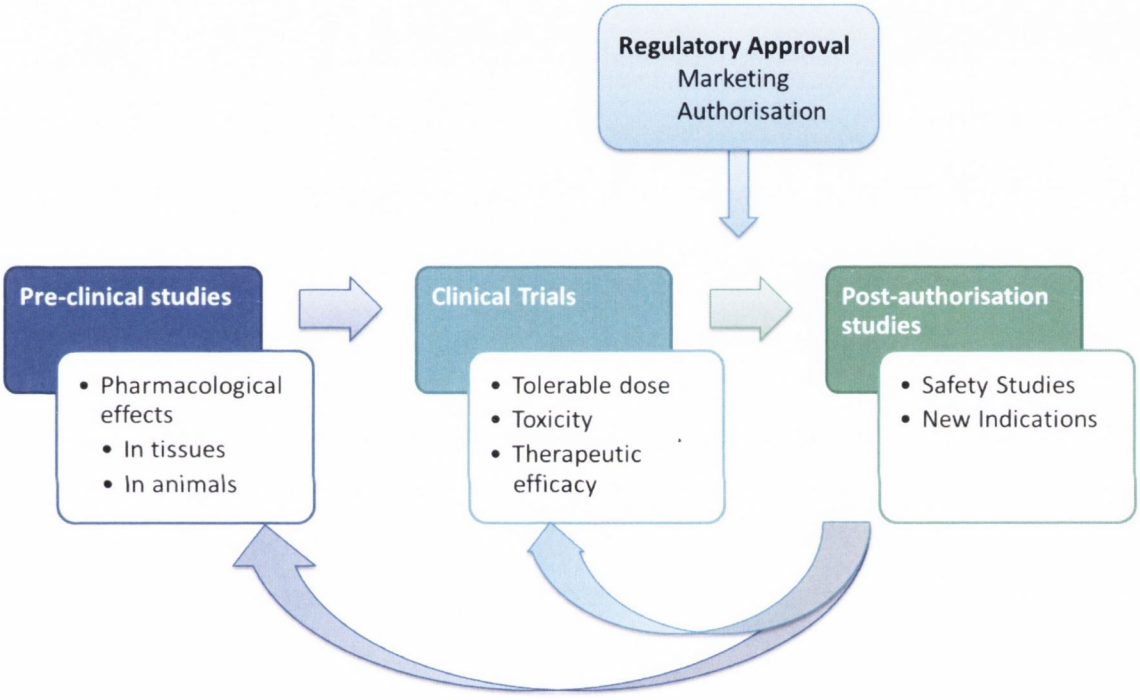

Figure 1-1: Medicinal product life cycle

Randomised controlled trials, first used in evaluating medicines in the 1940s,[9] are considered the gold standard in evidence based medicine; they have however, some limitations. These trials are designed to assess the specific effects of a medicine with the objective of proving its efficacy for a particular indication under ideal conditions. These trials do not have the statistical power to determine all of the effects a medicine may have, such as rare side effects; another limitation is that it is not always feasible to conduct trials in a patient population which is representative of the general population who will receive the medication in practice. Thus possible unintended or off-target effects of medicines, either beneficial or harmful, are not always identified in clinical trials. Drug utilization research, defined by the WHO as "the marketing, distribution, prescription, and use of drugs in a society, with a special emphasis on

the resulting medical, social and economic consequences";[10] must therefore continue after a medicine has received a marketing authorisation.

Post-authorisation surveillance and pharmacoepidemiology studies are essential to learning more about medicines in a large treated patient population. These studies examine the effectiveness of medicines; this is to establish whether, in the usual clinical setting, a drug achieves the intended effect.[1] These studies are of importance in that their findings may prompt further studies or have regulatory implications for a medicinal product (Figure 1-1). These include (i) pre-clinical studies i.e. cardiac glycosides investigated in cancer cells following early observational research; (ii) clinical trials for new indications for a medicine i.e. trials for aspirin as an anti-thrombotic following observations that patients receiving it for pain had higher risk of bleeding; or (iii) regulatory warnings about adverse events i.e. increased risk of bladder cancer associated with pioglitazone exposure.[11]

1.1.2 Evolution of Observational Research

The evidence of Doll and Hill, reporting the association between smoking and lung cancer incidence was refuted by Ronald Fischer, one of the most eminent statisticians at the time. Fischer and others believed that these studies did not have statistical standing, and biases existed in how the findings were presented.[12] Indeed many of these early studies were biased, and would not comply with standards set for observational studies today.[12] The Harvard statistician William Cochran in 1965 described the observational study as an empirical investigation with the objective of elucidating cause-and-effect relationships in settings in which it is not feasible to use controlled experimentation.[13] Experimental intervention involves clinical trials which may not be feasible i.e. investigation of long-term exposures; practical, i.e. the investigation of rare outcomes; or morally justified, when the exposure is hypothesised or known to be harmful i.e. randomising to smoking/non-smoking groups. Therefore, observational studies are used instead.

Following the controversy surrounding the publication of the studies linking smoking to lung cancer incidence, Sir Austin Bradford Hill published a list of causal criteria.[3] This is a list of factors to be considered in determining whether an observed association may be causal or correlative. This list includes: strength of an association; consistency; specificity; temporality; biological gradient; plausibility; coherence; experimental evidence and analogy.[3] This is not an exhaustive list nor is it a requirement that all factors be satisfied before causal association may be proposed, however, the more criteria that apply the stronger the argument for causation rather than merely association. The Bradford-Hill criteria have, for various reasons,

been criticised and other methods of interpreting cause-and-effect relationships have since been proposed, however epidemiologists have not as yet arrived at a definitive set of causal criteria.[3]

Observational studies as a means of answering medical research questions by "natural experiments" have evolved since the 1960s. The data sources used to conduct observational studies have improved significantly, e.g. Clinical Practice Research Datalink (CPRD) in the UK and public and private health insurance databases in North America. Methodological guidelines have also improved the recognition of observational research e.g. Strengthening The Reporting of Observational studies in Epidemiology (STROBE) guidelines.[14] As pharmacoepidemiology studies can form the premise for expensive randomised controlled trials or lead to regulatory changes e.g. new indications or warnings for a medicinal product, they must be carried out using transparent methodology. In 2011 the European Medicines Agency (EMA) established the European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) which aims to promote the quality of Pharmacoepidemiology studies in Europe.[15] The Pharmacoepidemiology group in the Department of Pharmacology and Therapeutics in Trinity is recognised as an ENCePP centre, and one of the studies undertaken in this thesis has been registered with ENCePP.[16] (See Appendix 6)

1.1.3 Pharmacoepidemiology in Cancer

1.1.3.1 The Understanding of Cancer

Cancerous tumours and their vasculature were first described by Hippocrates, as having the appearance of crab, and he named the disease "*karkinos*" which is Greek for crab.[17] Despite centuries of medical study, it is only within the last 50 years that the clinical understanding of cancer has developed to recognise cancer as a universal term, describing uncontrolled cell growth, and encompassing multiple diseases of different aetiology, epidemiology, histology, morphology and genetics. The future of epidemiology, in cancer particularly, is set to be transformed in how it will overlap with other scientific disciplines through combinations of pharmaco-, molecular- and patho-epidemiologic studies.[18]

Cancer is deregulated cell growth and there are specific cellular characteristics which differentiate cancerous cells from other cell types. To aid the understanding of the biology and development of human tumours Hanahan and Weinberg have identified "Hallmarks of Cancer", described as capabilities that enable tumour growth and metastatic dissemination.[19] The hallmarks identified are (i) sustaining proliferative signalling; (ii) evading growth

suppressors; (iii) resisting cell death; (iv) enabling replicative immortality; (v) inducing angiogenesis; (vi) activating invasion and metastasis (vii) deregulating cellular energetics; and (viii) avoiding immune destruction. Two enabling characteristics of these hallmarks have been described, these are tumour-promoting inflammation and genome instability and mutation.[19]

Initial treatments of solid cancers were focused on radical removal of the tumour or irradiation;[20] however, newer, more innovative approaches to treatment focus on interfering with these "hallmarks". In cancer pharmacoepidemiology, the interpretation of findings includes consideration of how the biological and pharmacological mechanisms of the drug exposure examined may modify the tumour development, growth or progression.

1.1.3.2 Potential for Pharmacoepidemiology Research

One of the key determinants of many cancers including prostate cancer is increasing age. Older age is associated with increased comorbidity and prescribed medication. An estimated 72% (95% Confidence Interval (CI) 71-73%) of the Irish population aged 50 years or over takes some medication on a regular basis and 21% (95% CI 20-22%) take more than 5 regular medications.[21] Thus medication exposure is an important factor to consider in older adults, and understanding the associations between prescribed medicines and related and unrelated disease incidence and outcomes is becoming increasingly important.

Associations observed between exposure to medicines and disease incidence and outcomes may also provide information on the molecular pathways involved in the progression of diseases, based on the existing knowledge of the medicine's pharmacological properties. Many medications, including digoxin and aspirin, which have shown potential as anti-cancer agents in pre-clinical studies, have been shown to impede some of the processes identified by Hanahan and Weinberg[19] as being integral to tumour growth and spread. Thus there is a need to understand more about whether these medicines are associated with improved outcomes in population-based studies of cancer patients.

Pharmacoepidemiology studies are, relative to large randomised controlled trials, an inexpensive means of determining the nature of associations between medication exposure and health related states i.e. adverse events, disease incidence or disease outcomes. In many cases, pre-clinical studies will provide the biological and pharmacological rationale for carrying out population-based pharmacoepidemiology studies of the associations between these medicines and cancer risk or outcomes. Additionally, pharmacoepidemiological evidence regarding existing drugs which impede tumour development, or improve patient outcomes

may identify clinically relevant molecular or pharmacological pathways as targets for new cancer therapies.

Pharmacoepidemiological studies, have formed the premise for randomised controlled trials for repositioning of medication commonly prescribed as anti-diabetic or cardiovascular agents in prostate cancer therapy i.e. metformin and simvastatin (US Trial number: NCT01561482), digoxin (NCT01162135). An added benefit of this drug repositioning is that the safety profile of these drugs, as well as tolerable doses in humans, is already understood, unlike new chemical entities, which require significant investment in clinical trials and post-marketing studies. Thus the development costs, and ultimately the cost to the healthcare payer may be reduced by drug repositioning.

1.2 PROSTATE CANCER

1.2.1 THE PROSTATE GLAND

The prostate is a walnut-sized gland of the male genitourinary tract, located below the bladder and in front of the rectum.[4] The urethra and ejaculatory ducts perforate the prostate.[22] See Figure 1-2. The glandular acini within the prostate form a ductal system which discharges into the urethra. The epithelial cells of the acini secrete prostatic fluid, which is a component of seminal fluid.[4] Prostate Specific Antigen (PSA), a serine protease, is also secreted from the glandular acini, and its function is to cleave proteins in semen to maintain the fluidity of seminal fluid.[4]

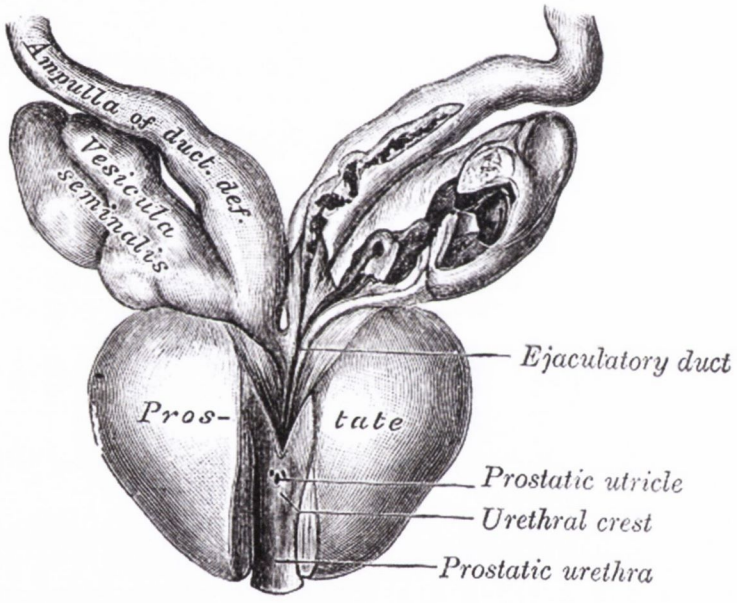

Figure 1-2: Image of the prostate, seminal vesicles and urethra; taken from Gray[22]

The growth, development and function of the prostate are controlled by the androgen testosterone.[23] Approximately 90% of testosterone is produced in the testes and the remainder in the adrenal glands.[17] Testosterone is produced in response to gonadotropin releasing hormone (GnRH) produced in the hypothalamus, stimulating the release of follicle stimulating hormone (FSH) and luteinising hormone (LH) from the pituitary, which regulate the Leydig cells of the testes.[24]

Within the prostate, testosterone is converted to di-hydrotestosterone (DHT) by the enzyme 5α-reductase. DHT binds with the androgen receptors (AR) in the nucleus of prostate cells.[23] Growth factors, such as fibroblast growth factor (FGF), transforming growth factor-β (TGF-β),

epidermal growth factor (EGF) and insulin-like growth factors (IGF) are involved in prostatic development;[23] Several of these are also implicated in prostate cancer development (TGF-β, EGF, IGF).[25]

1.2.2 Benign Conditions of the Prostate

A number of benign conditions of the prostate may precede the development of prostate cancer; or increase the likelihood of prostate cancer being diagnosed. Many of these involve inflammation. Inflammation has been described as an enabling characteristic in tumour growth and development, as it mobilises many mediators to the tissue including growth factors, survival factors, pro-angiogenic factors, and inductive signals.[19] There is therefore a hypothesis that anti-inflammatory agents may have chemo-preventative as well as therapeutic potential in prostate cancer.[26]

1.2.2.1 Benign Prostatic Hyperplasia

Benign Prostatic Hyperplasia (BPH) is a non-cancerous enlargement of the prostate, consisting of excess glands and stroma.[27] It usually presents in the transitional zone of the prostate, close to the urethra.[28] See Figure 1-2. Therefore BPH is associated with symptoms of urinary incontinence, frequency or urgency developing gradually over a period of years.[29] Increased numbers of chronic inflammatory cells are detectable in BPH tissue.[27] Treatments indicated for BPH include the 5α-reductase inhibitors (finasteride, dutasteride), which impede prostate growth and reduce prostate volume;[28] and α-adrenoceptor antagonists (i.e. alfuzosin, tamulosin) which improve urinary flow-rate.[30] Trans-urethral resection of the prostate (TURP) may also be carried out to remove obstructive tissue. While BPH is not understood to be a precursor to prostate cancer, the presence or treatment of BPH (TURP) may increase the detection of prostate cancer.[31,32]

1.2.2.2 Prostatitis

Prostatitis is an inflammatory condition of the prostate, often due to infection. It is not thought to be a direct cause of prostate cancer. Although there are associations between chronic prostatitis and prostate cancer,[33] it is difficult to quantify the association as the incidence of prostatitis is uncertain as the condition is often asymptomatic and its rate of incidence is uncertain.[27] Furthermore men with diagnosed prostatitis are more likely to be followed up for prostate assessment and associations with prostate cancer could potentially be due to detection bias.[34] Nevertheless, chronic inflammation and the presence of inflammatory cells and mediators such as in prostatitis may precipitate cancerous states.[27]

1.2.2.3 Proliferative Atrophy

Proliferative atrophy and proliferative inflammatory atrophy, (PIA) are characterised by lesions with greater proliferation than normal.[4] These lesions may be caused by infection, hypoxia or auto-immunity.[4] This proliferation may be indicative of genomic damage or genomic instability;[27] and PIA may indicate a microenvironment conducive to carcinogenesis.[4] Increased expression of cyclooxygenase-2 (COX-2) enzyme, which is induced in response to inflammation, has been reported in PIA.[35] Transitional areas of atrophic epithelium and adenocarcinoma have been observed.[27]

1.2.2.4 Prostatic Intraepithelial Neoplasia

Prostatic Intraepithelial Neoplasia (PIN) occurs when the acini become lined with malignant cells,[4] without invasion of the stroma.[36] High-grade PIN has been reported to precede prostate cancer in most cases by about a decade.[37] The epidemiology, morphology and genetic characteristics of high-grade PIN are similar to prostate cancer. PIN is recognised as a pre-cancerous state in animal models of prostate cancer.[36] PIN is androgen dependent and androgen deprivation will cause regression of PIN, however the associated adverse effects are too severe for this to be indicated as treatment. Currently PIN is not treated.[36]

1.2.3 Prostate Tumours

Prostate cancers are almost exclusively (>95%) adenocarcinomas of the glandular acini.[29,38] The remainder of cancers are comprised of transitional cell carcinomas, squamous cell carcinomas, and sarcomas.[38] Atypical hyperplasia especially PIN frequently occur with adenocarcinoma.[27,36] Unusually for solid tumours, prostate tumours develop from a number of foci; it is not understood whether this may be due to migration of the tumour cells through the ductal system within the prostate.[4]

1.2.3.1 Tumour Detection

A digital rectal examination (DRE), may be carried out by palpitation of the prostate gland through the rectum, to detect any abnormality in the size or shape of the gland.[17] Prostate cancer typically (70%) is located in the peripheral zone (lobes) of the prostate,[17] whereas BPH is usually in the transitional zone, close to the urethra.[28] While BPH is frequently associated with urinary symptoms, a prostate tumour can have advanced within or beyond the prostate gland before such symptoms are noticed.[29] In prostate cancer patients, these symptoms have a more rapid onset and may be accompanied by haematospermia.[29]

The concentration of PSA in the serum is normally very low 0.2-4.0ng/ml, as PSA is usually confined within the prostate.[28] When BPH, or prostate cancer disrupt the integrity of the glandular acini, PSA leaks into the serum; thus elevated levels of serum PSA are correlated with prostate disease.[28] PSA reference ranges vary according to age and race (Caucasian/African)[39] however PSA has been extensively used as a marker in the detection of prostate cancer.[17,28] Other factors such as ejaculation, prostatic massage, prostatitis, trans-rectal ultrasound (TRUS), and TURP may also elevate PSA levels,[28] and medication including finasteride and dutasteride may reduce PSA levels.[39] Therefore, PSA is not a very specific test for prostate cancer. However, the significant increase in detection of prostate cancers over the past two decades has been attributed to the extensive use of the PSA test in men without symptoms, especially in more developed countries.[40]

Current guidelines issued to General Practitioners (GPs) in Ireland state that "PSA testing of asymptomatic men or PSA screening is not national policy".[39] Where asymptomatic men request an examination, PSA testing should only be carried out after full advice and provision of information regarding the potential implications of a positive result and prostate assessment should consist of a DRE and a PSA test.[39] Men aged 50-70 years (or 40-70 years if of African ethnicity or with a first degree relative with prostate cancer) at increased risk of prostate cancer presenting with urinary tract symptoms or unexplained back pain are recommended to have a full assessment (PSA, DRE, urinalysis, creatinine and haemoglobin).[39] All men with an abnormal DRE should be referred to a urologist.[39] In the case of an abnormal DRE, or elevated serum PSA, a prostate biopsy, carried out guided by TRUS, is indicated for a definitive diagnosis of prostate cancer, and patients should be informed about the risks of prostate biopsy prior to prostate assessment.[17,28,39]

1.2.3.2 Staging of Prostate Cancer

The degree of differentiation of glands within the prostate tissue cores (at least 10)[28] sampled at biopsy is classified according to the Gleason scoring system.[41] Each core is assigned a grade 1-5 according to the degree of differentiation or non-uniformity of the glands in the tissue; the two scores which are most prevalent are summed.[28] The Gleason score may range from 2-10. Gleason Score is critical in treatment decision making as it is the most significant predictor of prostate cancer mortality. However, men with prostate cancer of Gleason score ≤6 are generally at low risk of death from prostate cancer.[42]

The Tumour, Node, Metastases (TNM) method of tumour staging was established by Denois in 1941[17] and adopted by the American Joint Committee on Cancer (AJCC) in 1975.[28] The current

staging system for prostate cancer incorporates the TNM stage, Gleason score and serum PSA at diagnosis;[43] the National Cancer Registry Ireland (NCRI) use this staging system in tumour records.

Table 1-1 AJCC prostate cancer staging

Tumour Stage	Tumour	Node	Metastasis	PSA (ng/ml)	Gleason Score
I	T1a-T2a	Negative	Negative	<10	≤6
II*	T1a-T2c	Negative	Negative	≥10<20	≤7
III	T3	Negative	Negative	Any	Any
IV	T4	Positive	Positive	Any	Any

*Stage IIB if T2c tumour, or PSA>20 and T1-2 tumour, or Gleason score ≥8 and T1-2 tumour

1.2.3.3 Tumour Growth and Progression

The uncontrolled growth of the tumour can cause disruption to the vasculature in the prostate and hence interfere with the supply of oxygen and nutrients. In order to survive the tumour must adapt; this is achieved through inducing angiogenesis as well as reprogramming energy metabolism.[19] Hypoxia triggers a number of cellular effects including the stabilisation of hypoxia-inducible factor 1-alpha (HIF-1α) which, following dimerization with HIF-1β, forms the transcription factor HIF-1.[44] This stimulates the expression of proteins involved in angiogenesis, such as vascular endothelial growth factor (VEGF) and the glucose transporter (GLUT 1, GLUT 2), as well as enzymes such as hexokinase (HK1, HK2) which enable the cell to adapt its energy needs with reduced oxygen.[44] HIF-1α is not expressed in normal prostate cells, but up-regulation of HIF-1α occurs in pre-neoplastic lesions and prostate carcinogenesis.[45] The cardiac glycoside digoxin has been identified, in high-throughput screening, as an inhibitor of HIF-1α expression. Pre-clinical studies have investigated prostate cancer cell lines and prostate cancer mouse models treated with digoxin and reported reduced tumour growth and dissemination.[46]

1.2.3.4 Tumour Dissemination

Adenocarcinoma of the prostate extends locally to the seminal vesicles and the base of the bladder; it also disseminates through the lymphatic system and vasculature.[17] Within the vasculature, circulating tumour cells cause activation of platelets and elevation of patients' platelet count in many types of cancer.[47] Activation of platelets, leads to P-selectin and glycoprotein activation on the platelet surface; platelets can then adhere to cancer cells and "cloak" the tumour cells as they move through the vasculature.[48] The cancer cells, through

this mechanism, have the ability to avoid immune destruction.[49] Platelet activation also induces the release of pro-angiogenic and tumour promoting substances including thromboxane-A_2 (TX-A_2) and serotonin; and growth factors such as platelet derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor β (TGF-β), IGF-I and VEGF.[47] The anti-platelet mechanism of aspirin therefore has been proposed as an anti-cancer mechanism.[47]

The majority (90%) of prostate cancer metastases are to the bone (lumbar spine or pelvis); other metastatic sites include the lungs, liver, adrenal glands, testes and breast.[17] Growth factors and cytokines implicated in the formation of osteoblastic lesions include bone morphogenic proteins, endothelien-1, urokinase plasminogen activator (uPA), IGF, and TGF-β. Prostaglandin-E_2 (PG- E_2), a product of the COX-2 pathway also plays a role in bone formation, bone repair and may play a role in the progression of bone metastases.[50]

1.2.4 Risk Factors for Prostate Cancer

The associations between prostate cancer, host factors, environmental factors and lifestyle exposures are not as strong as for some other cancers i.e. the associations between alcohol consumption or cigarette smoking and prostate cancer risk are not particularly strong.[17] Studies have examined many potential risk factors as well as chemo-preventative agents, and the following is not an exhaustive discussion of factors associated with prostate cancer risk. Platz and Giovannuci provide a thorough review of this topic.[4]

1.2.4.1 Familial and Racial Factors

The host factors which have been conclusively identified as risk factors for prostate cancer are older age, Afro-Caribbean ethnicity, and a family history of the disease.[4,17] A man has a 2-3 fold increased relative risk of prostate cancer if one first degree relative (brother/father) has had prostate cancer, and a 3-5 fold increased relative risk of the disease if more than one first degree relative has a history of prostate cancer.[4] Familial prostate cancer is thought to account for 5-10% of cases and these cases are more likely to be diagnosed in younger men.[51] There is a genetic association, and associations have been made between prostate cancer and breast cancer incidence in families who carry the mutated BRCA gene.[52] Men with Lynch Syndrome are pre-disposed to colorectal cancer and have recently been reported to also have a 2-fold increased risk of prostate cancer.[53]

1.2.4.2 Hormones

Hormonal factors, especially those relating to the sex-hormones, have also been associated with increased risk of prostate cancer. Higher circulating levels of testosterone and its metabolites, have been positively associated with prostate cancer, whereas oestradiol and sex-hormone binding globulin have been inversely associated with the disease.[4]

Oestrogens appear to have a conflicting role in prostate cancer development as high doses of oestrogens have been reported to result in the development of inflammation, hyperplasia, and dysplasia or PIN through the oestrogen receptor-alpha (ERα).[27] Some of the ethnic differences in prostate cancer risk may be partially explained by hormonal factors i.e. polymorphisms in the Cytochrome P (CYP)-450 enzymes which metabolise the sex steroid hormones can vary by race.[4]

1.2.4.3 Diet and Nutrition

Stresses throughout life may also have a role in the development of prostate cancer. Some studies have reported associations between reduced prostate cancer risk and consumption of foods containing lycopene (found in cooked tomatoes), glucosinolates (found in brassicas), carotenoids (orange-yellow vegetables); as well as dietary supplementation with selenium, zinc and vitamins A, C, E and D.[4] The consumption of soy products, containing isoflavones, has been inversely associated with prostate cancer, and the high consumption of soy products in Asian countries, may contribute to the lower incidence of prostate cancer in these regions.[4]

Associations have been made between the high incidence of prostate cancer in western and developed countries and high caloric diets as well as diets high in saturated fatty acids.[28] Obesity has been correlated with the development of higher-grade cancers.[4] It has been proposed that dietary fat may alter serum androgen levels, cause oxidative stress or increase IGF levels.[28] Fatty acids are a diverse family of substances; some have been associated with prevention of prostate cancer while others have been associated with increased incidence of and mortality from prostate cancer.[28] In particular linoleic acid and di-homo-gama linolenic acid (DHGLA) derived from dietary fatty acids and arachadonic acid from cell membrane phospholipids may be of importance, as they are substrates of the COX-1, COX-2 and lipoxygenase (LOX) enzymes. The products of these enzymes are the leukotrienes, prostaglandins and thromboxanes, which are implicated in inflammation, and may be involved in the development of PIN and its progression to prostate cancer.[28] See Figure 1-3.

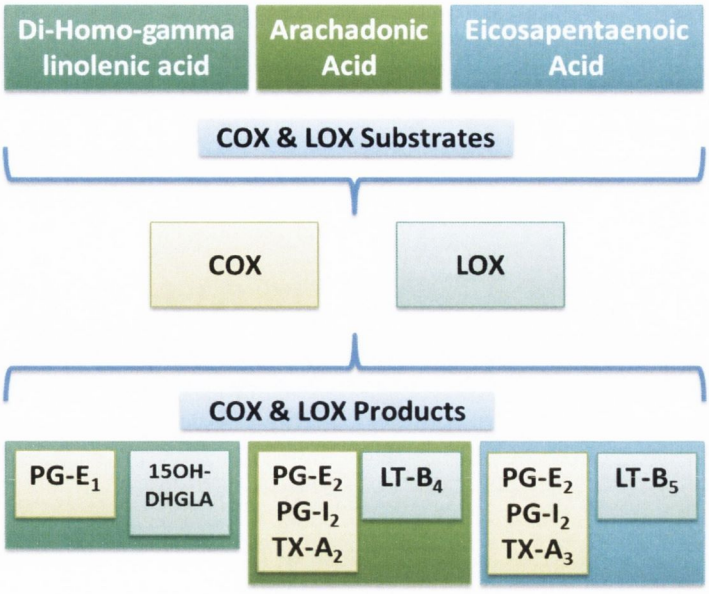

Figure 1-3: Schematic of some COX and LOX substrates and products which may have a role in prostate cancer progression[28]

Oxidative stress, which leads to the production of reactive oxygen species (ROS), may also play a role in the development of prostate cancer from PIA or high-grade PIN.[54] ROS is produced as a by-product of prostaglandin biosynthesis by COX. Foods rich in anti-oxidant properties (vitamin E, lycopene, selenium and isoflavones) have been associated with a reduced risk of prostate cancer.[28,54] However, it has also been proposed that anti-oxidants and other free radical scavengers may prevent cancer cell apoptosis triggered by ROS,[55] which suggests that anti-oxidants may not be as beneficial as some studies have suggested.

1.2.4.4 Infection

A history of sexually transmitted infections[56] or prostatitis[33] (often caused by bacterial infection) may be associated with prostate cancer incidence; although studies examining these risk factors may be subject to recall bias. Serological evidence of *Trichomonas vaginalis* infection, a sexually transmitted asymptomatic bacterial infection which spreads to the prostate has been associated with prostate cancer incidence,[57] more advanced prostate cancer and poorer outcomes.[58] The immune response to infection i.e. the secretion of inflammatory cytokines and other markers may contribute to the aetiology of prostate cancer.[4,27] Interestingly in one of the studies examining a history of *Trichomonas vaginalis*, this increased risk of prostate cancer was not observed in regular users of aspirin; whereas infrequent or never use of aspirin (over the participant's life-time) was associated in a significantly increased risk (OR=2.05, 95% CI 1.05, 4.02). This may suggest aspirin has a role in mediating the inflammation associated with this infection.[57]

1.2.4.5 Environmental Factors

Environmental stresses have been associated with prostate cancer risk. These include occupational exposures to pesticides, electromagnetic fields and cadmium.[4] It has been hypothesised that these stresses lead to the production of inflammatory cytokines, such as Interleukin-6, which is a prostate cancer growth factor; the downstream effects of this inflammatory response may lead to the development and progression of prostate cancer.

1.2.4.6 Medication Exposures

A number of medicines have been investigated for their association with prostate cancer incidence. The testosterone 5α-reductase inhibitor finasteride, used to treat BPH, has been associated with a reduction in prostate cancer incidence in observational studies,[59] and compared to placebo in a randomised trial.[60] However, cancers diagnosed in the finasteride arm were more frequently of Gleason score >7, and thus these patients had a poorer prognosis.[60]

Long-term exposure to digoxin[61] and aspirin[62] has been associated with reduced incidence of prostate cancer in observational studies and meta-analyses of randomised controlled trials. These will be discussed in later chapters (Chapter 3, Section 3.2.2 and Chapter 4, Section 4.2.1 respectively). A number of other medicines for cardiovascular indications have been associated with reduced risk of prostate cancer. The cholesterol-lowering statins have been reported to be associated with reduced incidence of prostate cancer in meta-analyses of observational studies.[63] The anti-thrombotic warfarin has also been found to be associated with reduced incidence of prostate cancer in observational studies.[64,65] Biological mechanisms have been proposed through which angiotensin II may have a role in proliferation, angiogenesis and inflammation.[66] However, the anti-hypertensive medicines which act on the angiotensin system, angiotensin II receptor antagonists and angiotensin converting enzyme (ACE) inhibitors, have been associated with a slightly increased risk of prostate cancer.[67] Beta-blocker use of four years or more has been associated with reduced risk of prostate cancer.[68] Adrenalin-signalling has been associated with tumour progression, which can be blocked by beta-blockers.[69]

Due to their anti-inflammatory activity, non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin have been hypothesised to reduce the risk of prostate cancer. In studies which have examined the association between use of these medicines and prostate cancer incidence, aspirin, but not non-aspirin NSAIDs, was found to be associated with reduced risk of prostate cancer.[70,71] However other studies have reported NSAIDs or COX-2 inhibitors to be

associated with reduced risk of prostate cancer.[72] The NSAIDs and their association with prostate cancer are discussed further in Chapter 4, Section 4.5.

Given the role of IGF in prostate development and the progression of prostate cancer, anti-diabetic agents have been examined in relation to prostate cancer risk. Patients with type 2 diabetes mellitus, and who have lower levels of circulating insulin are at reduced risk of prostate cancer. Type 2 diabetes mellitus patients also have lower testosterone levels.[73,74] By contrast, men with diabetes who are diagnosed with prostate cancer have poorer outcomes.[74] Exposure to the hypoglycaemic agent metformin, which does not increase insulin levels, has not been associated with a significant reduction in prostate cancer incidence;[75] however it can reduce the growth of prostate tumours and is being investigated in a clinical study as a potential treatment for prostate cancer (NCT01561482).

1.2.5 Challenges of Prostate Cancer Research

Some unique characteristics of prostate cancer, described by Reid and Hamdy,[51] make this disease a challenge for researchers and clinicians alike. The slow growing nature of the disease makes the detection of prostate cancer difficult; the multifocal nature of cancerous lesions in the prostate also makes it difficult to assess on biopsy. The age-related aetiology of prostate cancer means that the environmental factors or accumulation of exposures which may lead carcinogenesis are poorly understood; also treatment decisions are affected by the age at which patients are diagnosed and their other health conditions as will be discussed in Section 1.4. Finally prostate cancer has the highest prevalence of any non-cutaneous human cancer in men; this makes it a continuing public health issue.

1.3 The Burden of Prostate Cancer

International data from GLOBOCAN 2008[76] was used for comparison of prostate cancer incidence and mortality in Ireland to that globally. The GLOBOCAN is a project co-ordinated by the International Agency for Research on Cancer, to provide estimates of cancer incidence and mortality for all cancers globally.[76] The data from cancer registries is weighted according to the population it covers and used to generate population-weighted average estimates of incidence rates, mortality rates and disability adjusted life years for major cancer types in 184 different countries. Corrections were applied to prostate and breast cancer incidence rates to account for screening-related increases in incidence rates. The methodology used is described in more detail on the GLOBOCAN website.[76] There is considerable variation in the incidence rates of prostate cancer globally, varying 25-fold between the more developed countries, and those of Asia and North Africa, whereas prostate cancer mortality rates vary 10-fold globally.[76]

1.3.1 Global and European Prostate Cancer Incidence and Mortality

In males the incidence of prostate cancer is second to lung cancer; the Age-Standardised Rate (ASR, world standard population) is 27.9 cases per 100,000 person years. Higher incidence rate estimates of prostate cancer were observed in Europe and more affluent and industrialised regions such as North America, Europe and Australia.[76] Prostate cancer incidence is, in fact, correlated with Gross National Product.[4] Prostate cancer incidence rates globally reflect the influence of race on prostate cancer risk i.e. high incidence in Caribbean nations and lower incidence in Asian countries.[4] In Europe (WHO European region, 40 countries) prostate cancer accounted for 21.8% of all cancer cases diagnosed and is the most commonly diagnosed cancer in men, ASR=59.3 cases per 100,000 person years. PSA testing practices have contributed significantly to the increase in prostate cancer incidence and the variation in prostate cancer incidence rates globally.[77] The increase in use of the TURP procedure to treat BPH has also been attributed to the increased detection of prostate cancer.[32]

Globally, prostate cancer has the sixth highest cancer mortality rate in men. In Europe, the mortality rate of prostate cancer, 12 deaths per 100,000 person years, accounts for 9.4% of cancer deaths in men, making it the third most common cause of cancer death in European men. Comparison of incidence and mortality rates reveal that high-resource countries with high incidence rates of prostate cancer don't have high prostate cancer mortality rates.[77] This is potentially because of the increased detection of many non-aggressive tumours in some

western countries, due to PSA testing of asymptomatic men. The highest mortality rates are in the Caribbean and West African countries.[76] In these poorer resource regions, prostate cancer incidence rates are also high and the population is at high risk of the disease.[77]

The Nordic countries of Norway, Sweden and Iceland are the only European countries to have both high incidence and mortality rates. However mortality rates from prostate cancer in these countries have begun to decline significantly.[40] Bray *et al.* reported a strong correlation between incidence rates of prostate cancer from 1986-1990 and the mortality rates in 1995. The statistics from the following decade however had a much poorer correlation.[40] This inflated incidence of prostate cancer is attributed to the over-detection of indolent tumours, many of which are detected through PSA testing.[40]

1.3.2 Prostate Cancer in Ireland

The Republic of Ireland had the highest prostate cancer incidence rate estimate in Europe in 2008,[76] ASR=126.3 cases per 100,000 person years, and the third highest incidence rate estimate worldwide. The rise in prostate cancer incidence in Ireland has been particularly noteworthy,[78] and similar to other developed countries, has been attributed to the wide-spread use of PSA testing and subsequent prostate biopsy.[79] The crude incidence rate of prostate cancer in the Irish population has increased by 4% annually from 1994-1999; with the sharpest rise from 1998-2004.[78] A small decrease in prostate cancer incidence has been observed in recent years.

On the island of Ireland, the age-adjusted risk of prostate cancer (1994-2007) has been determined to be substantially (29%) lower in Northern Ireland compared to the Republic of Ireland.[5] PSA testing is far less prevalent in Northern Ireland, and this is thought to explain the differences between the incidence rates in the two jurisdictions. Figure 1-4 illustrates these regional variations in a cancer map. The more affluent areas of south county Dublin have a much higher relative risk than those areas in the centre or north of the city. This corresponds with income-related differences observed in uptake of PSA testing with higher uptake amongst more affluent men.[80]

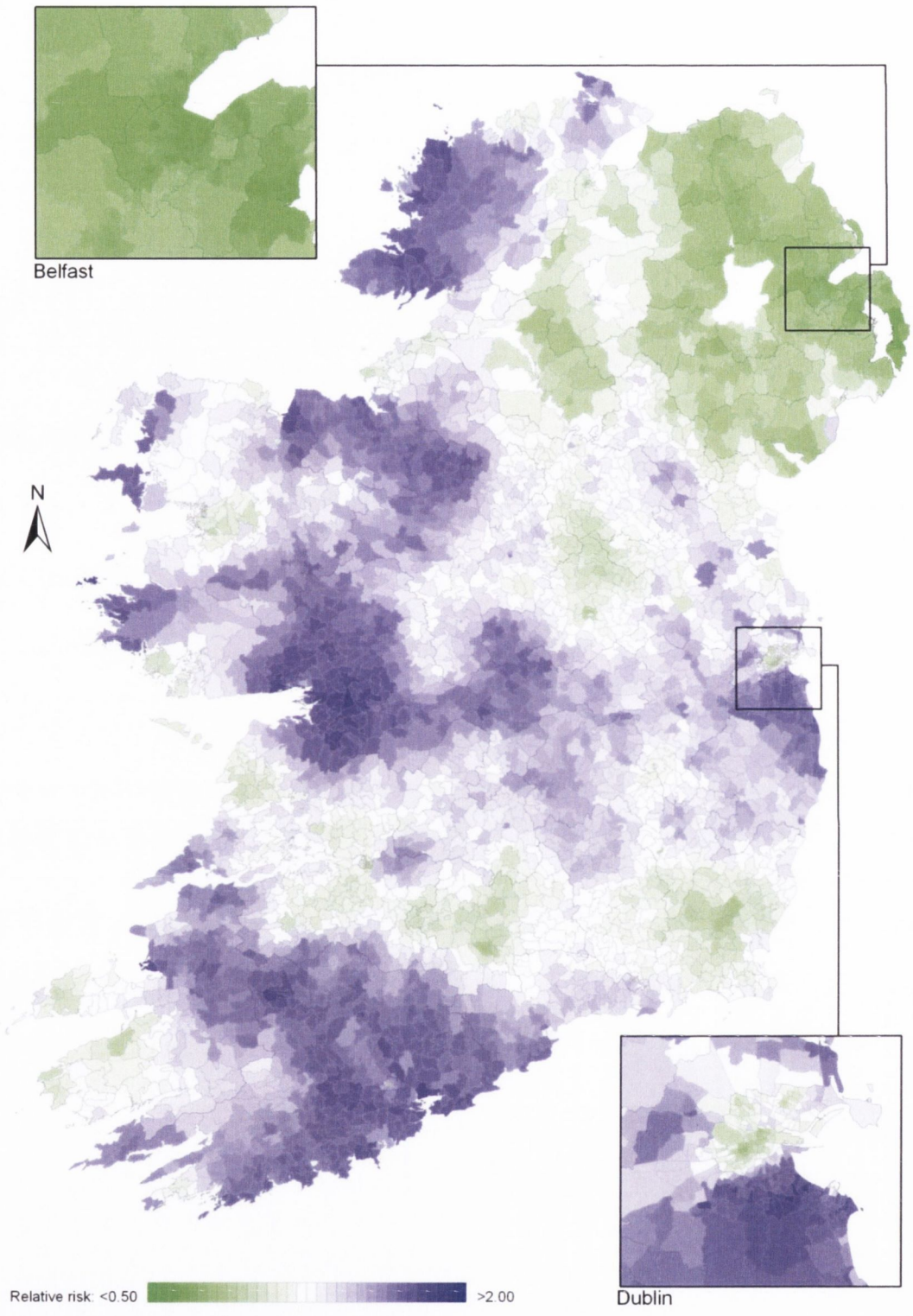

Figure 1-4: Smoothed relative risk of prostate cancer on the island of Ireland for years 1995-2007

(Reproduced with permission; All-Ireland Cancer Atlas)[5]

Population-based PSA testing has been associated with a downward migration in prostate cancer stage and grade at diagnosis in a British study.[81] This is similar to that observed in the Republic of Ireland where the increase in prostate cancer incidence has been driven by the increased detection of smaller tumours (T1, T2).[78] The majority of T1 cases are T1c, identified by needle biopsy, usually as a result of an elevated PSA.[78] The incidence of T3 and T4 tumours has not changed significantly.[78] The age at diagnosis is younger in the Republic of Ireland compared to Northern Ireland (median age 71 years, and 73 years respectively).[82]

In Ireland prostate cancer mortality began to decline in 1997.[40] Despite the prostate cancer incidence rate in Ireland being the highest in Europe in 2008, the age-standardised mortality rate estimate, 12.98 deaths per 100,000 person years, ranks sixteenth, which is close to the European average.[76] By the end of 2008, over 17,000 men in Ireland were living up to fifteen years after their prostate cancer diagnosis.[5] The prevalence of prostate cancer is likely to increase in the future as the population ages.

1.3.3 PSA Testing and Prostate Cancer Incidence in Ireland

PSA testing has been widely acknowledged as the driving factor behind the observed increase in prostate cancer incidence both nationally and internationally.[77,79] There is no organised population-based prostate cancer screening programme in Ireland, or any other country.[83] In 2006, The National Cancer Forum (the then national advisory body to the Minister for Health on cancer policy) advised against population-based prostate cancer screening.[84] Subsequent meta-analyses have failed to show the benefits of screening programmes on reducing mortality from prostate cancer.[85,86] Drummond *et al.* reported 78% of baseline PSA tests carried out in Ireland (1994-2005) were in men aged less than 70 years, with 26% of all tests in men aged under 50 years.[79] There were no Irish guidelines in place, however those in the UK and US clearly advised against PSA testing in men under 50 years and over 70 years[87] and 75 years[88] respectively. However PSA testing is often included in occupational health checks carried out by GPs,[89] and more men in a survey study by Hevey *et al.* responded that they would have a PSA test following their doctor's recommendation than would initiate undergoing a PSA test themselves.[90] A survey of Irish GPs found them to broadly support PSA testing and their knowledge of this area was based on personal clinical experience rather than the evidence base regarding the test.[89] National guidelines[39] as discussed above (Section 1.2.3.1) have been introduced since the studies by Drummond *et al.*[79,89] were carried out.

The factors that influence PSA testing in the Irish population were investigated and a paper investigating the associations between prostate specific antigen testing and men's healthcare utilisation and their physical, mental and emotional health, was prepared by Flahavan, Drummond *et al.* (Appendix 3). This cross-sectional study was carried out in The Irish Longitudinal Study on Ageing (TILDA) cohort. TILDA is a study investigating the health, lifestyle and financial situation of a population-representative sample of over 8,000 people aged 50 years and older living in the community.[91] This study found that men eligible for the state-funded General Medical Services (GMS) scheme were less likely to have ever had a PSA test. Eligibility for the GMS scheme is based on age and an income threshold. This is consistent with the socioeconomic differences observed in prostate cancer incidence in Ireland.[5] Higher educational status was also associated with increased likelihood of PSA testing. This corresponds with findings regarding prostate cancer incidence in the All-Ireland cancer atlas, where areas with the lowest proportion of the population educated to third level have a 17% reduced relative risk of prostate cancer than those areas where the highest proportion of the population have degree level education.[5]

1.3.3.1 Uses of the PSA Test

Despite the poor sensitivity and specificity of the PSA test in tumour detection, PSA is used as a prognostic marker in prostate cancer staging, risk-stratification, and as a measure of treatment success.[92] Various age- and race-related PSA normal ranges have been identified and several PSA-indices have been studied. PSA velocity, the change in total PSA over time, may be used to differentiate between patients and their risk factors. Men with BPH can be expected to have a linear PSA velocity, while those with prostate cancer will eventually have an exponential PSA velocity.[51] PSA doubling time, the time required for the PSA to double in value, is used as a surrogate for rapid tumour growth. It is also used to monitor disease recurrence and progression following primary treatment.[51] In patients who have metastatic disease, high baseline PSA, high PSA nadir and shorter PSA doubling time are predictors of poorer overall and progression free-survival.[93]

1.4 Prostate Cancer Treatment

Treatment for prostate cancer depends on the prognosis of the disease and the life expectancy of the patient. For almost every stage of disease there are multiple treatment options, however there is no definitive optimum therapy.[83] In the treatment decision-making process, the potential side-effects of various treatments are a strong consideration.

1.4.1 Localised Prostate Cancer

Stratification of patients into risk categories (low, intermediate, high) according to tumour characteristics is used in decision making regarding treatment. The risk category stratifications defined by D'Amico *et al.*[94] and used by the European Association of Urology (EAU)[92] are presented in Table 1-2.

Table 1-2: Risk stratification of prostate cancer patients[92,94]

Risk Category	AJCC Tumour size*	Gleason Score	PSA (ng/ml)
Low	T1c-T2a	2-6	<10
Intermediate	T2b	7	10-20
High	>T2c	8-10	>20

*EAU specify clinical staging of tumour size

1.4.1.1 Conservative Management

In the treatment of many cancers i.e. breast cancer, surgical excision of the tumour, radiation treatment, chemotherapy or hormonal therapy is regularly used as first line treatment. Many prostate cancers however, are slow-growing and a more conservative approach to treatment may be appropriate. Conservative management is often used in low-risk localised prostate cancer.[83] Conservative management may fall under two categories, watchful waiting or active surveillance.[28]

Watchful waiting is the decision not to treat the tumour with curative intent. However patients may receive palliative treatment such as androgen deprivation therapy (ADT) if and when required.[28,83] This is considered suitable for older men with shorter life expectancy, because the overall survival benefit to be gained from surgical or pharmacological intervention may not outweigh the consequences of potential side-effects of treatment.[95] The objective of watchful waiting is to minimise both disease and treatment-associated morbidity.

Active surveillance is a suitable treatment option for men with low-risk localised prostate cancer and a life expectancy of at least 10 years.[92] Initially the tumour is not treated

aggressively; the patient and their tumour are reassessed regularly by PSA test and biopsy. The decision to commence treatment with curative intent (i.e. surgery or radiation) will be made when disease is deemed to have progressed i.e. PSA or Gleason score progression.[28,92]

1.4.1.2 Surgery

Prostatectomy may be carried out by a variety of methods such as open radical retropubic prostatectomy, perineal prostatectomy or robotic or laparoscopic surgery.[28] Prostatectomy is considered suitable in men with tumours confined to the prostate gland which can be removed simply; pelvic lymph nodes may also be removed.[83] Depending on tumour extent men may experience side effects such as impotence and incontinence due to damage to nerves and vasculature during the procedure. It is generally not the therapy of choice in men with less than 10 years of life-expectancy.[92] Another localised treatment for low-risk prostate cancer is cryotherapy, where the cancerous tissue is frozen, however destruction of other tissue may result in more complicated side-effects.[17] Following radical prostatectomy, men should not have detectable PSA, and a serum PSA (> 0.2ng/ml) defines biochemical failure.[96]

1.4.1.3 Radiation

The use of radiation treatment of prostate cancer has increased over the past number of years in Ireland.[78] External beam radiation therapy may be used to treat the prostate exclusively, or additionally the seminal vesicles, and/or the entire pelvis; depending on the clinical extent of the disease, the Gleason score and PSA level.[17] Intensity modulated radiation therapy may be used also for the treatment of pelvic nodes, and is often used in the treatment of high-risk disease.[28] External beam radiation therapy may be used in combination with ADT for the treatment of intermediate-risk or high-risk disease.[92] Brachytherapy is a procedure where radioactive seeds are implanted into the prostate using ultrasound guidance. The seeds emit low-dose radiation for a period of weeks/months and thus kill cancerous cells. Brachytherapy is suitable as monotherapy in patients with low-risk disease,[92] or in combination with external beam radiation therapy in men with intermediate-risk disease.[28] Radiation may be indicated following surgery,[28] or if PSA recurrence (>0.2ng/ml but <0.5ng/ml) is detected (salvage radiation).[96] Similar to surgical treatment the success of radiation treatment is measured by the post-treatment PSA, or PSA nadir. Biochemical failure, in patients who receive radiation, is characterised by a rise in PSA nadir (>2ng/ml).[96]

The specific toxicity associated with radiation treatment depends on the treatment type; intensity modulated radiation therapy can be more localised than whole pelvic external beam

radiation, thus some side effects may be minimised.[28] The main side effects are those of the genitourinary and gastrointestinal tracts; the latter include rectal bleeding.[28]

1.4.2 Advanced Prostate Cancer

1.4.2.1 Androgen Deprivation Therapy

As the growth and development of the prostate is controlled by testosterone, the majority of prostate tumours are androgen dependent, and therefore sensitive to the withdrawal of circulating androgens i.e. androgen deprivation therapy (ADT). This may be done surgically (bilateral orchiectomy), but medical ADT is more common.[38] ADT as monotherapy is usually indicated in locally advanced and metastatic disease; in addition to this radiation may be indicated in the treatment of locally advanced disease.[96]

The classes of drugs used in ADT are GnRH analogues (i.e. buserelin, goserelin, leuprorelin); anti-androgens (i.e. bicalutamide, flutamide) and the GnRH antagonist (degarelix).[30] GnRH analogues, and GnRH antagonists act similarly, the former down-regulate the GnRH receptor in the pituitary, the latter antagonise the receptor; resulting in inhibition of the secretion of LH and FSH from the pituitary, which suppresses testicular production of testosterone.[24] These are usually administered by depot injections. Initially an increase in testosterone production (androgen flare) may occur in men receiving treatment with GnRH analogues; therefore anti-androgens may be indicated in the first month.[83] Anti-androgens are androgen receptor antagonists, which may be used prior to or in combination with GnRH analogues. Combined androgen blockade (CAB) can be used however it has only modest (5%) survival benefit compared to GnRH analogues.[96]

ADT is usually indicated long-term; it is non-curative, however it does slow disease progression. Men with androgen dependent prostate cancer which responds to ADT will eventually progress to androgen independent or castrate resistant prostate cancer. The median time to progression is two years,[38] and ADT is still indicated.[83] Secondary hormonal therapy may include oestrogen therapy.[38] The novel CYP17 inhibitor abiraterone, which inhibits androgen synthesis is indicated in combination with prednisolone in castrate resistant prostate cancer, prior to, or following chemotherapy.[83]

The main side effects of androgen deprivation therapy are hot flushes[83] which occur as a result of the hormonal imbalance (increased oestrogens, relative to androgens). Gynaecomastia may also occur with anti-androgens and GnRH antagonists. Sexual dysfunction is also a side effect of treatment. Long-term treatment with GnRH antagonists can result in

cardiovascular disease, and these agents are also likely to cause increased adiposity, and increase the risk of osteoporosis.[83]

1.4.2.2 CHEMOTHERAPY

Chemotherapy is only indicated in disease which has spread beyond the prostate, or in castrate resistant prostate cancer. Docetaxel, in combination with prednisolone, is the chemotherapy regimen of choice in castrate resistant disease which has metastasised; it has been shown to improve pain control and survival.[96] However it is associated with side effects such as hair loss, alopecia, neuropathy, bone marrow suppression and cardiovascular disease. The novel agent cabazitaxel, from the same class as docetaxel, is used in patients who have not responded to docetaxel.[92]

1.4.3 SUMMARY

As described above, treatment depends on the prostate tumour stage and Gleason score, as well as the patient's health status and life expectancy. Prostate cancer treatments in Ireland have been shown to vary considerably according to patients' area of residence (health-board area), even after adjustment for age and comorbidity.[97] While there are now national guidelines for referral of patients for a prostate cancer diagnosis to specialist clinics, there are no definitive national guidelines for treatment, and summary guidance[83] of European[92,96] or other guidelines must be consulted.

Curative treatment of localised prostate cancer places considerable financial burden on health services in comparison with conservative management.[98] In the treatment of localised prostate cancer, only radical prostatectomy shows survival benefit over conservative management.[92] New therapies, such as abiraterone indicated for metastatic castrate resistant prostate cancer, have shown marginal improvements in survival (4 months).[99] The approval of reimbursement of these agents and availability to patients has been hindered by their poor cost-effectiveness.[100]

1.5 DIGOXIN

1.5.1 THE HISTORY OF DIGOXIN AND THE CARDIAC GLYCOSIDE FAMILY

Cardiac glycosides, of the cardenolide family, are compounds with an aglycone composed of a steroidal nucleus with a five-membered lactone ring at Carbon 17, and glycosidic linkage to sugar molecules at carbon 3.[101] The related bufadienolide compounds have a six-membered lactone ring.[101] Plants of the *Digitalis* species *Digitalis lanata* and *Digitalis purpurea* or foxglove, are sources of the most commonly used cardiac glycosides, digoxin and digitoxin respectively. These are illustrated in Figure 1-5.

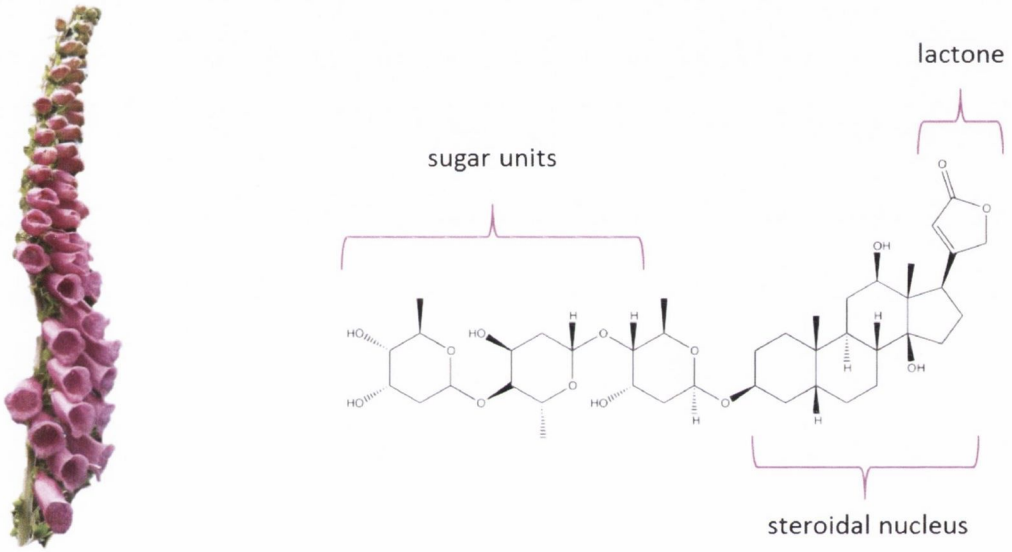

Figure 1-5: Digitalis flower and the chemical structure of digoxin

The cardiac glycosides have an interesting therapeutic history; in 1785, Sir William Withering described the therapeutic benefit of foxglove in patients with a condition called dropsy, (an accumulation of fluid in the body).[102] The use of the cardiac glycosides has been maintained to the present day in treatment of congestive heart failure and atrial fibrillation; albeit now as add-on therapy in patients with heart-failure.[103] Similarly in the treatment of atrial fibrillation, cardiac glycosides have been superseded by newer anti-arrhythmic agents as the mainstay of therapy.[104]

Some reports exist of other cardiac glycosides such as oleander being used in the Middle Ages for the treatment of cancer,[105] and more recently studies have been carried out investigating Anvirzel™, an aqueous oleander extract in the treatment of solid tumours. Phase I trials are complete[106] however phase II trials have not proceeded.

1.5.2 Pharmacology of Digitalis Glycosides

The digitalis glycosides (digoxin and digitoxin) are positive inotropes; in congestive heart failure this enhances the contractility of the cardiac muscle without increasing heart rate. In atrial fibrillation digitalis acts as a rate control agent.[24] Cardiac glycosides are sodium/potassium ATPase (Na^+/K^+ ATPase) ligands; they bind to the α-subunit of the Na^+/K^+ ATPase pump and inhibit the hydrolysis of ATP, thus preventing sodium ion transport. This reduces the concentration gradient for calcium efflux and causes intracellular calcium concentration to increase, which results in enhanced contractility of the cardiac muscle.

1.5.3 Indications for use of Digoxin

Digoxin is the only cardiac glycoside licensed for use in Ireland. It is indicated for the treatment of supraventricular arrhythmias, such as atrial fibrillation and atrial flutter to reduce ventricular rate. It is also indicated for chronic heart failure, where systolic dysfunction is the dominant problem and heart failure accompanied by atrial fibrillation.[107]

The prevalence of heart failure in Europe in 2008 was estimated at 2-3% in the total population, and 10-20% in the population between 70-80 years. It is more common in men than women.[103] The prognosis for heart failure is poor, 30-40% of people die within one year[108] and 50% die within four years.[103] Digoxin is recommended in symptomatic heart failure in addition to angiotensin converting enzyme (ACE) inhibitors and diuretics.[24] It can be used to control heart rate prior to, or in addition to a beta-blocker. Digoxin can improve ventricular function, and reduce hospital admission due to worsening atrial fibrillation but it has no effect on survival.[103] Digoxin may be used alone or in combination with verapamil or diltiazem to control ventricular rate.

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia.[109,110] The prevalence is estimated to be approximately 1-2% in the general population.[104] It is associated with increasing age, with prevalence of approximately 0.5% in persons under 60 years,[104,111] increasing to 5-15% at 80 years.[104] The incidence of AF is reported to have increased by 13% in the past two decades.[104] Men are more likely to develop AF than women, (OR=1.8, 95% CI 1.2, 2.8).[112] AF can be a cause and also a consequence of heart failure. Heart failure has been found in 30% of patients with AF, and AF is found in 30-40% of heart failure patients.[104] AF is attributed to be the cause of one in five strokes.[104,113] Anti-thrombotic therapy is recommended for all patients with AF and atrial flutter; anti-platelet therapy with aspirin is sufficient in those at very low risk of stroke.[114]

1.6 Aspirin

1.6.1 The Origins and History of Aspirin

The ancient Greeks were reported to have chewed willow bark for its analgesic properties. Salicin was identified as the active compound in willow bark in 1826. Aspirin, or acetyl-salicylic acid, a modification of salicylic acid, was commercialised by Bayer, one of the world's oldest pharmaceutical companies, in 1897.[115] The chemical structure of aspirin is illustrated in Figure 1-6.

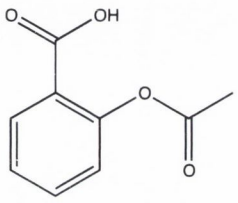

Figure 1-6: Chemical structure of aspirin

Aspirin was initially indicated and marketed for treating fever and pain. In the early 1950s Dr Lawrence Craven, a US physician noted increased bleeding in patients who chewed aspirin gum following surgical removal of tonsils and adenoids. He proposed aspirin had anticoagulant properties and reported personal observations of patients at-risk of myocardial infarction who having taken aspirin, even at low doses, had not suffered a cardiac event.[116] In the 1970s Vane *et al.* discovered that aspirin blocked the formation of prostaglandins and thromboxanes, the latter which play an important role in platelet aggregation and clotting. Vane *et al.* were subsequently awarded the Nobel Prize in Physiology or Medicine in 1982.[117] Trials of aspirin as secondary prevention for myocardial infarction and stroke also carried out in the 1970s led to its subsequent licensing for this indication.[118] A significant advantage of aspirin as prophylactic pharmacotherapy for cardiovascular disease is its low cost. Aspirin is the most commonly prescribed agent on the GMS scheme; over 2.5 million prescriptions for aspirin were dispensed in 2011.[119]

1.6.2 Pharmacology of Aspirin

The pharmacological target of aspirin is the COX enzyme channel; this is the active site of the COX enzyme, where arachidonic acid and other substrates are converted to prostaglandins and thromboxanes as described already (Section 1.2.4.3). Aspirin, through covalent bonding with a serine residue in the COX enzyme channel, prevents arachadonic acid from accessing the channel, thus reducing the production of prostaglandins.[24]

The COX enzyme exists in two isoforms, COX-1 and COX-2, with differing effects. COX-1 is constitutively expressed throughout the body; COX-1 products are responsible for maintaining various tissues i.e. protection of gastrointestinal mucosa, regulation of renal blood flow; and haemostasis i.e. regulation of platelet aggregation and adhesion.[24]

COX-1 in platelets produces TX-A_2, a platelet aggregation factor, and COX-1 in the vascular endothelium produces PG-I_2 which inhibits platelet aggregation. Aspirin irreversibly blocks COX-1, which cannot be regenerated by circulating platelets as they lack a nucleus. Cells of the endothelial tissue can regenerate COX-1, and also require higher doses of aspirin for inhibition. Aspirin is thus effective in altering the balance of these platelet aggregation factors and reducing the formation of thrombi.[24]

COX-2 is an inducible enzyme expressed in response to inflammatory cytokines (i.e. Interleukin-1α, Interleukin 6, NF-κB)[120] growth factors (i.e. IGF-I, EGF),[121] hypoxia[122] and tumour promoters. COX-2 production of prostaglandins such as PG-E_2, PG-D_2 and PG-I_2 results in localised pain and inflammation. PG-E_2 mediates fever, and there are three known PG-E_2 receptors leading to a variety of effects such as vasodilation and hyper-algesia. PG-E_2, PG-D_2 and PG-I_2 through their vasodilator activity, synergise with other inflammatory mediators.[24]

1.6.2.1 Pharmacokinetics of aspirin

Aspirin is absorbed in the stomach and intestine. Metabolism of aspirin to salicylate begins in the gut wall. Following absorption aspirin binds to plasma proteins, however conversion to salicylate occurs rapidly (half-life 15-20mins). Although it does not inhibit COX enzymes, salicylate does have pharmacological activity; it may reduce the expression of COX-2 in some cell types.[123] Salicylate undergoes further hepatic metabolism or may be excreted in urine, depending on the dose of aspirin or the urinary pH.[124]

1.6.2.2 Adverse Effects of Aspirin

COX inhibition by aspirin, while critical to its pharmacological effects, also contributes to its side effect profile. PG-E_2, produced by COX-1 protects the mucosal membrane of the stomach.[24] Inhibition of COX-1, leads to gastric irritation and potentially ulceration and bleeding. Reduced production of prostaglandins can also have adverse effects on renal function, as PG-E_2 has vasodilator activity and works to compensate vasoconstriction mediated by noradrenaline or angiotensin II. The anti-platelet effect of aspirin may also cause cerebrovascular bleeding. Excessive dosing of aspirin may cause *Salicylism,* which may present as tinnitus, vertigo, impaired hearing, nausea and vomiting. Aspirin is contraindicated in

children aged less than 16 years, due to the risk of *Reye's syndrome*.[24] Aspirin should be used with caution in the elderly as the risks of gastric irritation or ulceration may outweigh the benefit. Its use should also be avoided in patients with severe impairment of renal, cardiac or hepatic function.[125]

Enteric coating of aspirin tablets, and co-prescription of anti-secretory drugs, (histamine (H2)-receptor antagonists and proton pump inhibitors) have been used as means of overcoming these adverse gastrointestinal effects, while still allowing patients to benefit from the antithrombotic activity of aspirin. Selective COX-2 inhibitors such as celecoxib and rofecoxib were also developed to treat pain and inflammation due to the unfavourable gastric side effects of aspirin and NSAIDs mediated by COX-1 inhibition.[24] The therapeutic use of selective COX-2 inhibitors has been restricted by their cardiovascular adverse effects. Selective COX-2 inhibitors have however been examined as potential treatment of cancer in preclinical[126] and clinical[127] studies, and are further discussed in Chapter 4.

1.6.3 Indications of Aspirin

Due to the fact that aspirin inhibits both COX enzymes, which have pleiotropic functions, aspirin has a number of clinical indications. The dosing of aspirin also differs according to the indication.

1.6.3.1 Analgesic, Anti-inflammatory and Antipyretic Activity of Aspirin

In dosage forms where aspirin is indicated for its anti-inflammatory, anti-pyretic and analgesic properties, it is licensed for sale over the counter, and on general sale in Ireland.[124] These medicinal products contain typically 200-500mg of aspirin, and are indicated for relief of mild to moderate pain i.e. headache, migraine, dental pain, sore throat, dysmenorrhoea, neuralgia, myalgia, rheumatic pain, sciatica, lumbago, fibrositis, muscular pains, sprains, strains, joint swelling, stiffness, fever and symptoms of the common cold or influenza.[124]

1.6.3.2 Anti-thrombotic Activity

As a cardio-protective agent aspirin is indicated following myocardial infarction, ischaemic stroke and in patients with unstable angina.[125] Dosage forms of aspirin licensed for long-term use for a cardiovascular indication are only available on prescription in the Republic of Ireland.[125] This includes the low-dose (75mg) dosage form, high dose (300mg) products and combination products (i.e. with dipyridamole, clopidogrel).

Aspirin is widely prescribed as an antithrombotic agent for the prophylaxis of stroke in patients with atrial fibrillation,[128] and is also commonly prescribed for the prevention of cardiovascular events in patients with type II diabetes mellitus.[129] Aspirin has been investigated as primary prevention of cardiovascular disease and results of a meta-analysis have shown that aspirin-treated patients have significantly reduced serious vascular events, in particular non-fatal myocardial infarction.[130] However, in primary prevention of vascular disease, aspirin is not recommended as the increased risk of bleeding outweighs the benefit.[130]

1.7 Chapter Summary and Thesis Objectives

Pharmacoepidemiology is a valuable method of investigating whether exposure to commonly prescribed medicines may alter disease outcomes. Firstly there is the potential to elucidate whether the effects of these drugs observed in pre-clinical studies are observed at the population level; secondly, associations between exposure to these drugs and cancer outcomes may identify biological pathways integral to tumour growth and progression, which may be used in the development of new therapies; and thirdly, there are potential public health issues regarding the association between cancer and commonly used drugs. In addition to this, should these drugs be repositioned in cancer treatment, the development costs are reduced, and the side-effects of existing agents are well understood.

The rise of prostate cancer incidence in Ireland, largely due to widespread PSA testing, has been outlined. Prostate cancer mortality began to fall in Ireland before the PSA-era,[40] therefore the number of men surviving following their diagnosis of prostate cancer is increasing.[5] The identification of other methods of reducing the burden of this disease on health services and increasing the survival of men with prostate cancer is a priority. Cancer pharmacoepidemiology, examining medicines usage and cancer outcomes explores this possibility. In this thesis, two of the oldest used medicines are examined with respect to their potential anti-cancer activity in men with prostate cancer. The next chapter will outline the linked database used to conduct this research.

1.7.1 Digoxin

Digoxin has been shown in pre-clinical studies to inhibit HIF-1α expression, which is crucial in the adaptation of prostate cancer cells to hypoxic states, and may inhibit tumour growth.[46] This is because HIF-1α is crucial to the induction of angiogenesis and reprogramming of energy metabolism in hypoxic cancer cells.[44] Digoxin has also been associated with reduced prostate cancer incidence,[61] but digoxin exposure has not as yet been examined in relation to prostate cancer survival.

A literature review of the pre-clinical studies which have proposed the anti-cancer potential of digoxin in prostate cancer is presented in Chapter 3, Section 3.1. Similarly a review of the observational research to date which has examined this hypothesis is provided in Chapter 3, Section 3.2.

The studies examining digoxin exposure in men with prostate cancer are also presented in Chapter 3. The aims of these studies were to:

I. Assess the association between digoxin exposure prior to diagnosis and prostate cancer tumour characteristics at diagnosis
II. Examine whether exposure to digoxin at the time of diagnosis is associated with prostate cancer-specific mortality.

1.7.2 ASPIRIN

The anti-inflammatory activity of aspirin may play a role in inhibiting the development of prostate cancer,[27] as inflammation has been identified as a pre-cancerous[27] and tumour-promoting[19] process. The anti-platelet effect of aspirin has also been proposed to impede tumour dissemination.[47] Existing studies have reported aspirin to be associated with reduced prostate cancer incidence,[62] and a number of studies have shown varying associations between aspirin exposure and prostate cancer mortality.[131-133]

The literature regarding pre-clinical studies examining aspirin and prostate cancer is reviewed and presented in Chapter 4, Section 4.1. The meta-analyses and observational research to date which have reported associations between aspirin and prostate cancer incidence and outcomes are discussed in Chapter 4, Section 4.2.

The studies examining aspirin exposure and prostate cancer mortality are presented in Chapter 4; the objectives of these studies were to:

I. Examine the association between aspirin exposure prior to diagnosis and prostate cancer-specific mortality in men diagnosed with localised prostate cancer.
II. Examine the association between pre-diagnostic aspirin exposure and prostate cancer-specific mortality in men with high grade (Gleason Score >7) prostate cancer.

The results of these studies are discussed in each chapter in the context of the existing pre-clinical and observational evidence. The concluding chapter summarises the findings, discusses the potential implications of this research and the potential scope for future pharmacoepidemiology research in prostate cancer.

Appended to the thesis are two additional manuscripts which are indirectly related to the objectives of this work. These studies also investigated aspirin exposure and cancer patient outcomes. Firstly, a nested case-control study conducted in the UK CPRD population in collaboration with Professor Liam Murray and Dr Chris Cardwell. This study investigated the

association between low-dose aspirin use following diagnosis and prostate cancer mortality in a cohort of British prostate cancer patients and is described in Appendix 4. This has been accepted for publication in *Cancer Causes Control*. Secondly, a study led by Dr T. Ian Barron investigating the association between pre-diagnostic aspirin use and lymph node involvement in a cohort of Irish women with stage I-III breast cancer; this manuscript is currently under review. (Appendix 5).

Chapter 2 DATA SOURCES

This chapter outlines the methodology employed in this thesis. Firstly it provides a description of the databases used, and the information available in these databases. The strengths and limitations of these resources are discussed and the linkage of the databases is described. Finally, a descriptive drug utilisation analysis of digoxin and aspirin is provided in the study population used to conduct the main analyses of the thesis.

2.1 DATABASES

2.1.1 PRESCRIPTION CLAIMS DATA

The Department of Health through the Health Services Executive (HSE) funds a number of health and medical care schemes which are delivered by the Primary Care Reimbursement Service (PCRS).[134] Community pharmacies are contracted with the PCRS to provide pharmaceutical care to patients under a number of the community drug schemes. Approximately 1,600 community pharmacy contractors submit monthly claims to the PCRS for medicines dispensed on the community schemes in order to be reimbursed.

The General Medical Services (GMS) database is generated from the prescription claims submitted to the PCRS by the pharmacy contractors for GMS eligible patients. This database is nationally representative, and has been used extensively for research purposes in studies examining treatment outcomes,[135] good prescribing practice,[136,137] changes in prescribing practice[138] and the cost-effectiveness of prescribing, i.e. prescribing of generics or branded drugs.[139,140]

2.1.1.1 THE GMS SCHEME

The GMS scheme is available to "persons who are unable without undue hardship to arrange general practitioner, medical and surgical services for themselves and their dependants".[141] Under the GMS Scheme, patients are provided access to a number of healthcare services free of charge including GP visits, community health, dental and hospital care as well as provision of medicines and some medical devices through community pharmacy contractors. The eligibility criteria for the GMS scheme have changed over time, but approximately one third of the population (1.4-1.6 million) were covered by the scheme during the period relevant to this thesis.[142] Eligibility is based on means test and age. All persons aged 70 years and over were eligible for the scheme from July 2001 to December 2008 regardless of means; therefore there is virtually 100% coverage of these patients during this time period.

Patients who are eligible for the GMS scheme are also eligible for the High-Tech Drugs scheme provided by the PCRS. This provides high-cost medicines, usually initiated in hospital, to patients in the community.[134] The PCRS covers the cost of the medicines (no mark-up), by paying the wholesaler directly and pays pharmacies a patient care fee. This scheme covers items such as anti-rejection drugs, biological agents, growth hormones and ADT medicinal products for prostate cancer. Data from the High-Tech scheme was available; however the

date of dispensing was not available from the PCRS and therefore this data was not used. Information on the use of ADT was however available from the NCRI database.

2.1.1.2 Covariates

The following covariates can be captured from the GMS database (i) patient demographics such as age-group (0-4 years, 5-11 years, 12-15 years, 16-24 years, 25-34 years, 35-44 years, 45-54 years, 55-64 years, 65-69 years, 70-74 years, ≥75 years), gender (male, female), HSE region (Dublin Mid Leinster, East, South, West) and Local Hospital Office (approximately one for each county); (ii) details about each prescription claim, date of claim, WHO Anatomical Therapeutic Classification (ATC) code,[143] quantity dispensed and drug-product code (GMS code number, which is unique for each medicinal product formulation); and (iii) the PCRS doctor number and pharmacy number which are unique for each PCRS prescriber and pharmacy contractor.

2.1.1.3 Strengths and Limitations

The GMS scheme can be considered a closed pharmacy system; therefore the data capture within this database can be considered reliable and accurate. This is because firstly patients receive their medicines for free, making it unlikely that they will obtain their medication through another source at cost to themselves; secondly, claims from pharmacies are usually submitted to the PCRS electronically; and thirdly, pharmacies also submit the original prescription form to the PCRS for reimbursement and where discrepancies exist between prescriptions and claims, the pharmacy contractor will not be reimbursed for the claim.

Some limitations of this resource must also be acknowledged. It should be noted that GMS patients may receive certain specified medication under other schemes, such as the High-Tech Drugs, Long Term Illness, Dental Treatment Services, Methadone Maintenance Therapy, or Psychiatric Schemes. Unlicensed medicines are not captured in the data either. The GMS database only has information on patient medication dispensed; there is no information on patient diagnoses or patient outcomes. This is a limitation in assessing comorbid conditions. Dispensing of a medication does not mean that the patient is compliant with the medication received; therefore some misclassification of exposure may occur. Due to the eligibility criteria for the GMS scheme, older people and people of lower socioeconomic status are over-represented in the database.

2.1.2 National Cancer Registry Ireland

The NCRI has actively collected detailed data on all incident cancers in the population normally resident in the Republic of Ireland since 1994.[144] Trained, hospital-based tumour registration officers collect information on patient characteristics, tumour details, treatment received and death from multiple sources including pathology laboratories, radiology departments, oncology departments, hospital administrative systems and individual medical records. The national death certificate register, from the Central Statistics Office, which includes patient cause of death and date of death, is linked to patient records at the NCRI.

2.1.2.1 Database and Covariates

A database of all prostate cancers (ICD-O, C61)[145] diagnosed 2001-2006, was provided by the NCRI for this research project. For each man diagnosed with prostate cancer, details of all other diagnosed cancers (1994-2009) other than non-melanoma skin cancers were provided.

The NCRI database contains information on the following patient demographics at diagnosis: patient age (years); smoking status (current, former, non-smoker, unspecified); marital status; occupational status; county and health-board of residence, and a census-based socioeconomic deprivation score[146] for the local electoral district of residence. Tumour characteristics are also captured including: tumour morphology; tumour grade (Gleason Score <5, 5-7, >7, unspecified);[43] and AJCC TNM Stage. Staging was recorded as clinical or pathological stage. Pathological tumour staging, where available, took precedence over clinical staging. Staging data available was tumour size (T1, T2, T3, T4, unspecified); nodal status (negative, positive, unspecified); metastases (negative, positive, unspecified); and overall tumour stage (I, II, III, IV, unspecified).[43] Where nodal status or metastases were unspecified or not assessed they were assumed negative for categorising tumour stage. This is the NCRI policy.

Treatment types and the dates of treatment receipt in the year following diagnosis are also captured in the NCRI data: surgery (yes, no), radiation (yes, no) hormonal therapy (yes, no) or chemotherapy (yes, no). Occasionally tumour registration officers will gather information on treatments received beyond one year following diagnosis, or records of distance metastases diagnosed. However as this data is not routinely collected, it is not consistent and is not used. Date and cause of death as recorded on death certificates coded by ICD-9, or ICD-10 codes are also included in the database.

2.1.2.2 Strengths and Limitations

The strengths of this database are that it is nationally representative and five-year tumour registration of prostate cancer is estimated to be in excess of 96% complete.[147] The main limitation of this prostate cancer dataset is incomplete staging data. This is a significant issue in analysis of routinely gathered data. Due to the age at which men are diagnosed with the disease, there is increased likelihood of them having other comorbid conditions at diagnosis. The subsequent complications which may arise with invasive prostate biopsy or aggressive treatment may mean that a conservative approach to tumour staging and subsequent treatment is taken.

2.2 LINKED DATABASE

The linked NCRI-PCRS database is the principal resource used to carry out this doctoral research. The establishment of this database including the data linkage process is described in this section. This linked database has been used for similar studies before.[148]

2.2.1 LINKAGE

Data linkage of the NCRI database to the GMS prescription claims was carried out by staff at the NCRI. Cancer diagnoses from January 1st 2001 to December 31st 2006 were linked to prescription claims for GMS eligible patients from January 1st 2000 to December 31st 2007. Follow-up data on deaths was provided up to December 31st 2009 initially, but this was updated to December 31st 2010 over the course of this project.

The NCRI developed a data processing application (DataPipe) to clean the data and re-format data fields such as names, dates of birth and addresses, prior to matching. Subsequently record linkage of patient records in the prescription claims database to those in the NCRI database was carried out using probabilistic matching. The programme used for this was AutoMatch, which uses a fuzzy matching algorithm to return the degree of similarity between the records. Of prostate cancer cases diagnosed from 2002-2006, 51.0% of men under the age of 70 were identified as having a GMS card and 89.9% of men aged 70 years and over were successfully matched to a GMS record. (NCRI, personal communication)

2.2.2 LINKED DATA COVARIATES

2.2.2.1 IDENTIFICATION OF INCLUSION AND EXCLUSION CRITERIA

In order to be eligible for these studies, all men had to have at least one year of GMS eligibility prior to their prostate cancer diagnosis. Pre-diagnostic GMS eligibility was identified from a combination of the start- and stop-dates of eligibility for each patient provided by the PCRS, and prescription claims. Eligibility for all studies was based on having continuous GMS eligibility for at least one year prior to the date of prostate cancer diagnosis.

Cancer diagnoses (other than non-melanoma skin cancer) recorded by the NCRI, since 1994, which preceded a prostate cancer diagnosis (2001-2006) were identified, for the purpose of excluding patients with a prior diagnosis of invasive cancer. Over the course of this work, the decision was taken within the research group to exclude patients who had another cancer diagnosed on or before the date of their prostate cancer diagnosis. This explains some differences in the numbers excluded on this basis between different studies.

2.2.2.2 Medication Exposures

Medications were identified in the GMS database by WHO-ATC code;[143] exposures for periods (either before or after diagnosis) were identified from prescription claims. WHO-ATC codes for all medicines referred to in this thesis are listed in Appendix 7. For the medications of interest in each study (i.e. digoxin, aspirin), the dose of the drug product received and dosing intensity were determined. Dosing intensity was calculated as the proportion of days covered (PDC)[149] i.e. proportion of days in which the patient had a supply of the medication available to them during the period of interest. The exposed group was subsequently stratified by levels of dosing intensity. Medication exposure was also stratified by the dose of the drug received.

2.2.2.3 Comorbidity Score

Prescription claims on the GMS scheme for the year prior to prostate cancer diagnosis were used to generate a medication based comorbidity score. The number of distinct medication classes (determined on the five-character ATC code), was used to calculate the score. This method is based on a validated medication-based means of prediction of mortality, hospitalisation and long-term care admissions.[150] This or any other medication-based comorbidity score is limited by not having complete capture of medication dispensed on other community drugs schemes.

2.2.3 Data Utilisation Agreements

The use of data held by the NCRI for research purposes is covered by the Health (Provision of Information) Act 1997. Data utilisation agreements have been established with the NCRI and PCRS. All potential patient identifiers have been removed from the datasets prior to receipt and data used in this project has been stored on an encrypted drive on a desktop computer for use only by the PhD researcher.

2.2.4 Strengths and Limitations of the Linked Data

The strengths and limitations of the individual GMS and NCRI databases hold for the linked database. The NCRI does not carry out active follow-up of tumour progression or recurrence, therefore this limits further study of patient outcomes; mortality is the only outcome which can be assessed.

2.2.5 Data Analysis

SAS® V 9.2 (SAS Institute, Cary, NC) was used for all analyses of the GMS data and linked datasets. Significance at $p<0.05$ is assumed unless stated otherwise.

2.3 External Validity of the Linked Dataset

The inclusion criteria for all studies carried out in the linked NCRI-PCRS database is eligibility for the GMS scheme for the entire year prior to diagnosis. As already discussed, eligibility for this scheme is based on means test and age; therefore these men differ somewhat from the general population. Healthcare utilisation including GP visits[151] and PSA testing (Flahavan, Drummond *et al.* Appendix 3) differs in GMS eligible men, to those who are ineligible for this scheme. The characteristics of the GMS eligible men diagnosed with prostate cancer have been compared to the full cohort of men diagnosed with prostate cancer to illustrate the representativeness of this cohort.

2.3.1 Comparison of Linked NCRI-PCRS GMS Eligible Cohort with Full NCRI Cohort of Men Diagnosed with Prostate Cancer

The characteristics of men diagnosed with prostate cancer in the entire NCRI population and those identified from the NCRI-PCRS database as having GMS eligibility for the year prior to diagnosis have been tabulated. See Table 2-1. The linked database was used to identify all prostate cancer cases diagnosed 2001-2006 and the men with continuous GMS eligibility for the year prior to diagnosis. Covariates tabulated were patient factors (age at diagnosis, smoking status at diagnosis, deprivation score of electoral district of residence at diagnosis) year of incidence, tumour characteristics (tumour stage; Gleason score) and treatment received in the year following diagnosis (radiation, surgery, ADT, chemotherapy, no active treatment).

13,824 prostate cancer cases were diagnosed from 2001-2006; 48.4% (N=6,688) were eligible for the GMS scheme for the entire year prior to diagnosis (Table 2-1). The mean age at diagnosis of GMS eligible men (74.0 years), was greater than that of the full cohort (69.4 years). There were more GMS eligible men (32.4%) living in socioeconomically deprived areas than men in the full NCRI cohort (27.9%). The trend in the number of prostate cancer cases diagnosed over the years 2001-2006 was similar in the GMS eligible population to the full NCRI cohort. More GMS eligible men were diagnosed with stage IV (14.9% Vs. 11.3%) and Gleason Score > 7 (19.7% Vs. 17.9%) disease, relative to the full cohort. The percentage of men with Gleason Score unspecified was also greater in the GMS population. Differences in extent of diagnoses and treatments received are likely to reflect the older age of men in the GMS population; more men in the GMS eligible cohort received ADT (47.7% Vs. 36.7%) or no treatment (24.5% Vs. 22.2%) and fewer received surgery or radiation (Table 2-1.).

Table 2-1: Characteristics of the full NCRI cohort of prostate cancer cases (2001-2006) and the NCRI-PCRS GMS eligible cohort

Characteristic			Full NCRI Cohort (N=13,824)	NCRI-PCRS GMS eligible cohort (N=6,688)
Patient Details				
Age/years	Mean (SD)		69.4 (9.4)	74.0 (8.0)
Smoking Status[A] (%)	Never		1,914 (13.8)	1,061 (15.9)
	Former		4,465 (32.3)	2,106 (31.5)
	Current		2,269 (16.4)	1,282 (19.2)
	Unspecified		5,176 (37.4)	2,239 (33.5)
Deprivation Score[B] (%)	Least Deprived	1	2,742 (19.8)	1,032 (15.4)
		2	1,729 (12.5)	793 (11.9)
		3	1,883 (13.6)	890 (13.3)
		4	2,337 (16.9)	1,204 (18.0)
	Most Deprived	5	3,861 (27.9)	2,165 (32.4)
	Unspecified		1,272 (9.2)	604 (9.0)
Year of Incidence (%)	2001		1,905 (13.8)	780 (11.7)
	2002		2,144 (15.5)	1,020 (15.3)
	2003		2,155 (15.6)	1,106 (16.5)
	2004		2,664 (19.3)	1,327 (19.8)
	2005		2,462 (17.8)	1,264 (18.9)
	2006		2,494 (18.0)	1,191 (17.8)
Tumour Details				
Stage[C]	I		409 (3.0)	214 (3.2)
	II		7,036 (50.9)	3,073 (45.9)
	III		1,187 (8.6)	478 (7.1)
	IV		1,569 (11.3)	994 (14.9)
	Unspecified		3,623 (26.2)	1,929 (28.8)
Grade[C]	Low (Gleason ≤7)		8,621 (62.4)	3,714 (55.5)
	High (Gleason >7)		2,746 (17.9)	1,317 (19.7)
	Unspecified		2,727 (19.7)	1,657 (24.8)
Treatment Received[D]				
	Surgery		4,270 (30.9)	1,632 (24.4)
	Radical Prostatectomy		1,876 (13.6)	215 (3.2)
	Other Prostatectomy		2,394 (17.3)	1,417 (21.2)
	Radiation		4,449 (32.2)	1,749 (26.2)
	ADT		5,071 (36.7)	3,190 (47.7)
	Chemotherapy		240 (1.7)	135 (2.0)
	No Treatment		3,069 (22.2)	1,638 (24.5)

A: *Smoking status recorded at diagnosis*
B: *Deprivation Score of electoral district of residence according to SAHRU*[146]
C: *AJCC Staging Manual 5th Ed.*[43]
D: *Treatment received in the year following diagnosis*

In summary, the GMS eligible men are older and a greater proportion of these men live in more socioeconomically deprived areas compared to the full NCRI cohort. Given the older age of this cohort, the differences observed in tumour stage, Gleason score at diagnosis and less aggressive treatments received are to be expected.

2.4 Digoxin Prescribing in the GMS Population

2.4.1 Introduction

This section describes the prevalence of digoxin prescribing in the GMS population. As already described in the previous chapter (Section 1.5), digoxin is prescribed as add-on therapy for atrial fibrillation and heart failure.

2.4.2 Methods

The database used for this analysis was the GMS database as described in Section 2.1.1. The Defined Daily Dose (DDD) is "the assumed average maintenance dose per day for a drug used for its main indication in adults".[10] The most accepted way of describing drug utilization in a population is as numbers of DDDs per 1000 inhabitants per day. The prescribing of digoxin (WHO ATC C01AA05: DDD=0.25mg)[143] in DDDs per 1000 GMS eligible population per day was calculated for each year (2002-2009). The prevalence of prescribing of digoxin by gender and age-group (≥55 years) was examined. Persons aged less than 55 years were excluded as the prevalence of digoxin prescribing in this group was <1%.

2.4.3 Results

Figure 2-1 illustrates that digoxin use in the entire GMS population has decreased over the years 2002-2009. Prescribing of digoxin is higher in males compared to females, 8.5% compared to 6.7% in females in 2002; the prevalence fell to 5.1% and 4.0% respectively by 2009. See Figure 2-2. Prescribing prevalence is also considerably higher in the older age-groups, in particular those aged 75 years and older. The decline in use over time by age-group is illustrated in Figure 2-3. Prescribing was 15.4% in patients aged 75 years and older in 2002, falling to 8.9% by 2009.

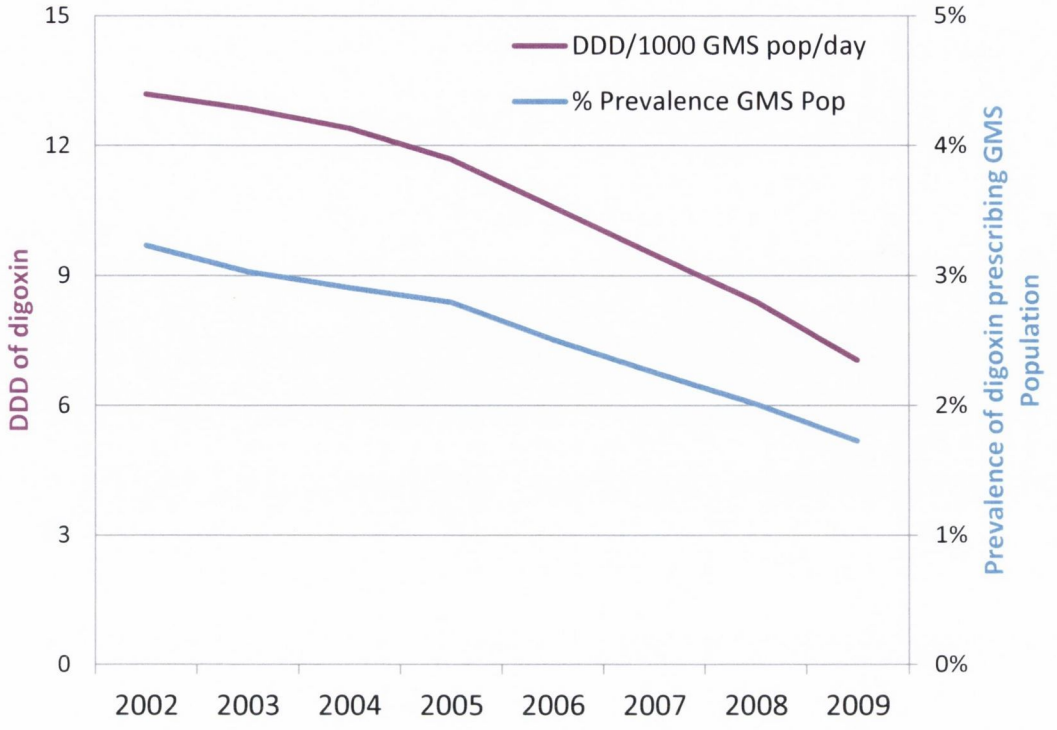

Figure 2-1: Digoxin prescribing in the entire GMS eligible population: 2002-2009, DDD and % prevalence of prescribing

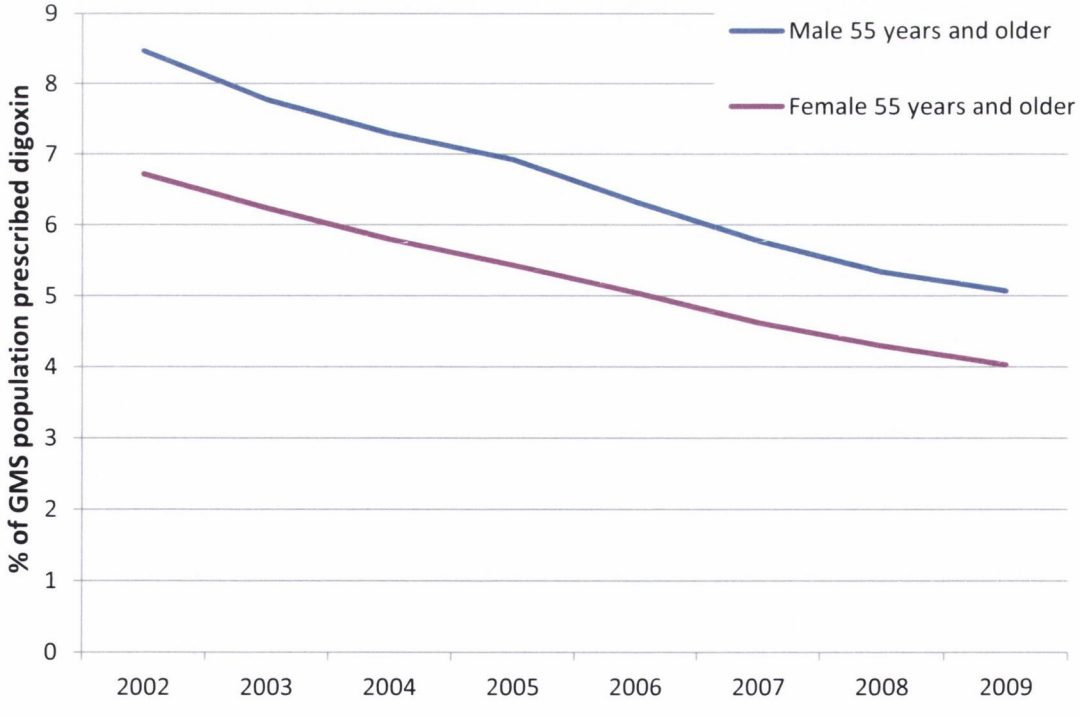

Figure 2-2: Digoxin prescribing in the GMS eligible population by gender

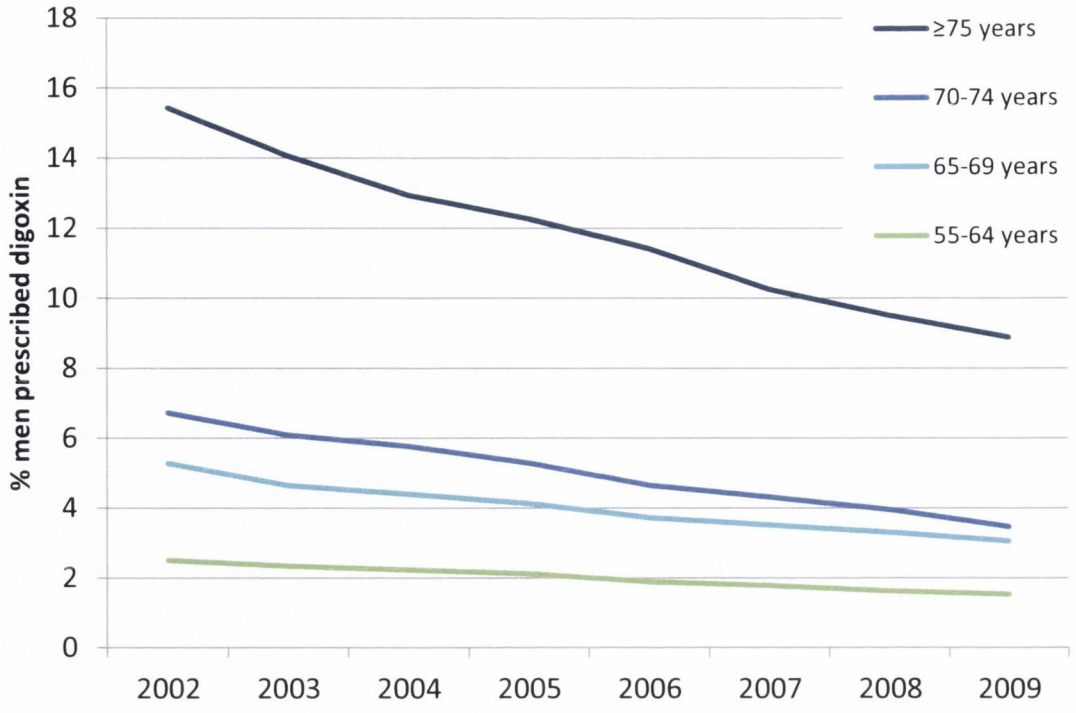

Figure 2-3: Prescribing prevalence of digoxin by age-group: males only

2.4.4 Discussion

The prevalence of digoxin prescribing is higher in males than in females, due to the higher prevalence of both heart failure and atrial fibrillation in males than in females. Digoxin prescribing is also higher in older age groups, again reflecting the epidemiology of these conditions (Chapter 1, Section 1.5.3). The use of DDDs as a measure of medication utilisation is a rather crude measure of medication consumption in a population, and its optimal use is in making comparisons between different populations. It must be recognised that although 0.25mg is the WHO recognised DDD of digoxin, many patients, especially elderly patients, receive far lower daily doses of digoxin.

Digoxin is regarded as one of the earliest therapies for cardiac illness; however, current guidelines state that, since the introduction of novel agents, digoxin is only the optimum therapy for a minority of patients. Beta-blockers have been shown to significantly improve outcomes of patients with heart-failure, and digoxin is indicated as add on therapy.[103] In atrial fibrillation, other anti-arrhythmic agents have become the mainstay of therapy.[104] Digoxin use is declining due to the evidence emerging in favour of these newer agents; however it is still prescribed in Ireland, particularly in older males.

2.5 Aspirin Prescribing in the GMS Population

Aspirin, in dosage forms licensed for long-term use for a cardiovascular indication is subject to a medical prescription in the Republic of Ireland.[125] This includes the low-dose (75mg) dosage form, high dose (300mg) products and combination products (i.e. with dipyridamole, clopidogrel). The prescribing of aspirin was assessed in the GMS eligible population for the years 2002-2009, again using the GMS database as outlined in the analysis of digoxin above.

2.5.1 Methods

The number of DDDs of aspirin used in the GMS eligible population per year was calculated (2002-2009). These were determined by aspirin indication (B01AC06: anti-platelet activity DDD=75mg; N02BA01: anti-inflammatory activity, DDD=3,000mg).[143] Trends of aspirin prescribing by gender and age-group (≥45 years) were also assessed over this period. Persons aged under 45 years accounted for <5% of aspirin prescriptions, and were excluded from analyses by age and gender. Within the cohort of patients receiving aspirin, the trend in average aspirin dose prescribed was also examined (low ≤75 mg; intermediate 75-150 mg high >150 mg).

2.5.2 Results

Aspirin prescribing has increased in the entire population over the period examined. See Figure 2-4. In men aged 45 years and older the prevalence of aspirin prescribing on the GMS scheme has risen from 32.2% in 2002 to 40.1% in 2009. The prevalence of aspirin prescribing by gender is illustrated in Figure 2-5. Aspirin is prescribed to more men than women in this population. The prevalence of aspirin prescribing is highest in the oldest age groups. See Figure 2-6. A consistent rise in the prescribing prevalence was seen from 2002-2009 in all age groups. Analysis of the dose of aspirin prescribed (Figure 2-7) illustrates that the majority of men who received aspirin only received low-dose preparations (≤75 mg).

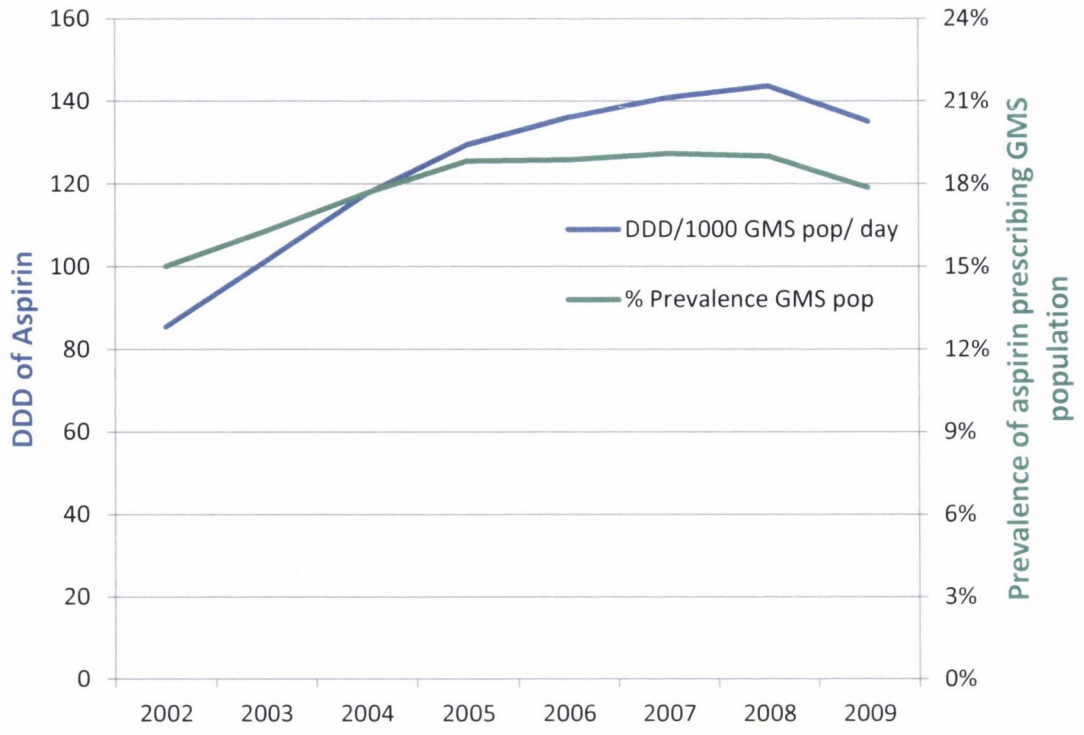

Figure 2-4: Aspirin prescribing in the entire GMS eligible population: 2002-2009, DDD and % prevalence of prescribing

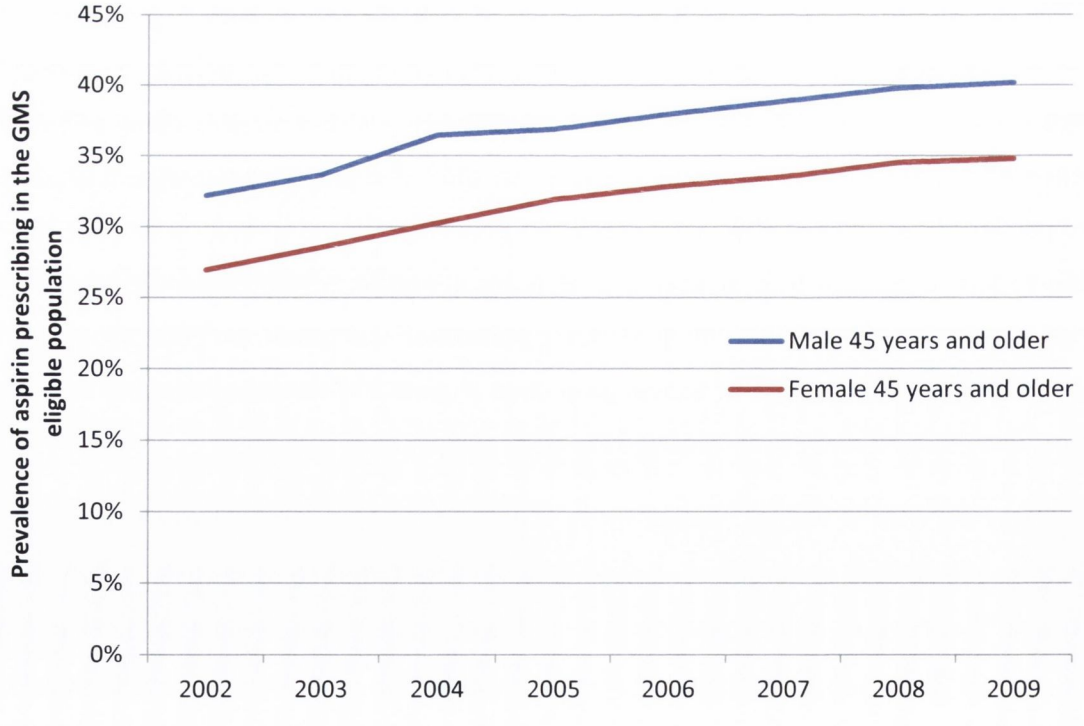

Figure 2-5: Aspirin prescribing in the GMS eligible population by gender

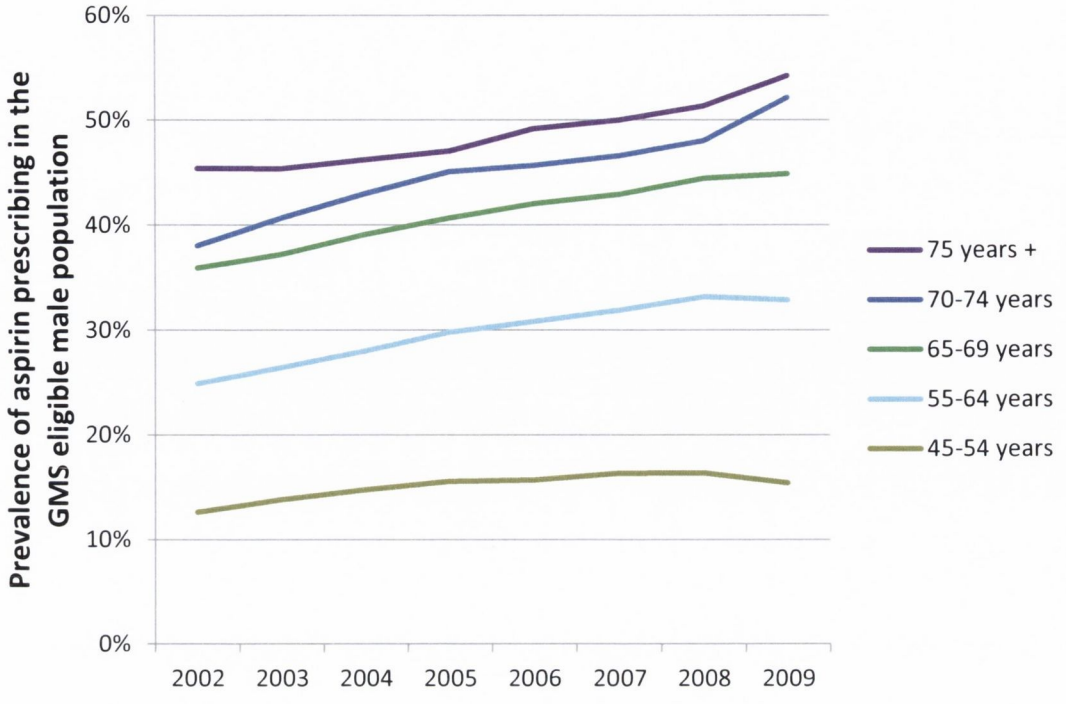

Figure 2-6: Prescribing prevalence of aspirin by age-group: males only

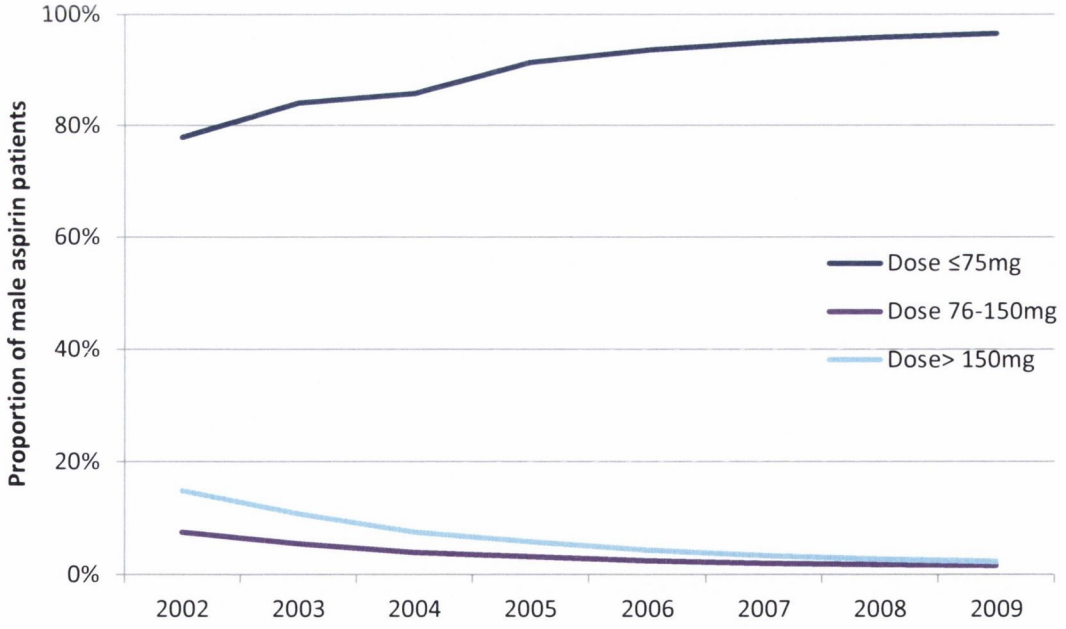

Figure 2-7: Prescribing prevalence of aspirin by dose: males only

2.5.3 Discussion

This analysis illustrates the increased prescription of aspirin to patients eligible for the GMS scheme. The overall prevalence of aspirin prescribing increased steadily over the years 2002-2008. The drop off in the prevalence of aspirin prescribing, and number of DDDs prescribed in the GMS population from 2008 to 2009 (Figure 2-4) can be attributed to changes in the denominator (number of GMS eligible patients). This is because (i) eligibility criteria changed during this year due to the introduction of means testing for patients aged over 70 years, and (ii) an increased number of people in younger age groups met the means-based eligibility criteria due to the economic circumstances at this time.[142]

The irregular trend noted in the DDD analysis highlights a limitation of this method. That is, that in a patient population which may fluctuate, trends need to be interpreted carefully. Another limitation regarding the use of DDDs which must also be acknowledged is that medical practice differs globally. Therefore the DDD of 3,000mg for aspirin for anti-inflammatory or analgesic indications, although not commonly used at this dose in Ireland (maximal daily dosing 3,600mg),[124] may be more commonly used in other countries. An increase in aspirin use was observed across all age-bands when the population aged 45 years and older was examined. On examination of the average dose of aspirin prescribed to males and females it is clear that the majority of aspirin prescribed was for low-dose (≤75 mg) aspirin; suggesting that the majority of aspirin prescribed is indicated for prevention of cardiovascular events. The highest prevalence of prescribing was in men in the oldest age-bands; this is consistent with the cardiovascular indications of aspirin as the prevalence of angina, stroke and myocardial infarction increase with increasing age.

2.6 Chapter Summary

The description of the databases and covariates used for the studies carried out in this thesis sets the scene for this research. The comparison of the GMS-eligible prostate cancer patients in with the entire cohort of men diagnosed with prostate cancer illustrates the representativeness of this cohort. The descriptive drug utilisation analysis of both digoxin and aspirin has shown that men who receive these medicines are of a similar age as those who are diagnosed with prostate cancer. There is mounting evidence from pre-clinical and observational studies that digoxin and aspirin have anti-cancer properties; therefore the study of the potential anti-cancer effects of these medicines in a population-based cohort such as the NCRI-PCRS database is therefore both feasible and warranted.

Chapter 3 DIGOXIN AND PROSTATE CANCER

The pre-clinical studies which have examined the potential for digoxin in prostate cancer, and proposed a number of anti-cancer mechanisms of action are reviewed in this chapter. The observational evidence which has assessed digoxin exposure and cancer incidence and outcomes is also presented. Based on this data two studies have been conducted, the first examining the stage and grade of prostate cancer at diagnosis in men who are exposed to digoxin compared to those who are not; the second examining prostate cancer survival in men receiving digoxin. The results from these studies are presented and discussed in light of the existing knowledge regarding digoxin and its potential mechanisms in cancer.

3.1 Preclinical Studies of Cardiac Glycosides in Cancer

The following section outlines the various mechanisms of anti-cancer activity of digoxin which have been investigated. Digoxin and other cardiac glycosides have been shown to have potential in the control of cancer cell growth in a variety of cancer cell lines. These are related to the well-documented pharmacological activity of digoxin on the sodium/potassium ATPase pump,[152] and more recently, the ability of digoxin to alter gene transcription which has been demonstrated through inhibition of HIF-1α expression.[46]

3.1.1 Sodium/Potassium ATPase in Cancer

Cardiac glycosides are sodium/potassium ATPase (Na^+/K^+ ATPase) ligands. The Na^+/K^+ ATPase plasma membrane pump is involved in cell adhesion and cancer progression. It has signal transduction properties and is a target for many substances both endogenous i.e. oestrogens and exogenous i.e. tamoxifen.[153,154] Each of the Na^+/K^+ ATPase pump α-subunit isoforms have differing affinity for the various members of the cardiac glycoside family; and have different isoform expression in different human cancer cell lines.[155,156] As a result different cancer cell lines have differing sensitivities to various cardiac glycosides.

Based on the knowledge that the inhibitory action of cardiac glycosides on the Na^+/K^+ ATPase causes disruption of intracellular calcium homeostasis, the role of increased intracellular calcium in the apoptotic pathway was one of the first to be investigated in cancer cells.[154] Concentration-dependent arrest of growth and apoptosis of prostate cancer cells treated with digoxin has been demonstrated; these effects were most pronounced in the androgen-dependent LNCaP cell line.[157,158] Caspase enzymes were determined to have a significant role in digoxin mediated apoptosis in prostate cancer cells due to the increased intracellular Ca^{2+} and reduced K^+ concentrations;[159] this was shown to cause DNA fragmentation.[157] These effects are illustrated in Figure 3-1. Digoxin also causes fibroblast apoptosis through this mechanism.[160]

Cyclin-dependent kinase 5 and p25 cleavage from p35, (cdk5/p25) are involved in cell cycle regulation and neuronal homeostasis and are distributed in cells of the male reproductive system.[159] Cleavage of p25 from p35 is a calcium dependent reaction, and increased expression of Cdk5/p25 was demonstrated in cells treated with digoxin. Cdk5/p25 were found to be required for digoxin-mediated prostate cancer cell death. This pro-apoptotic property of digoxin in cancer cells is an important anti-cancer mechanism as resisting cell death is one of the hallmarks of cancer.[19]

3.1.2 Extracellular Effects of Digoxin

Changes to the extracellular surface of the cancerous cell can affect the metastatic potential of the tumour. Digoxin has been shown to inhibit FGF-2 release, which is involved in tumour progression.[161] Reduced adherence of breast cancer cells to plates has been noted in the presence of digitalis.[154] Digoxin also sensitises cells to anoikis, a process whereby normal epithelial cells undergo apoptosis on detachment from the extracellular matrix. Digoxin has been demonstrated to reduce the viability of prostate cancer cells in vitro.[162] Cardiac glycoside inhibition of the Na^+/K^+ ATPase pump has also been shown to modify a biosynthetic pathway of extracellular N-glycan structures; this type of modification, can impede the migration and invasion of tumour cells, as well as the formation of distant metastases of prostate tumours in mice.[163]

3.1.3 Hypoxia Inducible Factor 1-alpha

Hypoxia Inducible Factor 1-alpha (HIF-1α) is not expressed in normal prostate cells;[45] however HIF-1α and HIF-1β are found in high-grade PIN a pre-cancerous state, thus highlighting the potential for HIF-1 targeting in both cancer treatment as well as cancer prevention.[164,165] There is an association between tumour grade and HIF-1α expression in breast and brain tumours.[166] This has not been reported in prostate tumours;[167,168] however HIF-1 activates VEGF expression which is associated with higher prostate cancer tumour grade.[168] HIF is involved in both inducing angiogenesis, and reprogramming energy metabolism in hypoxic prostate cancer cells.[174] In prostate cancer, biochemical failure[168] and reduced disease-specific survival have been associated with higher levels of HIF-1α expression.[169] One study reported androgen dependent prostate cancer cells had the lowest levels of HIF-1α expression and androgen independent prostate cancer cells the highest; suggesting that HIF-1α expression is associated with androgen independent prostate cancer.[170]

3.1.3.1 Inhibition of HIF-1α by Digoxin

In high-throughput screening studies, cardiac glycosides, as well as naturally occurring strophanthidin glycosides were found to be inhibitors of HIF-1α expression.[171,172] Cardiac glycosides were found to reduce the expression of HIF-1α and HIF-2α in a concentration-dependent manner and furthermore reduce the expression of the HIF-1 target genes in cancer cells under hypoxic conditions.[46] HIF-1 is a transcription factor formed when HIF-1α, expressed in response to hypoxia, binds with HIF-1β which is constitutively expressed. HIF-1 has been shown to augment the spread of metastases through the vasculature. The effects of HIF-1α

and its inhibition by digoxin are illustrated in Figure 3-1. Digoxin was found to prolong prostate cancer tumour latency, inhibit tumour xenograft growth and reduce the expression of HIF-1 target genes in mouse models.[46] Inhibition of HIF-1α expression by digoxin reduced primary tumour growth and lung metastases of breast cancer xenografts in mice; there was synergy of this effect in combination with doxorubicin.[173] The anti-angiogenic effects of digoxin have also been demonstrated in androgen independent prostate cancer xenografts, where significant inhibition of blood vessel formation and reduced blood vessel density, but not a reduction in tumour growth was reported.[174]

3.1.3.2 Phyto-oestrogenic Effects of Digoxin

Digitalis glycosides have been investigated in pre-clinical studies of breast cancer cell lines which reported the oestrogen receptor (ER) negative cell lines to have increased or equal susceptibility to cardiac glycoside-mediated cell death compared to the ER positive cell lines.[154,175,176] Digoxin has been reported to have phyto-oestrogenic properties,[177] and these properties have been implicated in associations between digoxin exposure and incidence of oestrogen sensitive cancers.[178] Oestrogens have been used previously in the treatment of prostate cancer,[179] and may be used as secondary hormonal therapy in addition to ADT.[38] Two oestrogen receptors ERα and ERβ have been identified in prostatic tissue, and have opposing effects.[180,181] ERα activation in both the epithelium and stroma of the prostate stimulates proliferation, malignancy and inflammation.[181] ERβ activation is pro-apoptotic and anti-proliferative in both BPH and prostate cancer. ERα antagonists, ERβ agonists[181] and agents with mixed agonist/antagonist activity such as raloxifene[182] have been suggested as potential treatment options in prostate cancer.[169] Digoxin has known oestrogenic side-effects, i.e. gynaecomastia, however it has not been determined which, if any, ER subtype digoxin has stronger affinity for.

Cross-talk exists between the HIF-1α and the hormone receptors (ER and AR). It has been proposed that ERβ1 destabilises HIF-1α in prostate cancer cells.[183] However, retained expression of ERβ with endothelial Nitric Oxide Synthase (eNOS), HIF-1α or HIF-2α in some hypoxic prostate cancer phenotypes has been associated with poorer prognosis. Transactivation of the AR can be enhanced by hypoxia in the presence of HIF-1α, in particular at low concentrations of DHT, which would mimic castration resistant prostate cancer.[184]

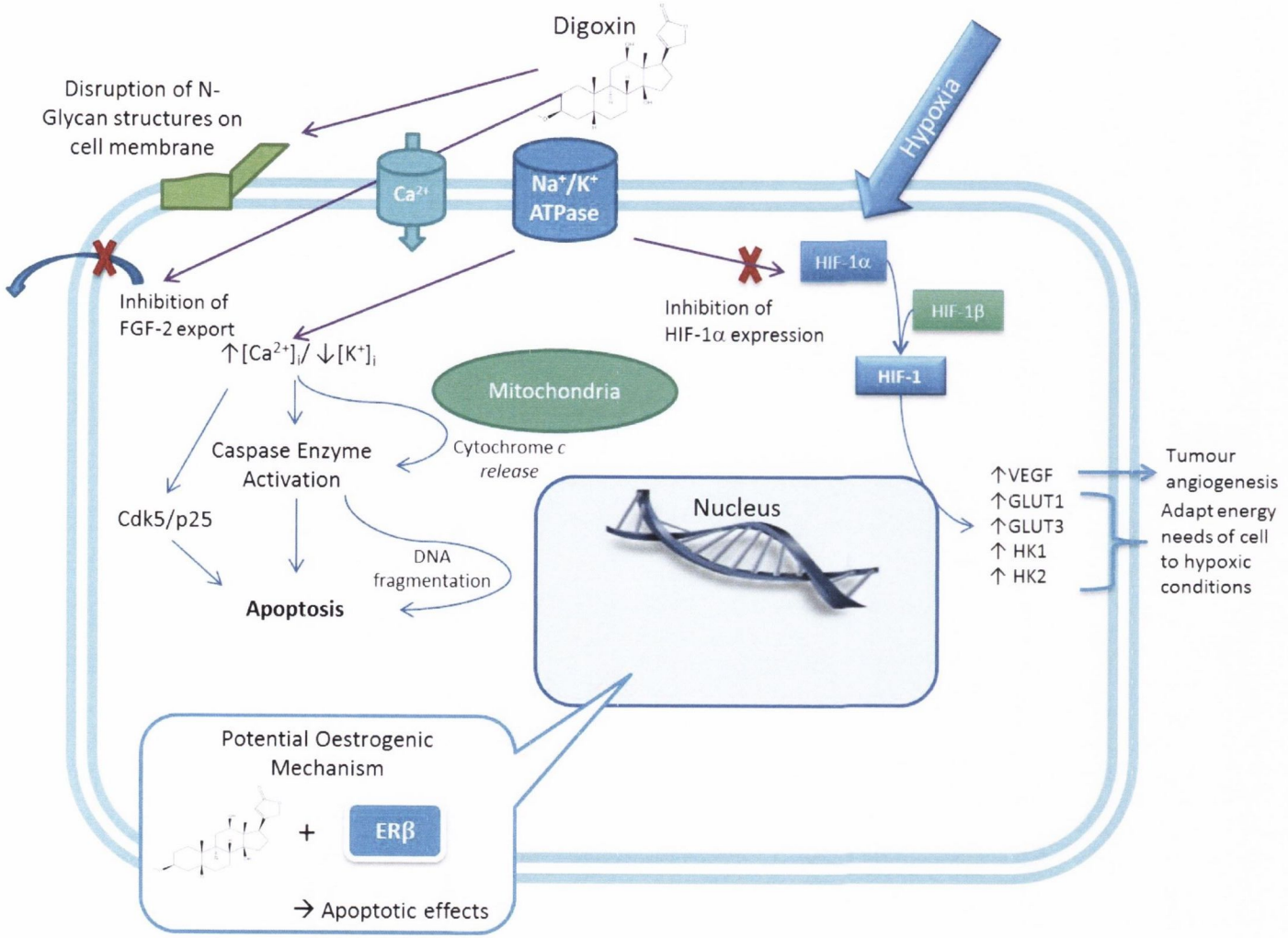

Figure 3-1: **Illustration of the proposed anti-cancer mechanisms of digoxin in prostate cancer cells**

HIF: Hypoxia-Inducible Factor; **GLUT:** Glucose Transporter; **HK:** Hexokinase; **VEGF:** Vascular Endothelial Growth Factor; **FGF-2:** Fibroblast Growth Factor; **ER:** Oestrogen Receptor; **Na^+/K^+ ATP-ase:** Sodium Potassium ATPase;

3.1.4 Potential Interactions of Digoxin

3.1.4.1 Prostate Specific Antigen expression

PSA, as discussed in Chapter 1, Section 1.3.3.1, has been used widely as a means of detection of prostate cancer and as a measure of disease recurrence or biochemical failure.[51] The Prostate Derived ETS Factor (PDEF) gene is a member of the ETS family of transcription factors which regulate cell cycles and are modulators of PSA expression.[185] Digoxin has been shown to reduce PDEF gene expression and hence reduce expression of PSA; however, it has not been elucidated as to whether this is solely through PDEF.[186]

3.1.4.2 Androgen Deprivation Therapy

There is evidence that digoxin inhibits testosterone biosynthesis and secretion in rat testicular cells.[187] This inhibitory effect of digoxin and digitoxin on $CYP450_{scc}$ was shown in rat luteal cells, and Leydig cells, reducing the biosynthesis of progesterone and testosterone.[188] Reduced levels of plasma testosterone and LH have been detected in patients receiving long-term digoxin therapy.[189,190] Furthermore, increased levels of serum oestrogen have been reported in men aged 25-40 years exposed to digoxin.[190] Although this has been refuted in other studies,[191] it is possible that these hormonal effects of digoxin may influence the outcomes of men with prostate cancer treated with ADT. Reported hormonal effects of digoxin on oestrogen dependent cancers will be discussed in Section 3.2.1.

3.1.4.3 Radiotherapy

Cardiac glycosides have been reported to have radio-sensitizing effects in breast cancer cell lines.[192] Cells treated with cardiac glycosides have been reported to accumulate in the G2M phase of the cell cycle which makes them more susceptible to radiation.[192] Topoisomerase II inhibition was also proposed as a possible mechanism for digoxin's anti-cancer activity in renal, breast and melanoma cell lines.[193,194] The lactone moiety of the cardenolide illustrated in Figure 1-5 is structurally similar to the active component of the topoisomerase inhibitor irinotecan.[154] Inhibition of topoisomerase II delays the repair of X-ray induced breaks in DNA and can hence increase radio-sensitivity. HIF-1α expressing cells have specifically been reported to be resistant to radiotherapy.[195] Radiation induces the formation of ROS in the cell, which is pro-apoptotic; ROS may however also induce HIF-1 activity.[195]

3.1.4.4 Chemotherapy

Synergy of digitoxin and digoxin with other chemotherapeutic agents has been demonstrated in breast and colon cancer cell lines.[176,196] In colon cancer cells, increased intracellular calcium

causes increased calmodulin kinase II activity and activation of HIF-1α. HIF-1 activates transcription of the mdr-1 (multiple drug resistance) gene, resulting in the expression of P-glycoprotein (PGP) which can confer multiple drug resistance.[197] Increased PGP expression was observed in colon cancer cells treated with digoxin resulting in reduced intracellular accumulation of doxorubicin (PGP substrate) and thus reduced efficacy. It has been suggested that the efficacy of doxorubicin or other PGP substrates may be reduced in patients treated with digoxin.[197]

Very few men with prostate cancer receive chemotherapy, which is usually indicated only at advanced stages of castrate resistant prostate cancer.[83] In a recent study of men with metastatic prostate cancer treated with docetaxel, digoxin exposure was associated with an increased risk of all-cause mortality (HR=1.43, 95%CI 1.01, 2.03).[198] The authors attributed this increased risk mainly to higher levels of cardiovascular comorbidity in the digoxin exposed group.

3.2 Observational and Clinical Studies of Cardiac Glycosides in Cancer

Preclinical studies have suggested a variety of mechanisms through which digoxin has anti-cancer activity. Despite the encouraging results, concerns have been raised as to the therapeutic relevance of these pre-clinical findings. The digoxin concentrations reported to have anti-cancer effects in these studies exceed those normally tolerated by humans in vivo. It has, however, been suggested that prolonged exposure to digoxin could be sufficient to have an anti-proliferative effect in humans.[46] Observational studies have investigated this hypothesis in relation to the incidence of breast, uterine, ovarian, cervical as well as prostate cancer.

3.2.1 Initial Observational Studies

The presence of an association between cardiac glycoside exposure and cancer outcomes was first hypothesised in the late 1970s, prior to the extensive pre-clinical investigations of cardiac glycosides in cancer which have been carried out in the last decade. In a study of 142 breast cancer patients Stenkvist reported that women receiving cardiac glycosides had tumours which were smaller and more uniform than those not exposed to cardiac glycosides.[199] Following this observation, Stenkvist *et al.* studied a cohort of breast cancer patients and found that the risk of recurrence within five years following mastectomy was lower in the group exposed to cardiac glycosides.[200] Other observational research regarding cardiac glycosides and cancer incidence at this time was inconclusive, mainly because these studies were of small size and limited by the data sources available at the time.[201-203]

Two case-control studies have shown cardiac glycoside exposure to be a risk factor for male breast cancer.[204,205] Another two Danish studies, one case-control,[206] one cohort,[207] reported approximately 30% increased risk of breast cancer in women exposed to digoxin compared to unexposed women, and approximately 40% increased risk when compared to other women receiving treatment for cardiovascular conditions (See Table 3-1). The relative risk was slightly higher for women with ER positive status, which the authors claim point to an oestrogen-mimicking mechanism.[207] A subsequent study reported a significantly increased risk of uterine cancer, but not ovarian or cervical cancer, associated with digoxin exposure; the risk of uterine cancer is increased by exogenous oestrogen exposure.[208]

Table 3-1: Reported associations between digoxin exposure and cancer incidence in observational research

Year	Author	Study Setting	Study type	Exposure	Cancer	Reported Result		(95% CI)
1990	Lenfant-Pejovic *et al.*[204]	France & Switzerland	Case-Control	Digitalis	Breast (male)	OR	4.10	(1.40, 12.40)
2001	Ewertz *et al.*[205]	Norway, Sweden, Denmark	Case –Control	Digoxin ≥5 years	Breast (male)	OR	2.00	(0.90, 4.40)
2008	Ahern *et al.*[206]	Denmark	Case-Control	Digoxin ≥1 year	Breast	OR	1.30	(1.14, 1.48)
2011	Biggar *et al.*[207]	Denmark	Cohort	Digoxin current use	Breast	RR	1.39	(1.32, 1.46)
				Digoxin former use	Breast	RR	0.91	(0.83, 1.00)
2012	Biggar *et al.*[208]	Denmark	Cohort	Digoxin current use	Uterine	IRR	1.48	(1.32, 1.65)
			Cohort	Digoxin current use	Ovarian	IRR	1.06	(0.92, 1.22)
			Cohort	Digoxin current use	Cervical	IRR	1.00	(0.79, 1.25)
2011	Platz *et al.*[61]	US, Health Professionals Follow-up Study	Cohort	Digoxin ever use	Prostate	RR	0.82	(0.72, 0.94)
				Digoxin <5 years	Prostate	RR	0.87	(0.72, 1.04)
				Digoxin 5-9.9 years	Prostate	RR	0.86	(0.70, 1.07)
				Digoxin ≥10 years	Prostate	RR	0.54	(0.37, 0.79)

OR: Odds Ratio; **RR:** Relative Risk; **IRR:** Incidence Risk Ratio;

Only one study in recent years has examined the outcomes of a large cohort of cancer patients exposed to digoxin. Risk of breast cancer relapse was reported to be higher in the first year after diagnosis in women who were exposed to digoxin and had ER positive tumours.[209] In the same study, digoxin exposure was associated with lower grade breast tumours at diagnosis; the authors again claim that this is due to the oestrogenic mechanism of digoxin.[209]

3.2.2 Studies Investigating Digoxin in Prostate Cancer

In a cohort study carried out in the Health Professionals Follow-up study, current digoxin use was associated with reduced prostate cancer incidence; adjusted OR=0.77 (95% CI 0.66, 0.90) compared to no digoxin use.[61] The reduction in prostate cancer risk increased significantly with increasing duration of digoxin exposure (p-trend <0.001). See Table 3-1. Additionally increased duration of digoxin exposure was associated with reduced odds of advanced and lethal prostate cancers, suggesting the potential for digoxin having a pre-diagnostic effect on tumour development.

A phase II single arm clinical study (NCT01162135) of digoxin in recurrent prostate cancer began recruiting patients in 2012. The outcome measure was PSA doubling time following six months of digoxin therapy, compared to baseline PSA doubling time (3-24 months). The findings of the pilot study were presented at the American Society of Clinical Oncology (ASCO) Annual Meeting in June 2013. At 6 months, 7 of the 15 patients had PSA doubling time more than 2-fold that of their baseline PSA doubling time. The authors suggest the need for controlled trials, to assess the potential benefit of digoxin in prostate cancer.[210] However digoxin has been shown to reduce PDEF gene expression and subsequent expression of PSA.[186] This may explain, in part, the results observed in the pilot study.

3.2.3 Summary and Hypothesis for Studies Carried out

Due to the extensive pre-clinical data investigating the effects of digoxin in cancer cell lines and mouse models (Section 3.1); the digoxin exposure and cancer incidence has been of interest to observational researchers, particularly in recent years (Table 3-1). The association reported regarding digoxin exposure and reduced risk of prostate cancer as well as suggestions that digoxin may influence the natural progression of prostate cancer are particularly important.[61]

Based on the hypothesis that digoxin exposure may alter prostate cancer development and may also be associated with differences in survival in digoxin exposed and unexposed men, the following two studies were conducted using the linked NCRI-PCRS dataset described in Chapter 2. Study I, a matched cohort study, examined the association between digoxin exposure in the year prior to prostate cancer diagnosis and prostate cancer tumour grade and tumour stage at diagnosis; Study II, also a cohort study examined the association between digoxin use at the time of prostate cancer diagnosis and prostate cancer-specific mortality.

3.3 Study I: Digoxin Exposure and Prostate Cancer Stage and Grade at Diagnosis

3.3.1 Introduction

Pre-clinical evidence has proposed that digoxin may have anti-cancer activity in a number of tumour types including prostate cancer (Section 3.1). Additionally, an observational study carried out in the US Health Professionals Follow-Up Study has reported digoxin use to be associated with reduced risk of prostate cancer.[61]

Digoxin, due to its indication for atrial fibrillation and heart-failure is more commonly prescribed to older men as illustrated in Chapter 2, Section 2.5. Due to these medical conditions, men who receive digoxin may visit their GP more regularly than men who do not receive digoxin and may therefore be more likely to be offered a PSA test or DRE. In an effort to guard against increased medical surveillance confounding the association between digoxin exposure and the stage at which prostate cancer may be detected, matching was used to identify digoxin unexposed men likely to have similar utilisation of healthcare to digoxin exposed men.

The objectives of this study were to examine in the matched cohort whether digoxin exposure in the year prior to prostate cancer diagnosis was associated with (i) prostate cancer grade at diagnosis or (ii) prostate cancer stage at diagnosis.

3.3.2 Methods

3.3.2.1 Study Cohort

Using the linked NCRI-PRCS database, described in Chapter 2 Section 2.2, men diagnosed with prostate cancer (ICD-O, C61.9)[145] between 1st January 2001 and 31st December 2006, and with eligibility for the GMS scheme in the full year prior to diagnosis were identified. Men with a prior invasive tumour were excluded.

3.3.2.2 Exposure definition

Prescriptions for digoxin (ATC: C01AA05) dispensed in the 360 days prior to diagnosis were identified from the GMS database. Men who had a supply of digoxin available to them in this period were defined as digoxin exposed. The intensity of digoxin dosing during this period was categorised into tertiles based on the proportion of days with a digoxin supply available (high, intermediate, low).

3.3.2.3 Outcome Definition

The outcome was prostate cancer tumour grade at diagnosis (low grade, Gleason Score ≤7; high grade, Gleason Score >7; unspecified); and tumour stage at diagnosis, (Stage I/II/III, Stage IV, unspecified).

3.3.2.4 Matching

It was not possible to adjust for GP visits or other health-care utilisation variables prior to diagnosis; therefore a combination of age and comorbidity score (Chapter 2, Section 2.2.2.3) was used to identify a digoxin-unexposed group with similar levels of healthcare utilisation. The cohort was matched (1:5), on age at diagnosis (± 5 years) and comorbidity score (± 2 agents) using the SAS® macro GMATCH; greedy matching, without replacement.[211] The quality of matching was assessed using standardised differences of means/proportions (*d*) to assess covariate balance between digoxin exposed and unexposed men following matching (d <0.1 considered acceptable). The standardised difference is preferred over other statistical tests for hypothesis, such as χ-squared or t-tests, because it is not as sensitive to smaller sample sizes.

3.3.2.5 Statistical Analysis

Multinomial Logistic Regression (SAS®, PROC LOGISTIC) was used to determine Odds Ratios (OR) and 95% CI for the association of digoxin exposure in the year prior to diagnosis with prostate cancer grade at diagnosis. (low grade, Gleason Score ≤7; high grade, Gleason Score >7; unspecified) and tumour stage at diagnosis, (Stage I/II/III, Stage IV, unspecified). The following covariates were assessed for inclusion in the multivariate model: age,[212] comorbidity score,[150] smoking status,[213,214] tumour stage,[212] (for analysis of associations with tumour grade), tumour grade,[212] (for analysis of associations with tumour stage), diabetes,[74] exposure to aspirin,[131] beta-blockers,[68,69] warfarin,[65,215] statins,[63] NSAIDs[72] and medication used in BPH.[216] Year of prostate cancer incidence was also considered. Backwards elimination with a 10% maximum change in the effect component of the fully adjusted OR was used to select the final multivariate model.[217]

3.3.3 Results

3.3.3.1 Cohort identified

From the linked database, 5,856 eligible men were identified. 375 of 468 patients exposed to digoxin in the year prior to diagnosis were matched to 1875 controls (See Figure 3-2). The characteristics of the matched population are presented in Table 3-2.

Despite matching, assessment of balance of covariates showed differences in the method of diagnosis, with more digoxin-unexposed patients having histologically diagnosed tumours compared to digoxin-exposed patients (84.7% and 77.1% respectively; *d*=0.20). This resulted in an imbalance in the numbers of patients with ungraded tumours, 27.0 % in the unexposed and 33.1% in the exposed (*d*=0.13). Exposure to cardiovascular medications differed between the groups, particularly those frequently co-prescribed with digoxin. Warfarin exposure was particularly imbalanced (*d*=0.81). Medication for BPH was less commonly prescribed to digoxin exposed patients in the year prior to diagnosis (27.6% vs. 33.7%, *d*=0.15).

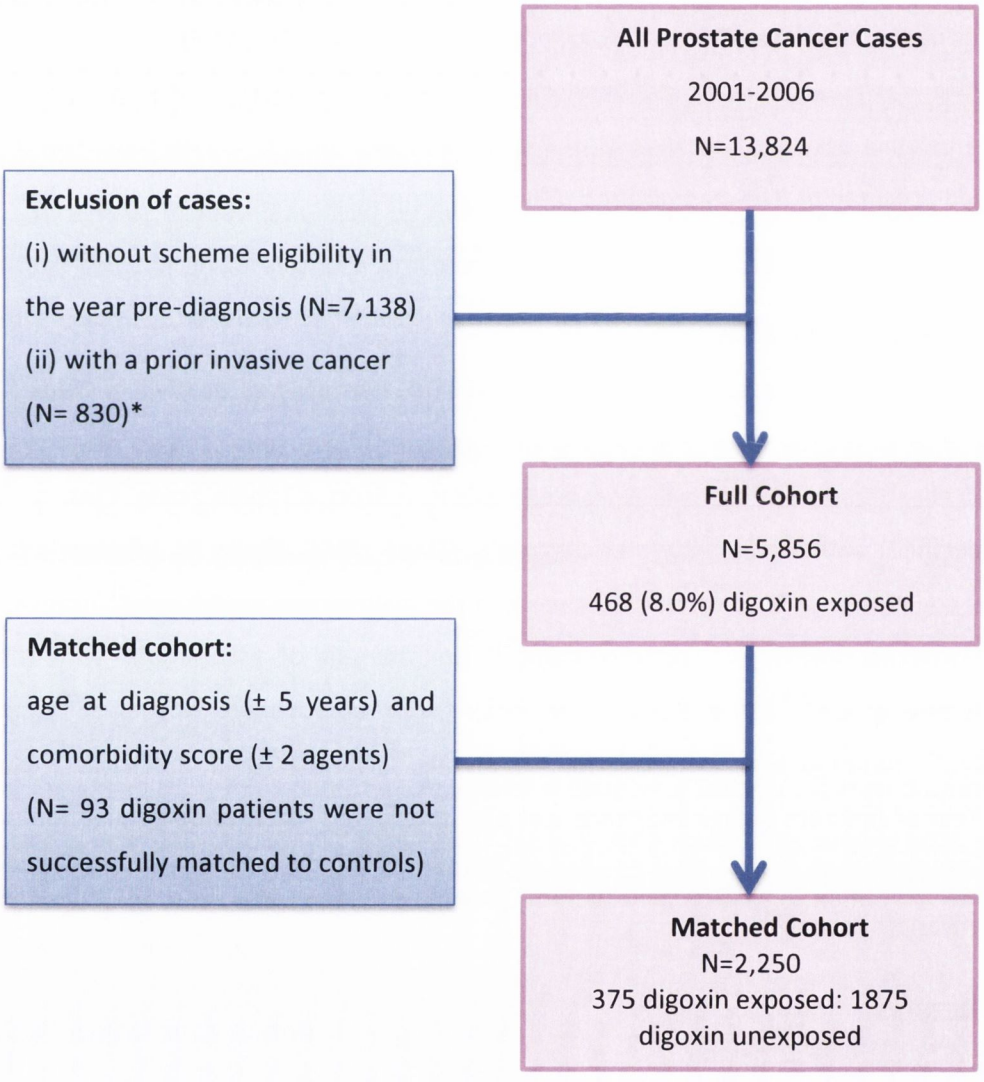

Figure 3-2: Study cohort selection for digoxin Study I: exclusion criteria and matching

*excluding non-melanoma skin cancer

Table 3-2: Cohort characteristics of matched cohort

Characteristic		Digoxin Unexposed (N=1875)	Digoxin Exposed (N=375)	
Patient Details				
Age/years	Mean (SD)	76.4 (6.7)	76.6 (6.8)	
Comorbidity Score[A]	Mean (SD)	11.2 (4.8)	11.3 (4.9)	
Smoking Status[B]	Never	605 (32.3)	120 (32.0)	
(%)	Former	392 (20.9)	74 (19.7)	
	Current	275 (14.7)	56 (14.9)	
	Unspecified	603 (32.2)	125 (33.3)	
Medication Exposures[C] (%)				
	Aspirin	945 (50.4)	195 (52.0)	
	Beta-blocker	551 (29.5)	104 (27.7)	
	Statin	556 (29.7)	93 (24.8)	*
	Warfarin	113 (6.0)	138 (36.8)	*
	Anti-diabetic	199 (10.6)	41 (10.9)	
	NSAID	938 (50.0)	154 (41.1)	*
	BPH medicines	631 (33.7)	100 (26.7)	*
Tumour Details				
Diagnosis	Histology	1588 (84.7)	289 (77.1)	*
	Clinical	144 (7.7)	48 (12.8)	*
	Radiology	104 (5.6)	24 (6.4)	
	Other/Unspecified	39 (2.1)	14 (3.7)	*
Stage[D]	I	65 (3.5)	11 (2.9)	
	II	805 (42.9)	143 (38.1)	
	III	135 (7.2)	24 (6.4)	
	IV	303 (16.2)	76 (20.3)	*
	Unspecified	567 (30.2)	121 (32.3)	
Grade[D]	Low (Gleason ≤7)	982 (52.4)	182 (48.5)	
	High (Gleason >7)	388 (20.6)	69 (18.4)	
	Unspecified	507 (27.0)	124 (33.1)	*

** Standardised differences between exposed and unexposed groups >0.10*

***A:** Number of distinct medication classes received in the year prior to diagnosis*

***B:** Smoking status recorded at diagnosis*

***C:** Medication exposures in the 360 days prior to diagnosis*

***D:** AJCC Staging Manual 5th Ed.*[43]

3.3.3.2 Prostate Cancer Grade at Diagnosis

Digoxin exposure was not associated with reduced odds of Gleason score >7 prostate cancer compared to Gleason score ≤7 prostate cancer, in unadjusted analysis (OR=0.96 95% CI 0.71, 1.30); the adjusted OR was similar (OR=0.93, 95% CI 0.67, 1.30). See Table 3-3. The highest dosing intensity tertile of exposed patients had a non-significant reduced odds of Gleason score >7 prostate cancer compared to unexposed patients, adjusted OR=0.70 (95% CI 0.39, 1.23). As there were significantly more digoxin exposed men with tumours of unspecified

grade in the analysis it is difficult to infer anything from this result. The trend across the tertiles of exposure was not significant (p-trend=0.50).

3.3.3.3 PROSTATE CANCER STAGE

Unadjusted analysis found digoxin exposure to be associated with increased odds of Stage IV prostate cancer, (OR=1.42, 95% CI 1.05, 1.91). The adjusted analysis showed a similar OR but was non-significant (OR=1.37, 95% CI 0.98, 1.90). Adjusted analysis of the tertiles of exposure showed a non-significant linear trend p-trend=0.28) in the odds ratio for advanced (Stage IV) versus localised prostate cancer (Table 3-3).

Table 3-3: Odds Ratios for the association between digoxin exposure in the year prior to diagnosis and prostate cancer tumour grade and stage at diagnosis

Digoxin Exposure		Total N	Prostate cancer Gleason Score >7 at diagnosis (Ref: Gleason Score <7)			Prostate cancer stage IV at diagnosis (Ref: tumour stage I-III)		
			N	Univariate OR (95%CI)	Multivariate OR[A] (95%CI)	N	Univariate OR (95%CI)	Multivariate OR[B] (95%CI)
Digoxin unexposed		1,875	386	Ref -	Ref -	303	Ref -	Ref -
Digoxin exposed		375	69	0.96 (0.71, 1.30)	0.93 (0.67, 1.30)	76	1.42 (1.05, 1.91)*	1.37 (0.98, 1.90)
Exposure response:[C] dosing intensity (%)								
Low	(0.5-74.4%)	133	26	1.07 (0.67, 1.71)	0.92 (0.56, 1.51)	28	1.63 (1.02, 2.61)	1.60 (0.98, 2.62)
Intermediate	(76.4-96.9%)	121	25	1.25 (0.76, 2.04)	1.24 (0.74, 2.09)	28	1.75 (1.09, 2.82)	1.49 (0.90, 2.47)
High	(97.2-100%)	121	18	0.66 (0.39, 1.13)	0.70 (0.39, 1.23)	20	0.98 (0.58, 1.63)	1.02 (0.59, 1.76)
P-trend				*0.45*	*0.50*		*0.14*	*0.28*

** p-value < 0.05.*

***A:** Adjusted for age, tumour size, smoking status at diagnosis, year of incidence, aspirin and warfarin exposure.*

***B:** Adjusted for age, year of incidence NSAID and warfarin exposure.*

***C:** Reference group: digoxin unexposed*

3.3.4 Discussion

3.3.4.1 Prostate Cancer grade at diagnosis

In this analysis of men diagnosed with prostate cancer, a significant association between digoxin exposure and tumour grade at diagnosis was not observed. Platz *et al.* reported current digoxin use to be associated with non-significantly reduced risk of Gleason Score >7 prostate cancer (adjusted RR=0.79, 95% CI 0.50, 1.24);[61] however this was only in patients who had prostatectomy or prostate biopsy. Similarly, in breast cancer, women exposed to digoxin were significantly more likely to be diagnosed with low-grade tumours.[209] The findings for prostate cancer grade at diagnosis in this analysis do not suggest such an association in this cohort. The lack of an association in this study does not corroborate with prior studies which have suggested that digoxin use may be associated with reduced odds of higher grade tumours.[61] However, significantly more digoxin exposed patients in this analysis did not have their tumours diagnosed histologically.

3.3.4.2 Prostate Cancer stage at diagnosis

Digoxin exposure was not significantly associated with increased odds of stage IV cancer, (compared to stage I/II/III). There are other confounders that could not be adjusted for such as GP visits, referrals to urological specialists or repeated PSA testing. These results conflict with those reported by Platz *et al.* where a significantly reduced risk of organ confined (T1b-T2b, and N0M0) prostate cancer associated with digoxin use (adjusted RR=0.70, 95% CI 0.52, 0.94) was reported.[61] The reduction in risk of advanced or lethal prostate cancer (≥T3b, or N1 or M1) at diagnosis associated with digoxin use was non-significant (adjusted RR=0.75, 95% CI 0.54, 1.06). However these subgroup analyses were based on very small numbers of patients.

The study by Platz *et al.* differs from this study in the Irish cohort. Firstly it was carried out in the Health Professionals Follow-up Study, a US cohort which would not be representative of the entire population; secondly medication exposures were self-reported, not ascertained from prescription claims. However, the study by Platz *et al.* covers a longer time period, and also has information on diet and other lifestyle factors, including PSA testing which can be adjusted for.

Concerns have also been raised about the bleeding risk associated with prostatic biopsy[218] and external beam radiation therapy[219] in men who are receiving anti-coagulant (warfarin) therapy.[220] Thus it is plausible that digoxin-treated men especially those co-prescribed warfarin may undergo more conservative prostate cancer diagnostic or staging procedures,

should they have an abnormal PSA or DRE. This may explain why despite matching on age and comorbidity score significantly fewer digoxin exposed men had their tumour Gleason score determined.

No significant trends were observed for increased dosing intensity in the year prior to diagnosis and prostate cancer grade or stage at diagnosis. Examination of a longer pre-diagnostic period may have been warranted; however as the cohort is a prevalent group of digoxin patients many of these patients were would have been exposed to digoxin for longer than just one year. Claims data over a longer period prior to diagnosis were not available for all patients. In the examination of a longer pre-diagnostic period, far fewer patients would have met the inclusion criteria and power would be substantially reduced.

An additional limitation of this study is that the matching criteria resulted in only 375/468 patients who had any digoxin exposure being included in the analysis. While this limits the statistical power of the study, broader matching criteria would have led to even more significant differences in the percentage of patients with unknown or missing tumour stage or tumour grade. Thus this could have potentially introduced other biases.

3.4 Study II: Digoxin Exposure and All-Cause and Prostate Cancer-Specific Mortality

3.4.1 Introduction

The findings of preclinical studies (Section 3.1) suggest that digoxin exposure may be associated with reduced prostate cancer tumour progression. To date, observational research has focused on associations between digoxin exposure and cancer incidence. The aims of this study were to assess the association between digoxin exposure at the time of diagnosis and mortality (prostate cancer-specific and all-cause mortality). This study has been accepted for publication. See Appendix 1 for the manuscript.

As observed in Study I, the extent of prostate cancer investigations may be influenced by cardiovascular comorbidity. Similarly, cardiovascular comorbidity associated with indications for digoxin use may confound associations between digoxin exposure and prostate cancer outcomes through differential effects on the selection and use of prostate cancer treatments.[219,221-223] Digoxin exposure at the time of diagnosis (based on having a digoxin supply available in the 90 days prior to diagnosis) was identified. To address possible confounding a propensity score model was then developed to predict digoxin exposure at the time of prostate cancer diagnosis and thus identify patients with similar cardiovascular comorbidity to digoxin exposed men. Secondary analyses of all-cause and prostate cancer-specific mortality were carried out using propensity score trimmed and matched cohorts.[224]

In light of the evidence of preclinical studies indicating potential for cancer treatment with radiation or ADT to interact with digoxin, the association between digoxin exposure and prostate cancer mortality was stratified by treatment receipt (radiation, ADT).

3.4.2 Methods

3.4.2.1 Study cohort

Using the linked NCRI-PRCS database, described in Chapter 2, Section 2.2 the study cohort was identified. Men were eligible for the study if they had a diagnosis of prostate cancer (ICD-O, C61.9)[145] between 1st January 2001 and 31st December 2006, and eligibility for the GMS scheme in the full year prior to diagnosis. Men with a prior invasive tumour and men in whom a prostate cancer diagnosis was made at death or autopsy were excluded.

3.4.2.2 Exposure definition

Prescription claims for digoxin (ATC: C01AA05) in the 90 days prior to diagnosis were used to identify men exposed to digoxin at the time of prostate cancer diagnosis. Digoxin exposure in the 90 days prior to diagnosis was stratified in two ways: (i) by dosing intensity tertiles (low, intermediate, high), based on the proportion of the 90 days with a supply of digoxin available; and (ii) by digoxin dose dispensed (low, ≤125 mcg; high, > 125 mcg). These stratifications were used to conduct exposure-response analyses.

3.4.2.3 Outcome Definition

Information from death certificates, linked to the NCRI database, was used to identify the date and cause of death. Cause of death was classified in two ways: (i) prostate cancer-specific deaths (ICD-9 185; ICD-10 C61) and (ii) deaths from all-causes. All men accrued follow-up time from the date of diagnosis to the first of death or the end of follow-up (31st December 2009).

3.4.2.4 Statistical Analysis

The frequency and proportion of digoxin exposed and unexposed men were tabulated by clinical and demographic variables. Unadjusted all-cause and prostate cancer-specific mortality rates were calculated for digoxin exposed and unexposed men. Univariate and multivariate hazard ratios (HR) and 95% CIs for associations between digoxin exposure and (i) prostate-cancer-specific mortality (ii) all-cause mortality were estimated using Cox proportional hazards models (SAS, PROC PHREG). Covariates were assessed for inclusion in the multivariate model based on prior knowledge of potential predictors of prostate cancer mortality (age;[212] comorbidity score;[150] smoking status;[213,214] tumour stage;[212] tumour grade;[212] diabetes;[74] and exposure to aspirin,[131] beta-blockers,[69,148] warfarin,[215] statins,[225] NSAIDS[225] or drugs used for the treatment of BPH[216]). The year of incidence, and treatments received in the year following diagnosis (surgery, radiation, ADT; time dependent covariates) were also assessed for inclusion. Backwards elimination of variables in a stepwise manner up to a 10% maximum cumulative change in the effect component of the fully adjusted HR, was used to select the final multivariate model.[217] A SAS® macro[226] was used to construct directly adjusted survival curves.

3.4.2.5 Tests for Interaction

Tests for interaction were carried out based on prior studies which have suggested that the oestrogenic effects of digoxin may have a role in its effects on cancerous tumours (Section 3.1.4 and 3.1.5.2), and that cardiac glycosides may sensitise cancer cells to radiation (Section 3.1.5.3). The association between digoxin exposure and prostate cancer-specific mortality is

examined across strata of patients who did and did not receive radiation therapy and ADT (yes, no; time-varying) in the year following diagnosis. Measures of interaction were estimated on a multiplicative scale (ratio of hazard ratios, rHR) with 95% CIs. These results are presented according to recommendations by Knol *et al.*[227]

3.4.2.6 Rationale for Propensity Score

A propensity score is defined as the probability of treatment assignment conditional on observed covariates.[228] Propensity score matching enables efficient matching of exposed to unexposed men, out-performing other matching methods, and balances the distribution of measured confounders in the exposed and unexposed groups.[229] The multivariate regression model used to develop the propensity score is not designed to be an accurate predictor of digoxin exposure in any population but is used as a means of balancing the distribution of confounders between the digoxin exposed and unexposed men. The dependent variable was digoxin exposure in the 90 days prior to diagnosis (yes/no); regressed on other baseline patient characteristics, such as patient age, comorbidity score, and co-prescribed medication using logistic regression.

A strength of this method is that it removes from the analysis patients who are not comparable to the men receiving digoxin,[229] however it can, in some situations, limit the external validity of the study. This is not an infallible method; matching on the propensity score does not necessarily balance unmeasured confounders in the same way as randomisation would.[229]

3.4.2.7 Propensity Score Development & Matching

Covariates were assessed for inclusion in the propensity score model based on prior knowledge of demographic covariates associated with cardiovascular comorbidity (age, comorbidity score) and exposure to cardiovascular medications commonly co-prescribed with digoxin.[230] See supplementary Table A2 in Appendix 1 for the full list of covariates included in the model. Logistic regression (SAS® PROC LOGISTIC) was used to estimate propensity scores for digoxin exposure using these covariates. Main effects, interaction terms and quadratic or cubic terms were included as appropriate.[231] Covariate balance within propensity score quintiles was assessed by standardised differences (d), with a $d<0.1$ being the desired limit.[232] It is acknowledged that in the building of propensity score models, it is not always possible for the standardised differences of all covariates to be below a particular threshold.[233] An iterative approach was used to arrive at the final multivariate model which achieved best balance between digoxin exposed and unexposed men across propensity score quintiles. The cohort

was then trimmed; men with a propensity score outside the 1st to 99th percentile for digoxin exposed men were excluded.[229] The propensity score was then re-estimated in this trimmed population.[231] Digoxin exposed and unexposed men were then matched (1:1)[224] within a calliper of 0.2 standard deviations of the propensity score logit[232,234] using greedy matching without replacement.[211] Covariate balance between digoxin exposed and unexposed men in the matched cohort was assessed by standardised differences (d <0.1). Survival analyses were repeated in both the propensity score trimmed and matched cohorts.

3.4.2.8 SENSITIVITY ANALYSES

Sensitivity analyses were conducted in two ways to assess the potential misclassification of prostate cancer-specific mortality on death certificates. Firstly mortality from prostate cancer was defined using ICD mortality site codes to include other potential causes of death, by which prostate cancer death may have been misclassified, as prostate cancer death.[235] (Table 3-4) The second sensitivity analysis classified as prostate cancer death, any deaths where prostate cancer was classified as the other or contributory cause of death on the death certificate.

Table 3-4: Potential other cancer sites which prostate cancer death may be misclassified:[235]

Cancer Site	ICD 9 Code	ICD 10 Code
Malignant neoplasm of prostate	185	C61
Malignant neoplasm of other male genital organs, site unspecified	187.9	C63.9
Malignant neoplasm of pelvis	195.3	C41.4
Secondary malignant neoplasm	196-198	C76-C80
Malignant neoplasm without specification of site	199	C80.9
Benign neoplasm of prostate	222.2	D29.1
Benign neoplasm of male genital organs, site unspecified	222.9	D29.9
Neoplasm of uncertain behaviour of prostate	236.5	D40.0
Neoplasm of uncertain behaviour of other and unspecified male genital organs	236.6	D40.9
Neoplasm of uncertain behaviour, site unspecified	238.9	D48.9
Neoplasm of unspecified nature of other genitourinary organs	239.5	D40.7, D41
Neoplasm of unspecified nature, site unspecified	239.9	D48.9

3.4.3 Results

3.4.3.1 Cohort Characteristics

A flow diagram outlining the study cohort selection is presented in Figure 3-3. The characteristics of digoxin exposed and unexposed men in the full cohort and the propensity score matched cohort are presented in Table 3-5.

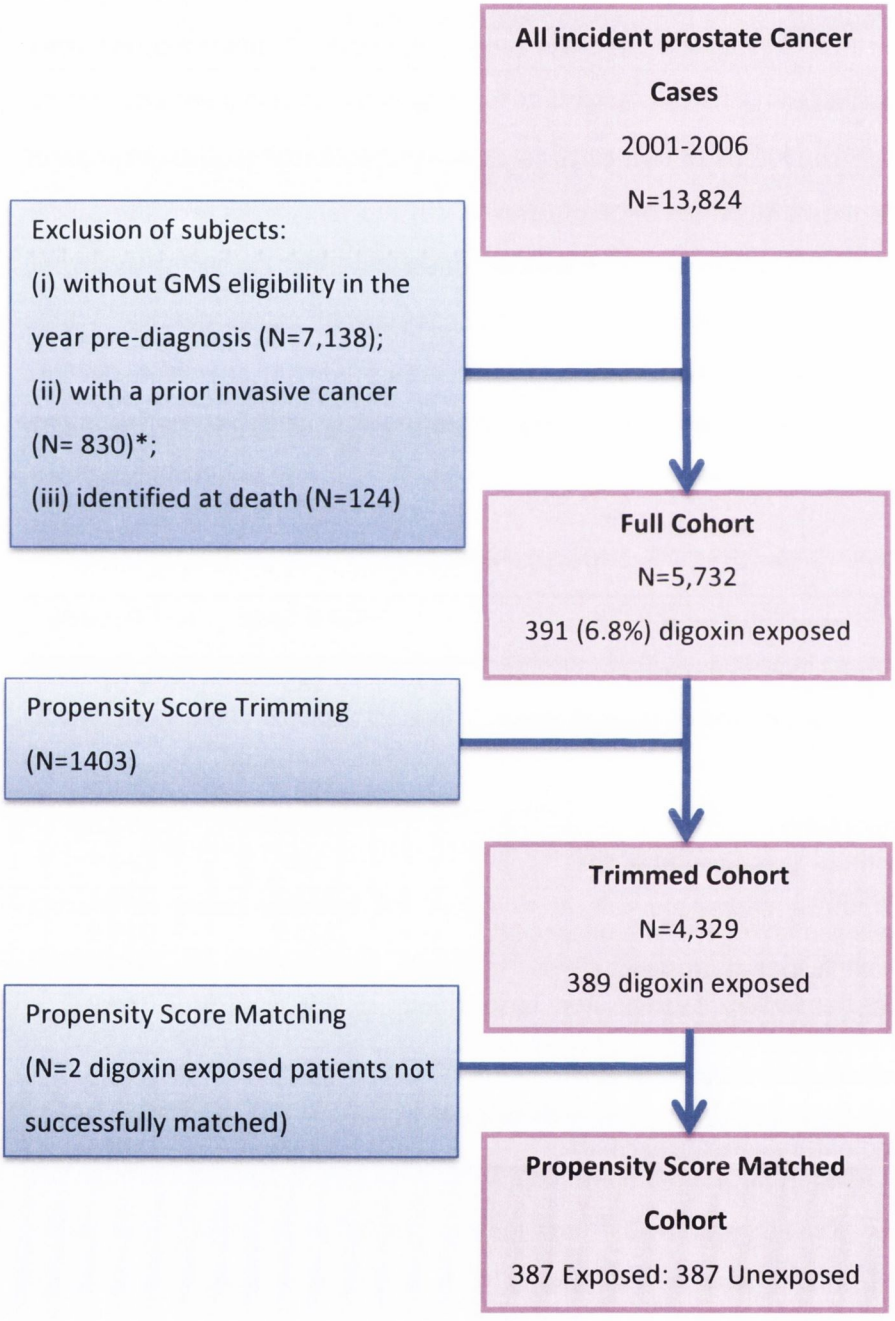

Figure 3-3: Study cohort selection for digoxin Study II: exclusion criteria and propensity score development and matching.

* other than non-melanoma skin cancer

Table 3-5: Characteristics of digoxin exposed and unexposed men for survival analysis

Characteristic		Full cohort			Propensity score matched cohort		
		Unexposed (N=5,341)	Exposed (N=391)		Unexposed (N=387)	Exposed (N=387)	
Patient details							
Age/years	Mean (SD)	73.1 (7.9)	77.5 (7.2)	*	77.8 (7.0)	77.5 (7.2)	
Comorbidity Score	Mean (SD)	8.9 (6.2)	13.2 (6.6)	*	13.0 (6.3)	13.2 (6.5)	
Smoking Status - (%)	Never	1,712 (32.1)	134 (34.3)		143 (37.0)	131 (33.9)	
	Former	1,020 (19.1)	85 (21.7)		93 (24.0)	85 (22.0)	
	Current	853 (16.0)	55 (14.1)		47 (12.1)	55 (14.2)	
	Unspecified	1,756 (32.9)	117 (29.9)		104 (26.9)	116 (30.0)	
Tumour details							
Stage - (%) [A]	I	166 (3.1)	12 (3.1)	*	19 (4.9)	12 (3.1)	
	II	2,589 (48.5)	147 (37.6)		151 (39.0)	145 (37.5)	
	III	410 (7.7)	22 (5.6)		17 (4.4)	22 (5.7)	
	IV	761 (14.2)	84 (21.5)		86 (22.2)	82 (21.2)	
	Unspecified	1,415 (26.5)	126 (32.2)		114 (29.5)	126 (32.6)	
Grade - (%)	Gleason score <5	334 (6.3)	30 (7.7)	*	23 (5.9)	30 (7.8)	
	Gleason score 5-7	2,769 (51.8)	155 (39.6)		167 (43.2)	152 (39.3)	
	Gleason score >7	1,061 (19.9)	70 (17.9)		75 (19.4)	70 (18.1)	
	Unspecified	1,177 (22.0)	136 (34.8)		122 (31.5)	135 (34.9)	
Treatment details							
Treatment - (%) [B]	Surgery	1,339 (25.1)	98 (25.1)		87 (22.5)	97 (25.1)	
	Radiotherapy	1,509 (28.3)	52 (13.3)	*	66 (17.1)	51 (13.2)	~
	ADT	2,617 (49.0)	198 (50.6)		202 (52.2)	195 (50.4)	
	Chemotherapy	111 (2.1)	6 (1.5)		7 (1.8)	6 (1.6)	
	No Treatment	1,174 (22.0)	106 (27.1)	*	103 (26.6)	106 (27.4)	
Medication Exposures - (%)	Aspirin	1,986 (37.2)	194 (49.6)	*	197 (50.9)	194 (50.1)	
	Beta-blocker	1,158 (21.7)	97 (24.8)		122 (31.5)	95 (24.5)	~
	Statin	1,293 (24.2)	95 (24.3)		91 (23.5)	95 (24.5)	

	Warfarin	214 (4.0)	150 (38.4)	*	91 (23.5)	148 (38.2)	~
	Anti-diabetic	417 (7.8)	48 (12.3)	*	32 (8.3)	48 (12.4)	~
	NSAID	1,833 (34.3)	139 (35.5)		151 (39.0)	136 (35.1)	
	BPH medicines	1,393 (26.1)	111 (28.4)		125 (32.3)	109 (28.2)	
Digoxin exposure (90 days pre-diagnosis)							
No of prescriptions dispensed		- -	1,030		- -	1,011	
Dosing intensity - (%)	Median (IQR)	- -	84.6 (75.6, 100)		- -	84.5 (75.6, 100)	
Digoxin exposure (1 year post-diagnosis)							
No of prescriptions dispensed		567	3,374		99	3,336	
Dosing intensity - (%)	Median (IQR)	0.01 (0.0, 0.0)	69.6 (36.4, 99.2)		1.8 (0.0, 0.0)	69.5 (36.4, 99.2)	

** p-value <0.05;*

~ Standardised differences between exposed and unexposed groups >0.10

***A:** AJCC Staging Manual 5th Ed.*[43]

***B:** Treatment received within one year following diagnosis (not mutually exclusive)*

In the full cohort digoxin exposed men (n=391) were significantly older than unexposed men (n=5,341); mean age 77.5 years and 73.1 years respectively. They also had higher comorbidity scores (13.2 compared to 8.9, $p<0.05$). Digoxin exposed men were more likely to have stage IV disease and less likely to have received radiation. For full cohort characteristics see Table 3-5. Men in the low, intermediate and high dosing intensity tertiles had mean post-diagnostic digoxin exposures in the year post-diagnosis of 53.8%, 70.8% and 80.6% respectively. The median follow-up was 4.3 years.

In the propensity score trimmed cohort, differences between exposed and unexposed men were reduced, but remained significant for some covariates, including age and comorbidity score. In the propensity score matched cohort acceptable balance for matched covariates was achieved between digoxin exposed (n=387) and unexposed men (n=387). Tumour stage, tumour grade and treatment received in the year following diagnosis were also comparable between the matched groups, although digoxin exposed men were marginally less likely to have been treated with radiation (13.2% versus 17.1%, d=0.11). More digoxin-exposed men received warfarin compared to digoxin unexposed men (d=0.32)

3.4.3.2 Survival Analyses

Estimated hazard ratios for the association between digoxin exposure and prostate cancer-specific mortality in the full, trimmed and propensity score matched cohorts are presented in Table 3-6. Adjusted cumulative probability plots for the association between digoxin exposure and prostate cancer-specific and all-cause mortality in the full cohort are presented in Figure 3-4.

A non-significant risk of prostate cancer-specific mortality was observed in the full cohort (multivariate HR=1.13, 95%CI 0.91, 1.42). The adjusted hazard ratios for prostate cancer-specific mortality for the trimmed (HR=1.12, 95%CI 0.90, 1.41) and propensity score matched cohorts (HR=1.17, 95%CI 0.88, 1.57) were not appreciably different. See Table 3-6. Adjusted cumulative probability plots indicate that associations between digoxin exposure and prostate cancer-specific mortality did not vary considerably over time (Figure 3-4). No trend was observed for associations between prostate cancer-specific mortality and increasing dosing intensity (*P-trend*=0.59) or dose (*P-trend*=0.19).

Table 3-6: Survival analysis results: univariate and multivariate Hazard Ratios for digoxin exposure and prostate cancer-specific mortality

			Prostate cancer-specific mortality		
Digoxin exposure	**N**	**Person Years**	**No. of deaths (rate)[A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI)[B]**
Digoxin unexposed	5,341	22,774	995 (43.7)	Ref -	Ref -
Digoxin exposed	391	1,277	103 (80.7)	1.77 (1.45, 2.17)	1.13 (0.91, 1.42)
Exposure response:[C] dosing intensity					
Dosing intensity 0%-85%	117	319	33 (103.5)	2.22 (1.57, 3.14)	1.18 (0.83, 1.68)
Dosing intensity 86%-99%	120	413	33 (79.6)	1.78 (1.26, 2.52)	1.39 (0.97, 1.98)
Dosing intensity 100%	154	544	37 (67.9)	1.50 (1.08, 2.08)	0.93 (0.65, 1.32)
P-trend				*<0.01*	*0.60*
Exposure response:[C] dose					
Dose ≤ 125mcg	241	720	65 (90.3)	1.95 (1.52, 2.51)	1.07 (0.82, 1.41)
Dose > 125mcg	150	557	38 (68.2)	1.54 (1.11, 2.12)	1.25 (0.89, 1.75)
P-trend				*<0.01*	*0.19*
Propensity score trimmed cohort analysis					
Digoxin unexposed	3,940	15,938	833 (52.3)	Ref -	Ref -
Digoxin exposed	389	1,272	102 (80.2)	1.49 (1.21, 1.82)	1.12 (0.90, 1.41)
Propensity score matched cohort analysis					
Digoxin unexposed	387	1,339	105 (78.4)	Ref -	Ref -
Digoxin exposed	387	1,269	101 (79.6)	1.02 (0.77, 1.34)	1.17 (0.88, 1.57)

A: *Deaths per 1000 person years*

B: *All multivariate hazard ratios are adjusted for age (years, continuous), comorbidity score (number of medication classes, continuous) tumour stage (I, II, III, IV unspecified), tumour grade (I, II, III/IV, unspecified), smoking status at diagnosis, year of incidence, warfarin exposure and statin exposure.*

C: *Reference group: digoxin unexposed*

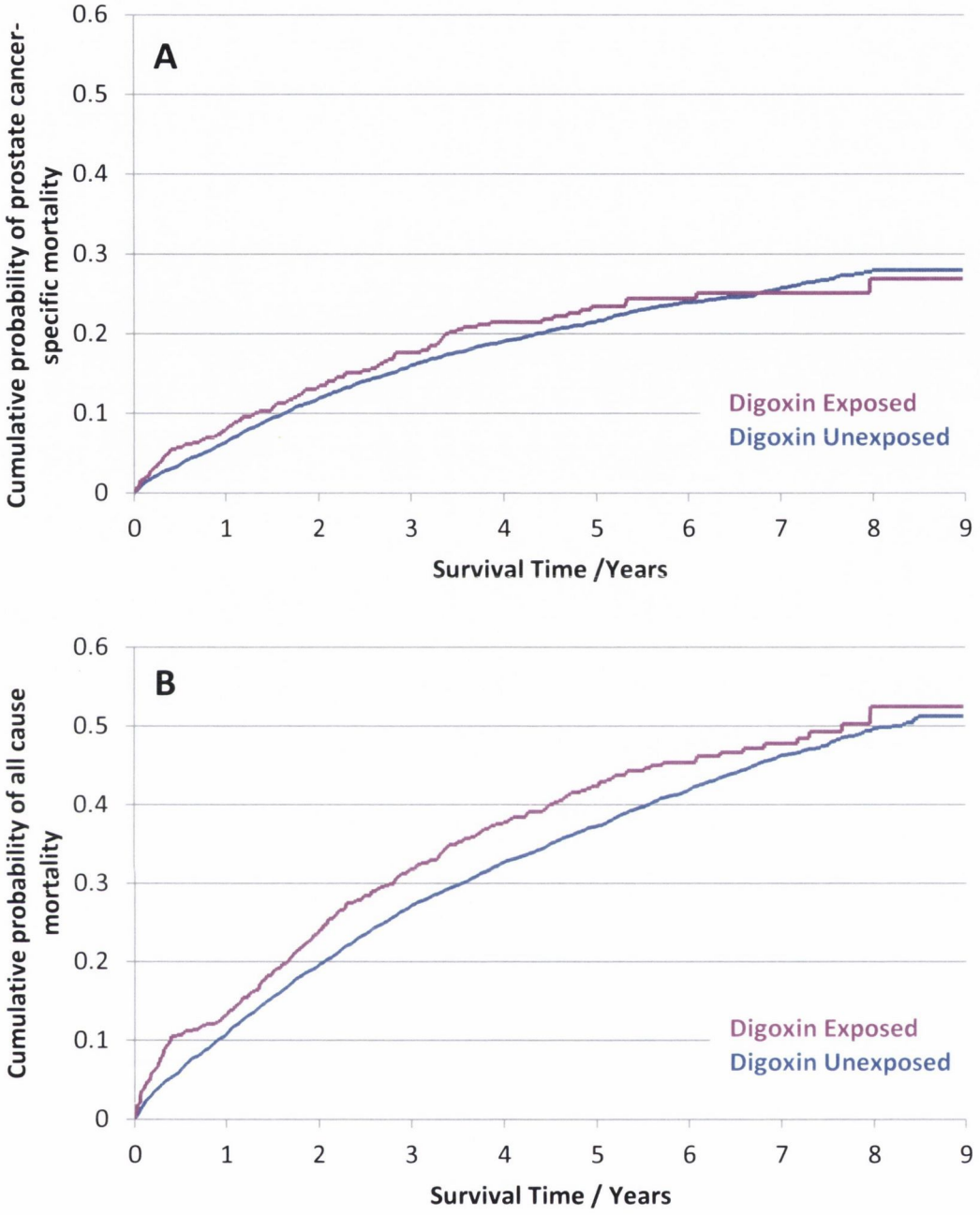

Figure 3-4: Adjusted cumulative probability curves of (A) prostate cancer-specific and (B) all-cause mortality

Table 3-7: Survival analysis results: univariate and multivariate Hazard Ratios for digoxin exposure and all-cause mortality

			All-cause mortality		
Digoxin exposure	**N**	**Person Years**	**No. of deaths (rate)[A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI)[B]**
Digoxin unexposed	5,341	22,774	2096 (92.0)	Ref -	Ref -
Digoxin exposed	391	1,277	253 (198.1)	2.11 (1.86, 2.41)	1.24 (1.07, 1.43)
Exposure response:[C] dosing intensity					
Dosing intensity 0%-85%	117	319	89 (279.2)	2.94 (2.38, 3.63)	1.59 (1.27, 1.97)
Dosing intensity 86%-99%	120	413	78 (188.9)	2.02 (1.61, 2.54)	1.33 (1.05, 1.67)
Dosing intensity 100%	154	544	86 (157.9)	1.69 (1.36, 2.10)	0.93 (0.74, 1.18)
P-trend				*<0.01*	*0.28*
Exposure response:[C] dose					
Dose ≤ 125mcg	241	720	168 (233.4)	2.47 (2.11, 2.89)	1.24 (1.04, 1.46)
Dose > 125mcg	150	557	85 (152.5)	1.65 (1.32, 2.04)	1.24 (0.99, 1.56)
P-trend				*<0.01*	*<0.01*
Propensity score trimmed cohort analysis					
Digoxin unexposed	3,940	15,938	1,780 (111.7)	Ref -	Ref -
Digoxin exposed	389	1,272	252 (198.1)	1.75 (1.53, 2.00)	1.23 (1.07, 1.43)
Propensity score matched cohort analysis					
Digoxin unexposed	387	1,339	234 (174.8)	Ref -	Ref -
Digoxin exposed	387	1,269	250 (197.1)	1.13 (0.94, 1.35)	1.20 (1.00, 1.45)

A: *Deaths per 1000 person years*

B: *All multivariate hazard ratios are adjusted for age (years, continuous), comorbidity score (number of medication classes, continuous) tumour stage (I, II, III, IV unspecified), tumour grade (I, II, III/IV, unspecified), smoking status at diagnosis, year of incidence, warfarin exposure and statin exposure.*

C: *Reference group: digoxin unexposed*

In the full cohort digoxin use was associated with a statistically significant increase in the risk of all-cause mortality (multivariate HR=1.24, 95%CI 1.07, 1.43). Adjusted estimates for all-cause mortality were similar in the propensity score trimmed (HR=1.23, 95%CI 1.07, 1.43) and matched populations (HR=1.20, 95%CI 1.00, 1.49). See Table 3-7. In multivariate exposure-response analyses a non-significant trend was seen for increasing dosing intensity associated with all-cause mortality (*P-trend*=0.28); however the trend for increasing digoxin dose was significant (*P-trend*=0.01).

3.4.3.3 Tests for Interaction

The results of the analyses examining the association between digoxin exposure and prostate cancer-specific mortality, stratified by treatment receipt, are presented in Table 3-8. Analyses of interaction between digoxin exposure and the receipt of radiation or ADT with prostate cancer-specific mortality were non-significant (Table 3-8, *P-interaction*=0.13, *P-interaction*=0.38 respectively). There was the suggestion of poorer prostate cancer-specific mortality for digoxin exposed men who received radiation and ADT compared to digoxin unexposed men (Table 3-8, HR=1.79, 95% CI 0.96, 3.33; HR=1.22, 95%CI 0.93, 1.59 respectively). However the number of digoxin patients who received radiation was small.

3.4.3.4 Sensitivity analyses

Sensitivity analyses of the potential misclassification of prostate cancer death did not show any appreciable differences in the full, trimmed or matched cohorts. Univariate and multivariate hazard ratios including prostate cancer deaths classified as in Table 3-4 (Sensitivity Analysis 1) and including prostate cancer deaths classified as secondary or contributory causes of death (Sensitivity Analysis 2) are presented in Table 3-9.

Table 3-8: Digoxin exposure and prostate cancer-specific mortality: tests for interaction by receipt of radiation therapy or ADT in the year following diagnosis

		Digoxin Unexposed		Digoxin Exposed		Exposed Vs. Unexposed	
Radiation							
No	**Death/Censored**	845/2987		92/247			
	Multivariate HR (95%CI)	1.00 -	-	1.08 (0.86, 1.37)	p = 0.51	1.08 (0.86, 1.37)	p = 0.51
Yes	**Death/Censored**	150/1359		11/41			
	Multivariate HR (95%CI)	0.95 (0.79,1.15)	p = 0.62	1.69 (0.92 3.10)	p = 0.09	1.77 (0.95, 3.30)	p = 0.07
		Multiplicative scale: rHR (95%CI)		Radiation (Yes Vs. No)		**1.64 (0.85, 3.14)**	**p = 0.14**
ADT							
No	**Death/Censored**	375/2358		35/158			
	Multivariate HR (95%CI)	1.00 -	-	1.00 (0.69, 1.43)	p = 0.98	1.00 (0.69, 1.43)	p = 0.98
Yes	**Death/Censored**	620/1988		68/130			
	Multivariate HR (95%CI)	1.06 (0.92, 1.21)	p = 0.46	1.29 (0.97, 1.70)	p = 0.08	1.22 (0.93, 1.59)	p = 0.14
		Multiplicative scale: rHR (95%CI)		ADT (Yes Vs. No)		**1.23 (0.80, 1.89)**	**p = 0.35**

All multivariate HRs are adjusted for age (years, continuous), comorbidity score (number of medication classes, continuous) tumour stage (I, II, III, IV unspecified), tumour grade (I, II, III/IV, unspecified), smoking status at diagnosis, year of incidence, warfarin exposure and statin exposure

Table 3-9: Sensitivity analyses: digoxin exposure and prostate cancer-specific mortality.

Sensitivity Analysis 1	N	Person Years	Prostate cancer-specific mortality			
			No. of deaths (rate)[A]		Univariate HR (95%CI)	Multivariate HR[B] (95%CI)
Digoxin Exposure						
Full cohort						
Digoxin unexposed	5431	22,774	1018	(44.7)	Ref -	Ref -
Digoxin exposed	391	1,277	106	(83.0)	1.78 (1.46, 2.18)*	1.14 (0.91, 1.42)
Propensity Score Trimmed Cohort						
Digoxin unexposed	3,940	15,938	852	(53.5)	Ref -	Ref -
Digoxin exposed	389	1,272	105	(82.5)	1.49 (1.22, 1.83)*	1.13 (0.90, 1.41)
Propensity Score Matched Cohort						
Digoxin unexposed	387	1,339	109	(81.4)	Ref -	Ref -
Digoxin exposed	387	1,269	104	(82.0)	1.01 (0.77, 1.32)	1.14 (0.86, 1.51)
Sensitivity Analysis 2						
Digoxin Exposure						
Full cohort						
Digoxin unexposed	5431	22,774	1068	(46.9)	Ref -	Ref -
Digoxin exposed	391	1,277	115	(90.1)	1.83 (1.51, 2.22)*	1.19 (0.96, 1.46)
Propensity Score Trimmed Cohort						
Digoxin unexposed	3,940	15,938	899	(56.4)	Ref -	Ref -
Digoxin exposed	389	1,272	114	(89.6)	1.53 (1.26, 1.86)*	1.17 (0.95, 1.45)
Propensity Score Matched Cohort						
Digoxin unexposed	387	1,339	113	(84.4)	Ref -	Ref -
Digoxin exposed	387	1,269	113	(89.1)	1.06 (0.81, 1.37)	1.24 (0.94, 1.63)

A: *Deaths per 1000 person years*

B: *All multivariate hazard ratios are adjusted for age (years, continuous), comorbidity score (number of medication classes, continuous) tumour stage (I, II, III, IV unspecified), tumour grade (I, II, III/IV, unspecified), smoking status at diagnosis, year of incidence, warfarin exposure and statin exposure.*

3.4.4 Discussion

In this study of 5,732 men with prostate cancer, digoxin exposure was not associated with a reduction in prostate cancer-specific mortality. These results remained unchanged in the propensity score matched analyses of men with similar cardiovascular comorbidities, suggesting that the lack of observed effect is not confounded by associations between high cardiovascular comorbidity and less aggressive treatment of prostate cancer in digoxin treated men. Additionally, no trend was observed for increasing digoxin dose or dosing intensity in exposure-response analyses. Although a short pre-diagnostic period of digoxin exposure were examined, these patients were prevalent users and many patients would have been exposed to digoxin for much longer periods.

Analyses of interaction between digoxin exposure and receipt of radiation in the year after prostate cancer diagnosis did not indicate that digoxin exposure was associated with additional clinical benefit for prostate cancer-specific mortality in men receiving radiation therapy. There was, instead, the suggestion that digoxin exposure in men receiving radiation therapy may be associated with increased prostate cancer-specific mortality but this was not significant. The reasons for this are unclear, but the analysis is limited by the small number of digoxin exposed patients receiving radiation, therefore strong conclusions cannot be drawn from this finding.

These findings show an increased risk of all-cause mortality among digoxin exposed men, similar to that in another study of men with metastatic prostate cancer treated with docetaxel (HR=1.43, 95%CI 1.01, 2.03).[198] In the propensity score matched cohort associations with all-cause mortality had wider confidence intervals due to smaller numbers.

The use of a propensity score in this study revealed similar hazard ratio estimates for prostate cancer-specific mortality in the full, trimmed and propensity score matched cohorts. The propensity score was effective in achieving a matched cohort with a similar balance of covariates. It was important to conduct these analyses in a well-matched cohort so as to ensure that the comorbidity associated with digoxin use was not a source of confounding, or that adjusting for categorical variables where significant differences in missing data existed between exposed and unexposed groups i.e. tumour stage, tumour grade, was not as source of additional bias. The fact that these results did not differ significantly also serves to strengthen the validity of the comorbidity score as an adjustment for potential confounding by comorbidity in this population.

3.5 Overall Discussion

Many preclinical studies have indicated a potential role for digoxin in prostate cancer through the apoptotic effect mediated through disruption of intracellular calcium concentration and also the inhibition of HIF-1α. However, it has been suggested that digoxin plasma levels achievable in humans may not be sufficient to exert these effects.[236,237] The validity of using mouse models in preclinical studies of cardiac glycosides has also been called into question as mouse cells are far more resistant to cardiac glycosides than human cells.[237,238] The findings of the studies presented here do not suggest an overall association or dose-response trend between digoxin exposure and prostate cancer stage or grade at diagnosis or prostate cancer-specific mortality.

This may be explained by the difference in digoxin concentration in pre-clinical studies and those tolerated in humans. Digoxin concentrations in the 1-10 μM range have been shown to reduce proliferation and cause apoptosis of prostate cancer cells.[158] Another study has shown reduced prostate cancer cell proliferation at digoxin concentrations of 23-255 nM. This study has also shown inhibition of HIF-1α by digoxin in prostate cancer cell lines at concentrations of 100 nM.[61] These digoxin concentrations are, however, considerably higher than the therapeutic plasma concentrations normally tolerated in humans, 1.6 ± 1.0 nM.[239] It has been suggested that prolonged exposure to digoxin, even at normal therapeutic concentrations, may successfully inhibit HIF-1α in humans.[46] Analyses stratified by digoxin dose dispensed (≤125mcg, >125mg) were carried out as it has been reported previously that many elderly patients receive sub-therapeutic doses of digoxin;[240] again no association was observed. It appears that digoxin concentrations in humans do not exert a clinically meaningful anti-cancer effect, given that digoxin exposure was not associated with improved prostate cancer outcomes in this study.

Elevated HIF-1α expression in prostate tumours has been associated with increased resistance to radiation[195] and it has been suggested that cardiac glycosides may have synergistic activity in combination with radiation therapy.[241] This was not observed in this study in relation to prostate cancer-specific mortality, possibly due to small numbers. Although HIF-1α expression has been shown to be associated with poorer outcomes for prostate cancer patients,[169] it should be noted that the clinical benefits of HIF-1α inhibition as a therapeutic target in prostate cancer, have yet to be demonstrated in randomised trials.[242] Digoxin has, however, been suggested as a potential combination treatment with other anti-cancer agents.[46,236] HIF-1α expression in non-small cell lung cancer (NSCLC) is also associated with poorer

outcomes.[243] However, a phase II trial (NCT00281021) examining the co-administration of digoxin with the tyrosine kinase inhibitor erlotinib as second line therapy in NSCLC patients has been terminated; interim results found only one patient had a partial response. As well as the study of digoxin in prostate cancer patients with recurrent disease discussed earlier (NCT01162135), digoxin as HIF-1α inhibitor is being examined in a "window of opportunity" study to examine the influence of digoxin on molecular markers of response in women with operable breast cancer, this trial commenced in January 2013 (NCT01763931).

The phyto-oestrogenic properties of digoxin (Chapter 3, Section 3.1.5) are hypothesised to be a potential reason for the increased risk of breast and uterine cancer and reduced risk of prostate cancer observed in digoxin patients.[178] Increased endogenous oestrogen levels, and alterations in the testosterone-oestrogen ratio have been weakly associated with prostate cancer risk.[244] ERβ signalling has been reported to have anti-proliferative effects that balance the proliferative action of androgens in prostatic tissue;[181] thus in the non-cancerous prostate ERβ agonists may have a beneficial effect. However in prostate cancer phenotypes where ERβ expression is retained, poorer outcomes have been reported.[169] ERα signalling in prostate cancer has been associated with increased tumour cell proliferation;[181] concerns have been raised about the proliferative effects of agents with ER-α agonist activity in prostate cancer.[180,181,244] While the exact ER-subtype that digoxin is proposed to act on has not been identified, this hypothesis may explain why digoxin does not appear to show survival benefit in men with prostate cancer. No effect modification by receipt of ADT was noted between digoxin exposure and prostate cancer-specific mortality.

Some negative effects of digoxin in cancer cell lines have been reported; at low concentrations (30 nM) digoxin can activate Mitogen Activated Protein Kinase (MAPK) which enhances proliferation of fibroblasts. Contrastingly at high concentration, (300 nM) digoxin can cause fibroblast apoptosis.[160] Such effects have also been reported at differing concentrations of ouabain (another cardiac glycoside),[245] and endogenous digitalis-like compounds.[246] These endogenous digitalis-like compounds are produced in response to acute stress, however following chronic stress plasma concentrations of endogenous digitalis-like compounds become depleted to the pM concentration range. More recently the hypothesis has been proposed that tumour cells are even more sensitive than non-cancerous cells to proliferative effects of endogenous digitalis-like compounds at very low concentrations.[247] In combination with tumour promoting cortisol concentration which increased in chronic stress conditions, these patients may be even more predisposed to tumour development.[247]

3.6 Conclusion

The pre-clinical evidence has strongly suggested digoxin could have a role in the prevention or treatment of prostate cancer, however these population-based studies do not agree with these claims. The caveat that therapeutic plasma levels of digoxin and digitoxin are significantly lower than the concentrations required in vitro for HIF-1 inhibition has been documented clearly.[236,237] However, other studies of the effects of endogenous digitalis-like compounds report that tumour cells are sensitive to extremely low concentrations of these substances, though these effects may not be beneficial.[247] Since digoxin's first reported use in humans it was noted to have powerful activity, as noted by Sir William Withering:

> *"The more I saw of the great powers of this plant, the more it seemed necessary to bring the doses of it to the greatest possible accuracy"*

Over 220 years later the same issues arise in relation to the use of digoxin as a potential anti-cancer agent. It has plausible anti-cancer activity based on pre-clinical research, though the dose may not be sufficient in terms of achieving this activity in humans.

Chapter 4 ASPIRIN AND PROSTATE CANCER

This chapter commences with an overview of the pre-clinical studies which have examined the anti-cancer effects of aspirin in prostate cancer. The observational studies assessing the association between aspirin exposure and prostate cancer incidence are summarised, and the studies which have examined aspirin exposure and prostate cancer survival are reviewed. The two studies carried out are then presented: the first examining the association between aspirin exposure and mortality in men with stage I-III prostate cancer, and the second study examining aspirin exposure and prostate cancer survival in men with high grade prostate cancer (Gleason Score >7). Finally the findings of these studies are discussed in the context of prior studies which have examined aspirin exposure and prostate cancer outcomes.

4.1 Aspirin in Prostate Cancer – Proposed Mechanisms of Action

A number of mechanisms of anti-cancer activity have been proposed for aspirin, including both its anti-inflammatory effects and anti-platelet properties. The inflammatory aetiology of pre-neoplastic lesions, such as PIA and PIN, (described in Chapter 1 Section 1.2.2) may suggest that the anti-inflammatory mechanism of aspirin has a potential application in prostate cancer.[27] Other research has proposed that the anti-platelet activity of aspirin may inhibit the dissemination of blood-borne metastases.[47] The findings of preclinical studies which have examined COX-2 inhibition, COX-1 inhibition and other potential anti-cancer mechanisms of action of aspirin are described in more detail in this section.

4.1.1 Cyclooxygenase Enzyme 2 in Prostate Cancer

COX-2 expression may be induced in response to inflammatory cytokines,[120] growth factors,[121] hypoxia[122] and tumour promoters. COX-2 expression and resultant PG-E_2 synthesis has been demonstrated to enhance proliferation and angiogenesis and inhibit apoptosis of prostate cancer cells.[248] Expression of COX-2 has been reported to be significantly higher in cancerous prostate cells compared to BPH cells; differences in COX-2 expression have not been observed between androgen dependent and androgen independent cell lines.[121] However not all studies which have examined COX-2 expression in prostate cancer have reported consistent findings. A meta-analysis of studies which have examined associations between COX-2 expression and clinico-pathological parameters by Shao *et al.*, found COX-2 expression to be significantly associated with larger tumour size (T3/T4 compared to T1/T2; OR=2.33, 95% CI 1.54, 3.53).[249] Although COX-2 expression has been associated with higher Gleason Score in a number of studies,[121,250,251] Shao *et al.* in their meta-analysis did not find high expression of COX-2 to be significantly associated with prostate cancer of Gleason score ≥7, compared tumours of Gleason score <7, (OR=1.16, 95% CI 0.74, 1.83).[249] A number of COX-2 single nucleotide polymorphisms have also been studied in relation to prostate cancer risk, however only one (rs2745557, G>A) has been found in the meta-analysis by Shao *et al.* (N=7 studies) to be associated with increased risk of prostate cancer.[249]

A number of studies have examined COX-2 expression and the role of COX-2 products (TX-A_2 and PG-E_2) in tumour growth and progression. Activation of the TX-A_2 pathway, via COX-2 in prostate cancer tissue has been associated with tumour progression and loss of differentiation in prostate cancer.[252] The vasculature close to the tumour and newly generated tumour vasculature has been shown to consistently express COX-2.[253] High levels of VEGF-C and COX-2

expression have been correlated with lymph-angiogenesis and lymph node metastases in prostate cancer.[254] The COX-2 product PG-E_2 is involved in the development of bone lesions, as it mediates osteoblasts and osteoclasts involved in bone formation and restructuring.[50] Poorer clinical outcomes have been associated with COX-2 expressing tumours; COX-2 expression in the epithelial tumour cell and tumour stromal areas has been associated with increased risk of distant metastases and death from prostate cancer.[255] Biochemical failure and treatment failure,[256] including resistance to radiation,[257] have been associated with COX-2 expression. In light of these pro-neoplastic effects of COX-2, inhibition of COX-2 has been proposed and investigated as a potential treatment in prostate cancer.[26]

4.1.1.1 COX-2 Inhibition

Inhibition of COX-2 has been shown to have a number of beneficial effects in prostate cancer cell lines; these include suppression of bcl-2, an anti-apoptotic oncoprotein,[258] as well as stimulation of prostate cancer cell apoptosis independent of bcl-2.[259] Additionally, COX-2 inhibition stimulates an increase in intracellular calcium concentration, which through caspase activation may trigger cancer cell apoptosis.[260] In murine prostate models, COX-2 inhibition has been shown to cause regression of PIN and prostate carcinogenesis corresponding with a decrease in VEGF, NF-κB, p65, AKT, bcl-2 and AR expression.[261] Furthermore COX-2 inhibition causes tumour cell apoptosis and inhibits growth of adenocarcinoma in the mouse prostate.[262,263] Induced prostate carcinogenesis in mouse models, has been demonstrated to involve activation of inducible nitric oxide synthase, NF-κB and p65 expression; COX-2 inhibition reduced the activation of these inflammatory mediators which are potentially pro-carcinogenic.[264] These pre-clinical findings have been attributed to the anti-inflammatory mechanism of COX-2 inhibition, impeding prostate carcinogenesis mediated by inflammation.[264] As PG-E_2 is involved in bone formation and restructuring, COX-2 inhibition has also been investigated in relation to metastatic progression; reduced formation of osteoblastic prostate cancer lesions was reported in mice treated with a COX-2 inhibitor.[50] COX-2 inhibition may therefore also have a potential role in prevention of disease progression.

4.1.1.2 Androgens and COX-2

DHT has been reported to potentiate the apoptotic effect of NSAIDs in androgen dependent prostate cancer cells.[265] DHT binding to the AR has been shown to supress the expression of COX-2 in BPH[266] and androgen dependent prostate cancer cells.[267] In androgen dependent prostate cancer cells, treated with very low concentrations of DHT and hydroflutamide to mimic combined androgen blockade (CAB), COX-2 expression was reported to increase.[267] This

resulted in increased proliferation of these cells, which was inhibited by a COX-2 specific inhibitor, suggesting that COX-2 inhibition may have a synergistic effect with CAB.[267] It is hypothesised that this regimen may delay progression of prostate tumours from the androgen dependent to the castrate resistant stage. In men treated with radiotherapy, Khor *et al.* noted an association between biochemical failure and COX-2 over-expression in men treated with short term CAB and radiotherapy compared with long term ADT and radiotherapy.[256] Pre-clinical research has demonstrated reduced tumour growth in mouse models treated with CAB and a COX-2 inhibitor,[268] supporting the findings of Khor *et al.* and suggesting COX-2 inhibition could be of therapeutic benefit in men treated with CAB.[267]

4.1.1.3 Angiogenesis

HIF-1α, the transcription factor expressed in response to hypoxia,[195] stimulates the expression of VEGF, which promotes angiogenesis.[269] COX-2 expression has also been associated with increased levels of VEGF.[269] Increased expression of COX-2 mRNA by prostate cancer cells has been reported in response to hypoxic conditions.[122] Some cross-talk exists between COX-2 and HIF-1α; HIF-1α stabilisation and nuclear localisation has been shown to be induced by PG-E_2 expression in prostate cancer cells and inhibition of COX-2 has been demonstrated to reduce hypoxia-induced VEGF expression.[270] The level of co-induction of COX-2 and VEGF, in response to hypoxia, has been correlated with the metastatic potential across prostate cell lines.[122] In addition to the potential COX-2 mediated effects of aspirin on angiogenesis,[48] inhibition of COX-1 in platelets by aspirin, which reduces platelet activation and thromboxane synthesis and also prevents the release of pro-angiogenic growth factors (i.e. VEGF) may also play a role in angiogenesis through this mechanism.[47]

4.1.2 Cyclooxygenase Enzyme 1 Inhibition and Platelet-Mediated Effects

Cancer patients have elevated platelet counts and high levels of circulating activated platelets.[271] Activation of platelets stimulates the release of various growth factors which support tumour growth, angiogenesis and metastasis.[47,272] Platelet adhesion to tumour cells circulating in the vasculature can protect them from immune surveillance and destruction, and facilitate the dissemination of metastases.[271] The association between reduced platelet count and impaired platelet function and reduced development of metastases has been known for many years.[49,273]

Aspirin irreversibly inhibits COX-1 in platelets, reducing the synthesis of TX-A_2, thus impeding platelet adhesion and aggregation; and reducing the formation of thrombi.[24] It has been

suggested that, by improving oxygen perfusion to tumours this could reduce tumour hypoxia, inhibit the progression of tumours and prevent tumours from becoming resistant to treatment.[195] COX-1 inhibition by aspirin has also been shown to inhibit the release of growth factors from platelets.[274] Thus aspirin could play an important role in platelet-mediated cancer progression, impeding the development of metastases and tumour spread.[47]

There are strong arguments for this anti-platelet activity being the mechanism of action of aspirin in cancer; platelets are the only significant pharmacological target for low doses of aspirin as it is rapidly metabolised and these low doses do not reach other tissues.[275] As will be discussed later (Section 4.2) aspirin use, even at low doses has been associated with reduced cancer incidence.[276]

4.1.3 Other Proposed Anti-Cancer effects of Aspirin in Prostate Cancer

Aspirin has been shown to inhibit growth of non-cancerous prostate epithelial cells at therapeutic plasma concentrations (0.5mM).[277] High concentrations of aspirin (1.5mM) had an inhibitory effect on advanced prostate cancer cells however lower concentrations did not. This is suggestive of a chemo-preventative effect of aspirin in the early stages of disease development.[277]

There are suggestions that medicinal salicylates i.e. aspirin and its metabolite salicylate, or dietary salicylates (found in fruits, vegetables, herbs and spices) could have a role in the inhibition of tumour development.[278] A number of studies have reported that both aspirin and salicylate may inhibit the enzyme I-κB kinase-β (IKK-β), responsible for the phosphorylation of the protein (I-κB); which, in its dephosphorylated state, inhibits activation of the transcription factor NF-κB.[123,279] The downstream effects of NF-κB inhibition include suppression of COX-2 expression and subsequent prostanoid synthesis.[280] Other reported effects of aspirin inhibition of NF-κB activation in prostate cancer cells are: reduced expression of the chromosomal protein survivin, which protects cells from apoptosis;[281] suppression of uPA secretion by aspirin, which supresses cell motility and impedes metastases;[282] and inhibition of bcl-2 expression.[283] Reduced expression of bcl-2 in cells pre-treated with aspirin (0.1-1mM), has been reported to sensitise prostate cancer cells to TNF-Related Apoptosis-Inducing Ligand (TRAIL)-mediated apoptosis.[281,283] Aspirin has also been shown to supress the adhesion of the invasive PC-3 prostate cancer cell line.[282]

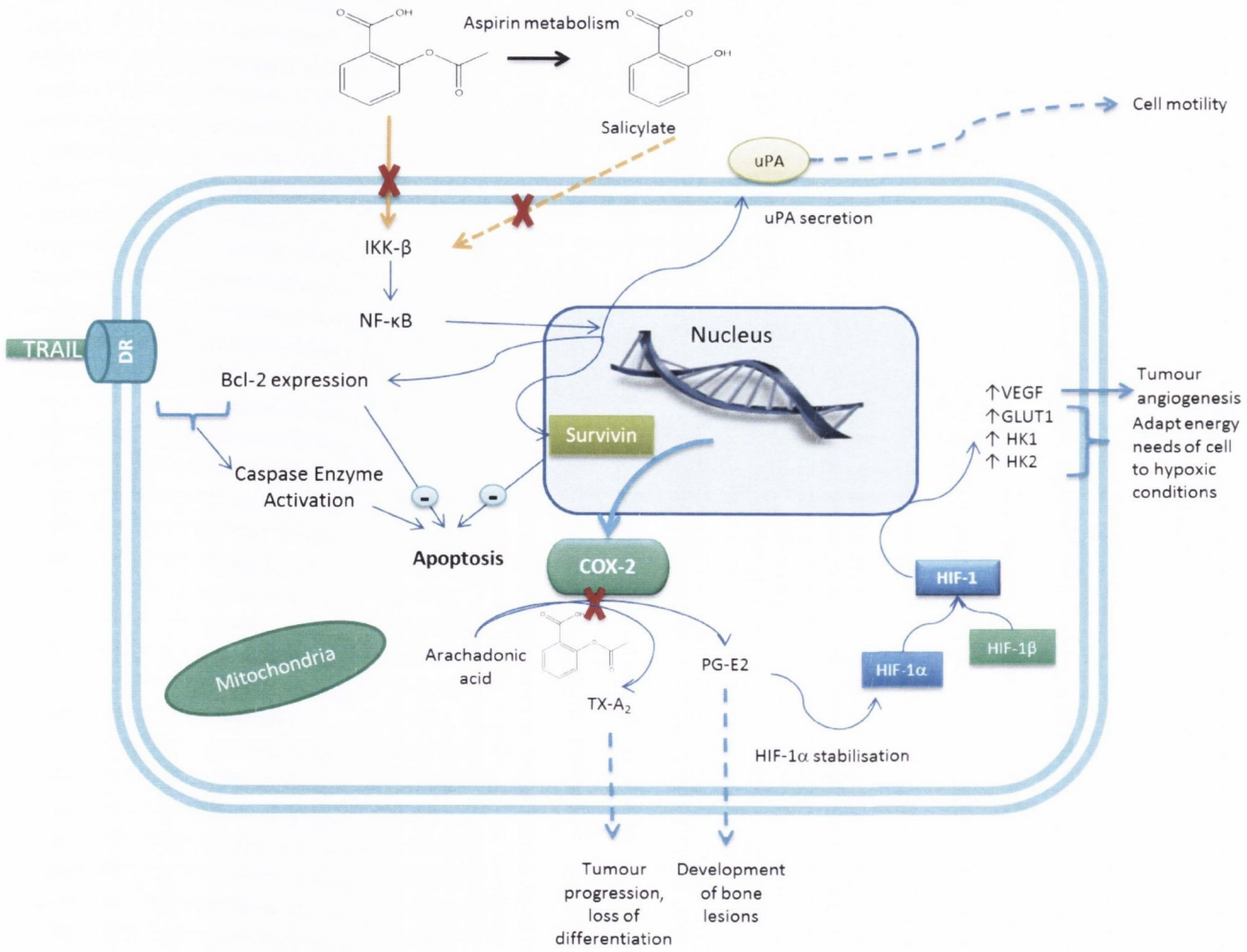

Figure 4-1: Illustration of the proposed anti-cancer mechanisms of aspirin in prostate cancer cells.

HIF: Hypoxia-Inducible Factor; **GLUT:** Glucose Transporter; **HK:** Hexokinase; **VEGF:** Vascular Endothelial Growth Factor; **uPA** urokinase Plasminogen Activator; **PG-E_2**: Prostaglandin- E_2; **COX-2:** Cyclooxygenase-2; **DR**: Death Receptor; **TRAIL:** Tumour necrosis factor- Related Apoptosis-Inducing Ligand; **IKK-β:** Inhibitory Kappa-B Kinase-β; **NF-κB:** Nuclear Factor κB; **TX-A_2:** Thromboxane-A_2;

4.1.4 SUMMARY

The main potential mechanisms of anti-cancer action of aspirin have been outlined above and illustrated in Figure 4-1; the potential for aspirin to interfere with the capabilities that characterise cancerous cells such as apoptotic mechanisms, tumour inflammation and angiogenesis[19] has been widely reported. These pre-clinical studies have predominantly focused on the effects of aspirin and other COX-2 specific inhibitors as tumours develop in mice, as opposed to in established prostate cancer. The role of inflammation in prostate cancer carcinogenesis and the demonstrated pre-clinical effects of COX-2 inhibition strongly suggest this anti-inflammatory effect as the principal mechanism of action. In addition to these proposed anti-inflammatory mechanisms, the role of platelets in facilitating the development of tumour metastases is receiving increasing attention,[272] and the anti-platelet effect of aspirin, mediated through inhibition of COX-1, may potentially play a role in preventing tumour dissemination.

4.2 Aspirin in Cancer: Existing Evidence from Trials and Observational Studies

4.2.1 Aspirin Use and Cancer Incidence

Many pharmacoepidemiological studies have established that exposure to aspirin is associated with reduced incidence of a number of solid cancers. Although this chapter focuses on the association between aspirin exposure and prostate cancer survival, a number of these studies investigating prostate cancer incidence can aid the interpretation of the anti-cancer mechanism of aspirin.

4.2.1.1 Any Cancer Incidence

Rothwell *et al.,* in a meta-analysis of randomised trials of daily low-dose (75-100mg) aspirin for primary prevention of cardiovascular disease, reported significantly reduced incidence of all cancers in persons randomised to aspirin with at least three years of follow-up (OR=0.76, 95% CI 0.66, 0.88).[276] The same group conducted meta-analyses of aspirin use associated with cancer incidence in observational studies. In this they reported any aspirin use to be associated with a smaller but still significant reduction in risk of all cancers (case-control: OR=0.88, 95% CI 0.84, 0.92; cohort: RR=0.87, 95% CI 0.83, 0.91).[284] The association between aspirin use and reduced cancer incidence was strongest for cancers of the gastrointestinal tract. A different meta-analysis of observational studies of aspirin and cancer risk across twelve cancer sites, by Bosetti *et al.* also concluded that aspirin is significantly associated with reduced risk of cancers of the gastrointestinal tract and associated with more modest, but still significant, reductions in the risk of breast and prostate cancer.[62]

4.2.1.2 Prostate Cancer Incidence

Bosetti *et al.* in their meta-analysis of observational studies (N=24), reported a 10% reduction in risk of prostate cancer associated with aspirin use (RR=0.90, 95% CI 0.85, 0.96).[62] By contrast, none of the pooled estimates in the meta-analyses of observational studies carried out by the Rothwell group found a significant reduction in risk of prostate cancer associated with aspirin use.[284] The findings of the meta-analysis by Bosetti *et al.*, possibly due to the heterogeneity of the studies, did not provide evidence of an association between the duration of aspirin use, or dose of aspirin associated with this reduction in prostate cancer incidence; thus definitive conclusions regarding causality cannot be made.[62]

Some individual studies have reported longer durations of aspirin use to be associated with reduced risk of prostate cancer. A number of these studies have reported aspirin use of five or more years to be associated with a reduction in risk of prostate cancer.[285-287] The meta-analysis of observational studies carried out by the Rothwell group reported slightly increased risk of prostate cancer associated with aspirin use of less than 5 years (N=3 studies; pooled OR=1.12, 95% CI 1.04, 1.20) and no association with aspirin use of greater than or equal to 5 years (N=3 studies; pooled OR=1.02, 95% CI 0.90, 1.15). The dose and dosing frequency of aspirin associated with reduced prostate cancer incidence has been examined in some observational studies. Many of these observational studies are based on self-reported aspirin use, and have examined a variety of different doses and dosing frequencies; this makes comparison difficult. Bosetti *et al.* determined similar relative risks of prostate cancer in studies which examined low (approximately 100mg) and regular/ high (300-500mg) aspirin dose.[62] Without considering the frequency or duration of aspirin use, simple comparisons may not be particularly informative. Overall these observational studies do not provide conclusive information as to the association between aspirin dose and prostate cancer incidence.

4.2.2 Aspirin Use and Tumour Characteristics at Diagnosis

In a meta-analysis of randomised controlled trials of daily aspirin use conducted by Algra and Rothwell, significantly reduced combined incidence of breast, colorectal and prostate cancers with distant metastases has been reported (OR=0.48, 95% CI 0.30, 0.75); for prostate cancer alone this was non-significant (OR=0.69, 95% CI 0.31, 1.51), although the number of prostate cancer cases in this analysis was small(N=43).[284] A number of observational studies have also reported non-significant reduced risks of metastatic prostate cancer at diagnosis.[288,289] A study carried out in the American Cancer Society Cancer Prevention Study II Nutrition Cohort considered duration of aspirin use; ≥5 years duration of aspirin use has been associated with a non-significant reduced risk of advanced (nodal/metastatic involvement) or fatal prostate cancer at diagnosis (OR=0.64, 95% CI 0.39, 1.05).[285] Other work carried out by our research group, investigating aspirin use and breast cancer progression, has found aspirin use to be associated with a reduced risk of presenting with node-positive breast cancer (Appendix 5). These findings suggest that aspirin exposure may be associated with reduced tumour progression.

Aspirin use may also be associated with reduced tumour grade. Norrish *et al.* reported aspirin use to be associated with a non-significant reduced risk of prostate cancer extending beyond the prostate capsule or Gleason Score ≥7 (OR=0.71, 95% CI 0.47, 1.08).[290] This suggestion that

aspirin exposure is associated with lower grade tumours at diagnosis has also been reported in a population of men at high-risk of prostate cancer; regular aspirin use was associated with a reduced odds of high-grade (Gleason Score ≥7) prostate cancer.[291] More recently, Dhillon *et al.* reported that six or more aspirin tablets per week was associated with a 28% reduction in risk of tumours with Gleason score >7 (OR=0.72 95% CI 0.54, 0.96), with a significant trend observed for increasing quantity of aspirin use.[292] These are interesting observations considering reported associations between COX-2 expression in large and high Gleason score prostate tumours.

4.2.3 Aspirin Use and Cancer Survival

4.2.3.1 Meta-analyses

Over twenty years ago, it was suggested that aspirin may be associated with reduced mortality from colorectal cancer.[293] Since then meta-analyses of randomised controlled trials as well as observational studies have shown associations between aspirin use and improved survival in a number of cancers, though for many cancers the magnitude of this association between aspirin use and mortality is not clear. Rothwell *et al.* in a meta-analysis reported significantly reduced mortality from cancer in patients who participated in randomised trials of daily aspirin.[131,276] The association between aspirin use and reduced death from all cancers was also shown to be greater with increasing duration of aspirin use, (follow-up 0-5 years HR=0.86, 95% CI 0.71, 1.04; follow-up ≥5 years HR= 0.66, 95% CI 0.50, 0.87).[131] Regarding aspirin dose, Rothwell *et al.* in a different meta-analyses of randomised trials examining aspirin use and cancer mortality, have reported similar results for doses of aspirin ≥300mg (OR=0.81, 95% CI 0.66, 0.99) and doses <300mg (OR=0.86, 95% CI 0.75, 0.99).[276] A separate meta-analysis of randomised controlled trials of aspirin (doses 75mg-325mg), by Mills *et al.* found similar results; they reported a 23% reduction in risk of cancer mortality associated with daily aspirin use (RR=0.77, 95% CI 0.63, 0.95); a significant association was observed after four years.[294]

The meta-analyses by Rothwell *et al.* examined the association between randomisation to aspirin and death from individual cancers. The overall association between daily aspirin use and mortality from prostate cancer was not significant in either study by Rothwell *et al.*; although these analyses were limited by small numbers of patients (HR=0.70, 95% CI 0.29, 1.73; HR=0.43, 95% CI 0.19, 1.01).[131,295] However in the latter study, men who were randomised to aspirin and had non-metastatic prostate cancer at diagnosis had reduced odds of death from prostate cancer which was just statistically significant (OR=0.34, 95% CI 0.12,

0.99).[295] The results of these meta-analyses as well as observational studies which have examined the association between aspirin use and prostate cancer-specific mortality are presented in Table 4-1.

4.2.3.2 Observational Studies

Since the publication of the Rothwell meta-analyses, a number of observational studies have been carried out investigating the association between aspirin use and cancer-specific mortality. Before meaningful comparison may be made it must be recognised that these observational studies differ in their design. These differences include: the populations in which they are conducted (prospectively enrolled cohorts in selected populations i.e. Health Professionals Follow-up Study; clinical trial cohorts and population-based cohorts i.e. CPRD), the ascertainment of aspirin exposure (self-reported by questionnaire; prescriptions issued; prescription refills), the dosing of aspirin (daily aspirin use; any aspirin use), the dose of aspirin used and the timing of aspirin use relative to cancer diagnosis (pre- or post-diagnosis, or both). In the interpretation of these studies it must also be considered that patients taking low dose aspirin for its anti-platelet effects are more likely to be taking aspirin on a long term daily basis whereas use of higher aspirin doses for analgesia is likely to be indicated for shorter periods of time.

Observational studies in large cohorts have not reported the association between aspirin and mortality from any cancer to be as strong as the meta-analysis above.[62] Current aspirin use in the Cancer Prevention Study II Cohort, of over 100,000 men and women, was reported by Jacobs *et al.* to be associated with an 8% reduction in risk of any cancer death (RR=0.92, 95% CI 0.85, 0.99).[132] This association (for all cancers) did not differ with increasing duration of aspirin use.

In the same study, Jacobs *et al.* reported a 23% non-significant reduction in risk of mortality from prostate cancer associated with current daily aspirin use,[132] similar to the meta-analysis by Rothwell *et al.* of randomised controlled trials.[131] A duration-dependent reduction in risk of prostate cancer-specific mortality was also observed.[132] See Table 4-1. A recently published abstract by Daugherty *et al.* describing a study carried out in the control arm of the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer screening trial (N=3,857), also reported a 23% non-significant reduction in risk of prostate cancer mortality in men who reported pre-diagnostic use of aspirin on a daily or more than daily basis.[296] Another conference abstract, presented at the ASCO Annual Meeting 2013, examined any aspirin use and the risk of prostate cancer-specific mortality in the CPRD.[297] A smaller, again non-significant, reduction in

risk of risk of prostate cancer-specific mortality was observed in this study by Assayag *et al.* for any aspirin use prior to diagnosis, compared to those which examined daily use of aspirin prior to cancer diagnosis. See Table 4-1 where the results of these studies are tabulated.

The studies mentioned above have all examined pre-diagnostic aspirin use; a number of studies have also examined post-diagnostic aspirin use and the association with prostate cancer-specific mortality. These studies have reported equivocal results, due to differences in the ascertainment of aspirin exposure, the entry of aspirin exposure into time-varying models and whether or not these studies adjusted for pre-diagnostic aspirin use.

Two studies of interest have been carried out in the Cancer of the Prostate Strategic Urologic Research Endeavour (CaPSURE) database; these were in a cohort of men with localised prostate cancer treated with radical prostatectomy and radiation.[133,225] The first examined NSAID (including aspirin) exposure and all-cause mortality,[225] the second examined anticoagulant (including aspirin) exposure and prostate-cancer-specific mortality.[133] Medication exposure was determined by self-reported questionnaire at baseline and approximately annual follow-up questionnaires. Both of these studies reported significantly reduced HRs of mortality associated with aspirin/NSAID exposure prior to or following a cancer diagnosis; univariate associations were significant in those with high-risk disease.[133,225] The reported association between aspirin exposure and prostate-cancer-specific mortality was particularly significant (HR= 0.43, 95% CI 0.21, 0.87);[133] this may be due to the select cohort of men, who received curative treatment, in which this study was conducted.

The study presented in Table 4-1 by Grytli *et al.* in a selected cohort of Norwegian men with high-risk of prostate cancer mortality (PSA <20 ng/ml or Gleason score >7 or clinical tumour stage ≥T3a),[92] also reported low dose aspirin exposure before and after prostate cancer diagnosis to be associated with a reduction in risk of prostate cancer-specific mortality.[298] Concerns have been raised regarding the identification of aspirin exposure which has potentially biased these results.[299] Aspirin exposure was identified on the basis of having an aspirin prescription filled before and after the date of prostate cancer diagnosis; patients who died within three months following diagnosis were considered exposed regardless of whether they filled a prescription after diagnosis; and patients who received aspirin following, but not before, their diagnosis were excluded. The number of patients excluded on this basis was not presented. The potential methodological issues are as follows: firstly, to be classified as aspirin-exposed, patients had to survive until they received a prescription after their diagnosis; this introduces immortal time bias.[300] Secondly, exclusion of patients on the basis of treatment

received during follow-up also introduces immortal time bias.[300] This means that aspirin exposed men may have a survival advantage over unexposed men and the association between aspirin use and prostate cancer-specific survival may be biased towards aspirin having a beneficial effect. Finally, the classification of patients who died in the first three months following diagnosis as exposed may confound the results presented by making aspirin exposure appear artificially detrimental in the period shortly following diagnosis.

Other researchers have focused their attention on whether use of aspirin following a prostate cancer diagnosis is associated with reduced disease progression or survival benefit. Dhillon *et al.* examined aspirin use following a prostate cancer diagnosis in the Health Professionals Follow-up Study;[301] their results did not show any association between aspirin use and lethal prostate cancer or the development of metastases; this analysis adjusted for aspirin use prior to diagnosis. The study was carried out in a cohort of whom 50% had a prostatectomy; the authors also lagged entry of aspirin into the model by two years, to guard against biases introduced by changes in aspirin prescribing close to death.[301]

In a collaborative project with Dr Chris Cardwell and Professor Liam Murray at the Centre for Public Health in Queen's University Belfast, the PhD candidate has prepared a manuscript entitled *"Low dose aspirin and survival in men with prostate cancer: A study using the UK Clinical Practice Research Datalink"* which is to be published in *Cancer Causes Control* (Appendix 4). This examined, in a case-control study, the association between low-dose aspirin use following diagnosis (up to the 6 months prior to death, for cases, or end of matched follow-up period for controls) and prostate cancer-specific survival. The use of low-dose aspirin was higher in men who died from prostate cancer than those who did not (52.1% compared to 38.7%). This corresponded to an unadjusted OR=1.51 (95% CI 1.19, 1.90). Oestrogen therapy is used widely in the UK to treat castrate resistant prostate cancer (32.8% of cases had received oestrogen therapy) and low dose aspirin is frequently prescribed to reduce the risk of thromboembolic events associated with oestrogen therapy.[302] No association between the use of low dose aspirin following prostate cancer diagnosis and the risk of prostate cancer-specific mortality was observed, when covariates, including prescription of oestrogen therapy, were adjusted for (adjusted OR=1.02 95% CI 0.78, 1.34). When the aspirin exposure period was varied to exclude aspirin use in the period one or two years prior to death the associations between aspirin use and prostate cancer-specific mortality were attenuated (adjusted OR=0.96, 95% CI 0.72, 1.28 and adjusted OR=0.81, 95% CI 0.57, 1.15, respectively). This finding highlights the potential for time-varying confounding to

occur, with changes in prostate cancer prognosis such as disease progression (and subsequent prescription of oestrogen therapy) influencing post-diagnostic aspirin prescribing.

Assayag *et al.* presented a study at the ASCO Annual Meeting 2013 which examined pre-, post- and pre-/post-diagnostic aspirin exposure and prostate cancer-specific mortality in men with non-metastatic prostate cancer. This study was also carried out using data from the CPRD. See Table 4-1 for results. These authors observed an apparent increased risk of prostate cancer-specific mortality in men with pre-/post-diagnostic aspirin use. The rate ratio for men who were only exposed to aspirin post-diagnosis was greatly increased (RR=1.69, 95% CI 1.43, 2.00). The authors stated that this was driven by new users of aspirin following diagnosis which was most likely related to changes in aspirin use associated with disease progression.[297] This confirms that time-varying confounding may influence observed associations between post-diagnostic aspirin use and prostate cancer outcomes.

Table 4-1: Tabulation of studies reporting associations between aspirin use and mortality in men with prostate cancer

Year	Author	Study Details		Aspirin Use			Other Details	Reported Result	
		Setting	Type	Classification	Dosing	Timing			(95% CI)
2011	Rothwell *et al.*[131]	RCTs of aspirin for cardio prevention	Meta-analysis	Randomised	Daily	Pre-dx	Follow-up 0-5 years	HR	0.70 (0.29, 1.73)
					Daily	Pre-dx	Follow-up≥ 5 years	HR	0.52 (0.20, 1.34)
2012	Rothwell *et al.*[295]	RCTs of aspirin for cardio prevention	Meta-analysis	Randomised	Daily	Pre-dx	All Cases	OR	0.43 (0.19, 1.01)
					Daily	Pre-dx	Non-metastatic	OR	0.34 (0.12, 0.99)*
2012	Jacobs *et al.*[132]	American Cancer Society, Cancer Prevention Study II	Cohort	Self-reported questionnaire	Daily	Pre-dx	Current	RR	0.77 (0.53, 1.12)
					Daily	Pre-dx	< 5 years	RR	0.88 (0.59, 1.33)
					Daily	Pre-dx	≥5 years	RR	0.64 (0.39, 1.03)
2012	Dhillon *et al.*[301]	HPFS: participants with Stage I-III disease at diagnosis	Cohort	Self-reported questionnaire	≥6 tablets /week	Post-dx	Aspirin use, time-varying covariate with 2 year lag; adjusted for Pre-dx use	RR	1.16 (0.74, 1.82)
2012	Choe *et al.*[133]	CaPSURE: cases who received radiation / radical prostatectomy	Cohort	Self-reported questionnaire	any	Pre- / post-dx	Aspirin use, time-varying covariate with 1 year lag	HR	0.43 (0.21, 0.87)*
2013	Grytli *et al.*[298]	Norwegian population: high-risk disease	Cohort	Prescriptions Filled	any	Pre- & post-dx	Low-dose	HR	0.81 (0.71, 0.93)*
2013	Daugherty *et al.*[296]	Control arm of the PLCO Cancer Screening Trial; men aged 55-74 years	Cohort	Self-reported questionnaire	Daily	Pre-dx	All cases	HR	0.77 (0.48, 1.25)
					Daily	Pre-dx	Stage I-II	HR	0.86 (0.47, 1.58)
					Daily	Pre-dx	Stage III-IV	HR	0.37 (0.15, 0.92)*
2013	Assayag *et al.*[297]	CPRD: men with non-metastatic disease	Nested case-control	Prescriptions Issued by GP	any	Pre- / post-dx	Non-metastatic	RR~	1.36 (1.18, 1.55)*
					any	Pre-dx	Non-metastatic	RR~	0.93 (0.76, 1.15)
					any	Post-dx	Non-metastatic	RR~	1.69 (1.43, 2.00)*

RCT: Randomised Controlled Trial; **HPFS:** Health Professionals Follow-up Study; **CaPSURE:** Cancer of the Prostate Strategic Urologic Research Endeavour; **PLCO:** Prostate, Lung, Colorectal and Ovarian; **CPRD:** Clinical Practice Research Datalink; **Pre-dx:** Pre-diagnostic; **Post-dx:** Post-diagnostic *p-value<0.05 pre-dx **HR:** Hazard Ratio; **OR:** Odds Ratio; **RR:** Relative Risk; **RR~:** Rate Ratio;

4.2.4 Summary of the Evidence Regarding Prostate Cancer

There is moderate evidence from observational studies and meta analyses of clinical studies of aspirin in cardiovascular disease that aspirin use may be associated with reduced incidence of prostate cancer.[62] Prostate tumours diagnosed in aspirin exposed men have been reported to be less advanced at diagnosis.[288-292] Studies have suggested that men with localised cancer at diagnosis, who are exposed to aspirin, have reduced risk of prostate cancer-specific death;[133,295] although one recent study has reported significant reductions in the risk of prostate cancer-specific death in men with advanced disease.[296]

A recent editorial has discussed the differences observed in the magnitude of the association between aspirin use and mortality from any cancer in trials and observational studies; concluding that there is an association between aspirin use and mortality from cancer and that the duration of aspirin use is a significant factor.[303] The most recent observational studies would suggest that the timing of aspirin use is important. Studies by Daugherty *et al.* and Jacobs *et al.* which have examined the association between daily aspirin use prior to prostate-cancer diagnosis have reported consistent, although non-significant, associations with reduced risk of prostate-cancer-specific mortality.[132,296] However, studies which have examined post-diagnostic aspirin exposure exclusively, have not found aspirin to be associated with development of metastases or prostate cancer death. Further studies of the dose and timing of aspirin use in relation to prostate cancer progression have been called for.[304]

On the basis of the pre-clinical studies which have suggested that aspirin may act in impeding the inflammatory processes which precede prostate cancer;[27] impede the transition of prostate tissue from benign to cancerous states;[277] prevent the dissemination of micro-metastases;[47] as well as the evidence from observational studies and meta-analyses, the following studies were carried out in the NCRI-PRCS database. The first study tests the hypothesis that aspirin exposure prior to diagnosis is associated with reduced risk of prostate cancer-specific mortality in a cohort of men with stage I-III disease at diagnosis. The second study examines the hypothesis that in men with high grade (Gleason score >7) prostate cancer of any stage, aspirin exposure may be associated with reduced risk of prostate cancer-specific mortality. As aspirin use is prevalent in this population, men were also excluded from these studies on the basis of age. Firstly men aged less than 50 years were excluded as they are not typical prostate cancer patients, nor are they typically prescribed aspirin. Secondly, men aged over 80 years were excluded as their tumours are less likely to be completely staged and their tumours are conservatively managed.

4.3 Study I: Aspirin Use and Mortality in Men with Localised Prostate Cancer

As outlined in Section 4.1 pre-clinical evidence suggests the pharmacological activity of aspirin may reduce tumour growth and/or impede tumour dissemination. Aspirin use, prior to a prostate cancer diagnosis, has been associated with a lower risk of advanced disease at diagnosis and reduced mortality from prostate cancer, in particular localised prostate cancer (Chapter 4, Section 4.2). The magnitude of association between aspirin use and prostate cancer mortality has varied considerably between studies. It has also been suggested that aspirin has shown greater benefit in men with higher grade or larger tumours.[133,296] Further clarity has been called for regarding the influence of dose, frequency and timing of aspirin use on prostate cancer outcomes.[304]

The aims of this study were to investigate, in men aged 50-80 years with incident localised (stage I-III) prostate cancer: (i) associations between aspirin exposure prior to diagnosis, and mortality; (ii) the influence of dose, frequency and duration of aspirin exposure on mortality, and (iii) whether tumour characteristics, such as tumour size or Gleason score, modify associations between aspirin exposure and mortality. Notable reductions in risk of prostate cancer-specific mortality have been observed in men who received aspirin and who were treated with radiation or prostatectomy,[133] therefore potential interactions by treatment received were also investigated.

4.3.1 Methods

4.3.1.1 Study Cohort

The NCRI-PCRS database was used to identify the study cohort. Men aged 50-80 years at the time of a diagnosis of localised (Stage I-III)[43] prostate cancer (ICD-O, C61)[145] between 1st January 2001 and 31st December 2006 were included in the study. Continuous eligibility for the GMS scheme in the full year prior to diagnosis was also required for inclusion. Prostate cancer cases diagnosed at death or autopsy were excluded and men with a prior invasive tumour were also excluded. Associations between duration of aspirin exposure and mortality were assessed in a smaller cohort of men with at least three years of GMS eligibility prior to diagnosis.

4.3.1.2 Exposure Definition

Aspirin exposure was defined as having a supply of aspirin (for WHO-ATC codes see Appendix 7) available in the year prior to prostate cancer diagnosis. The date, dose and number of days' supply on each prescription are recorded and these were used to stratify pre-diagnostic aspirin exposure by: (i) dosing intensity (high/low) split on the median proportion of days with a supply of aspirin available in the year prior to diagnosis;[149] (ii) dose prescribed (low: only received dose ≤75mg / high: any received dose > 75 mg); (iii) combination of dose and dosing intensity.

In the smaller cohort used to examine the duration of aspirin exposure pre-diagnosis and mortality from prostate cancer, the duration of pre-diagnostic aspirin use was categorized (0-2 years, and > 2 years). In this group aspirin dosing intensity was determined from the date of dispensing of the earliest aspirin prescription to the date of prostate cancer diagnosis. Dosing intensity was stratified (high/low) based on the median proportion of days which men had an aspirin supply available from the date of the earliest aspirin prescription to prostate cancer diagnosis.

4.3.1.3 Outcome Definition

All men were followed from the date of diagnosis to the first of either death (prostate cancer-specific: ICD 9 185; ICD 10 C61; or any cause) or the end of follow-up (31st December 2010).

4.3.1.4 Statistical Analysis

The frequency and proportion of aspirin exposed and unexposed men were tabulated by clinical and demographic variables. Cox proportional hazards models (SAS®, PROC PHREG) were used to estimate univariate and multivariate HRs and 95% CIs for associations between aspirin exposure and (i) prostate-cancer-specific mortality (ii) all-cause mortality. Similar to the previous survival analysis (Chapter 4 Section 3.4), a backward deletion method was used to select covariates in the multivariate model; with a 10% maximum change in the effect component of the fully adjusted HR used to select the final multivariate model.[217] Covariates strongly associated with prostate cancer outcomes in prior studies were fixed in the multivariate model (age at diagnosis, tumour size, tumour grade).[212] Based on prior knowledge of clinical and demographic predictors of prostate cancer mortality, the following additional covariates were then considered for inclusion: comorbidity score;[150] smoking status;[213] diabetes;[74] and exposure to statins,[225] non-aspirin anti-coagulants,[133] non-aspirin NSAIDs,[225] beta-blockers,[305] and medication for the treatment of BPH.[216] The year of prostate

cancer diagnosis (continuous) and treatment received in the year following diagnosis (time varying) were also assessed for inclusion.

The proportionality of the hazard functions were assessed by testing for the interaction between the exposure medication, and the logarithm of person-time (Wald test for product term). To examine whether the risk of death changed over time,[306] HRs were determined at 2, 4, and 8 years of follow-up.

4.3.1.5 Interaction Tests & Effect Modification

Based on the study by Choe *et al.* which reported significant association between aspirin use prior to or following prostate cancer diagnosis and prostate cancer-specific mortality in men treated with radiation or surgery,[133] the following analyses were conducted. The association between aspirin exposure and prostate cancer-specific mortality was examined across strata of patients who did and did not receive radiation therapy or prostate surgery in the year following diagnosis. Measures of interaction were estimated on a multiplicative scale (ratio of hazard ratios, rHR) with 95% CIs. Choe *et al.* also reported that men with higher grade and larger tumours had the most prominent reduction in risk of prostate cancer-specific mortality.[133] Associations between aspirin exposure and prostate cancer-specific mortality were assessed for effect modification by tumour grade and tumour size at diagnosis, with measures of interaction estimated on a multiplicative scale.

4.3.1.6 Sensitivity Analyses

As in the previous survival analysis, sensitivity analyses were conducted in two ways to assess the potential misclassification of prostate cancer-specific mortality on death certificates. Firstly mortality from prostate cancer was defined including other potential causes of death by which prostate cancer death may have been misclassified as prostate cancer death.[235] (Refer to Table 3-4 for list) The second sensitivity analysis classified as prostate cancer death, any deaths where the prostate cancer was classified as the other or contributory cause of death on the death certificate.

4.3.2 Results

4.3.2.1 Cohort Characteristics

A flow diagram outlining selection of the study cohort is presented in Figure 4-2. The characteristics of aspirin users and non-users are presented in Table 4-2. Aspirin exposed men (N=1,131) were significantly older than men (N=1,805) who did not receive aspirin (71.5 years compared to 69.5 years); they also had higher comorbidity scores (11.1 and 6.9 respectively,

p<0.0001). Aspirin exposed men were significantly less likely to be current smokers at diagnosis. Significantly more aspirin exposed men received ADT than unexposed men (48.1% Vs. 43.3%, p=0.01) and fewer received prostate surgery (18.7% Vs. 22.8%, p=0.01). The median duration of patient follow-up was 5.5 years.

1,807 of these men had GMS scheme eligibility for at least 3 years prior to their prostate cancer diagnosis and were eligible for the duration-response analysis. The characteristics of these men did not differ significantly from those of the larger cohort.

4.3.2.2 Survival Analysis

Mortality rates and hazard ratio estimates of the association between aspirin use and prostate cancer-specific mortality for the main analyses are presented in Table 4-3. Aspirin exposure was not associated with prostate cancer mortality in unadjusted analyses (Table 4-3: HR=1.01, 95% CI 0.79, 1.29) however it was associated with a small, non-significant reduced risk of prostate cancer-specific mortality in adjusted analysis (Table 4-3: HR=0.88, 95% CI 0.67, 1.15). Adjusted HRs for the association between aspirin use and prostate cancer-specific mortality at two, four and eight years follow-up were 1.02 (95% CI 0.61, 1.69); 0.90 (95% CI 0.64, 1.27); and 0.88 (95% CI 0.67, 1.17) respectively. The adjusted Cox model satisfied the proportional hazards assumption, p=0.75. Aspirin exposure was not associated with all-cause mortality (Table 4-4: adjusted HR=0.98, 95% CI 0.84, 1.15).

In stratified analyses (Table 4-3), high aspirin dosing intensity was associated with a non-significant reduced risk of prostate cancer-specific mortality (HR=0.73, 95% CI 0.51, 1.05). Men who received higher doses of aspirin (>75mg) had a statistically significant reduced risk of prostate cancer-specific mortality HR=0.61 (95% CI 0.37, 0.99). No significant association with prostate cancer-specific mortality was observed for low dose aspirin (≤75mg) although there was the suggestion of a lower risk of death in men with high dosing intensity of low-dose aspirin.

In the sub-group analysis examining duration-response, no association between increased duration of pre-diagnostic aspirin exposure and either all-cause or prostate cancer-specific mortality was observed (Table 4-3: *p*-trend=0.48 and Table 4-4: *p*-trend=0.59 respectively). Reduced HR of prostate cancer-specific mortality was observed in men with high dosing intensity relative to those with low dosing intensity, these results were non-significant.

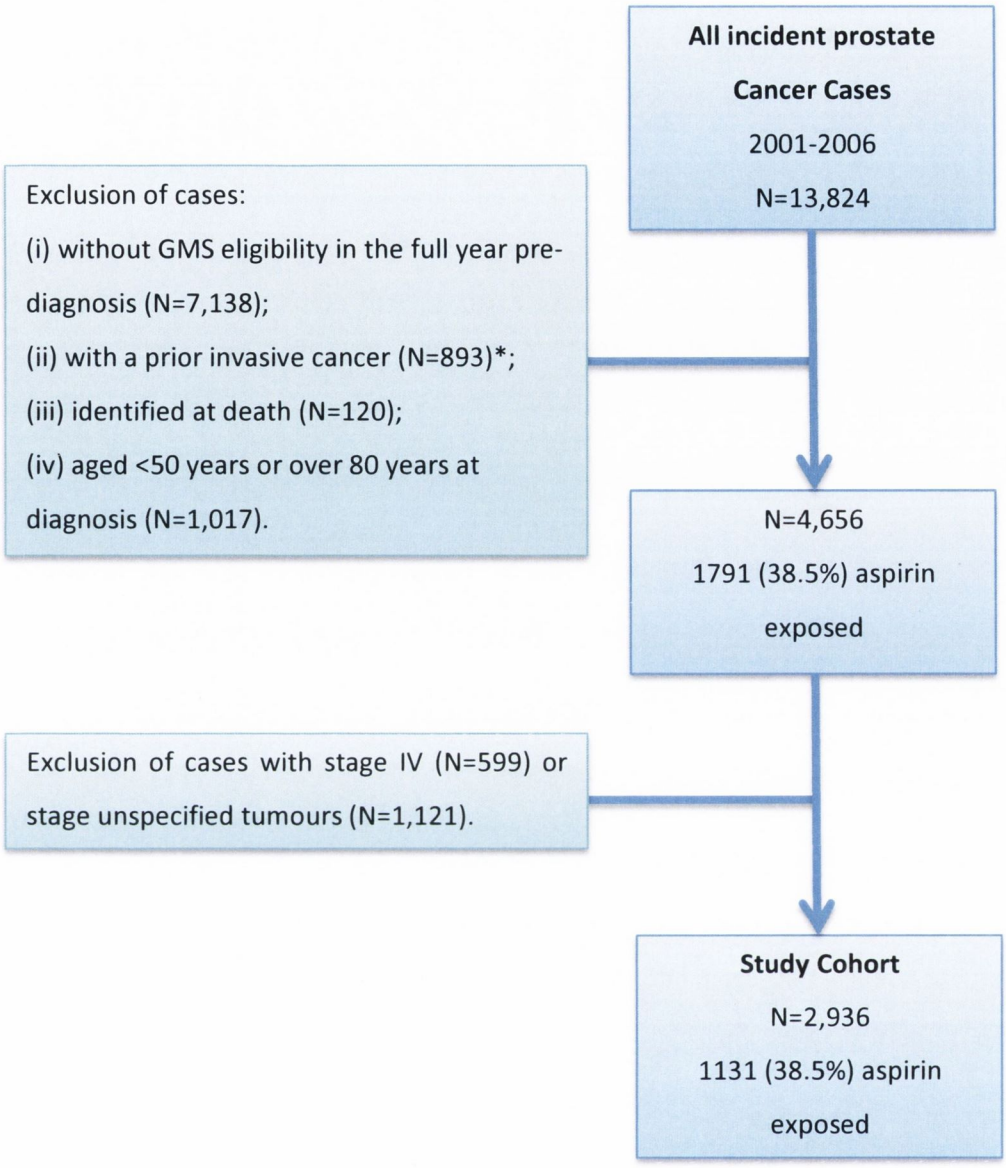

Figure 4-2: Study cohort selection for aspirin Study I: exclusion criteria

** excluding non-melanoma skin cancers;*

Table 4-2: Characteristics of aspirin exposed and unexposed men in the year prior to diagnosis

Characteristic		Aspirin Unexposed (N=1,805)	Aspirin Exposed (N=1,131)	
Patient details				
Age /years	Mean (SD)	69.5 (6.8)	71.5 (5.7)	*
Comorbidity Score	Mean (SD)	6.9 (5.7)	11.1 (5.6)	*
Smoking Status - (%)	Never	556 (30.8)	374 (33.1)	*
	Former	331 (18.3)	230 (20.3)	
	Current	325 (18.0)	161 (14.2)	
	Unspecified	593 (32.9)	366 (32.4)	
Tumour details				
Stage - (%)[A]	I	92 (5.1)	62 (5.5)	
	II	1478 (81.9)	918 (81.2)	
	III	235 (13.0)	151 (13.4)	
Tumour Size- (%)[A]	T1 / T1c	166 / 318 (9.2) / (17.6)	96 / 217 (8.5) / (19.2)	
	T2	1,086 (60.2)	667 (59.0)	
	T3	235 (13.0)	151 (13.4)	
Grade - (%)	Gleason Score <5	129 (7.1)	77 (6.8)	
	Gleason Score 5-7	1193 (66.1)	743 (65.7)	
	Gleason Score >7	289 (16.0)	176 (15.6)	
	Unspecified	194 (10.7)	135 (11.9)	
Treatment details				
Treatment - (%)[B]	Surgery	412 (22.8)	211 (18.7)	*
	Radiation	678 (37.6)	443 (39.2)	
	ADT	781 (43.3)	544 (48.1)	*
	Chemotherapy	16 (0.9)	10 (0.9)	
	No treatment	386 (21.4)	245 (21.7)	
Medication Exposures[C] - (%)				
	Beta-blocker	222 (12.3)	433 (38.3)	*
	Statin	271 (15.0)	622 (55.0)	*
	Non-aspirin anticoagulant	186 (10.3)	149 (13.2)	*
	Anti-diabetic	94 (5.2)	163 (14.4)	*
	NSAID	738 (40.9)	521 (46.1)	*
	BPH medicines	429 (23.8)	282 (24.9)	
Aspirin exposure details:				
Pre-diagnostic aspirin[D]				
No of prescriptions dispensed		- -	10,732	
Dosing intensity	(%) Median (IQR)	- -	86.0% (48.5, 98.4)	
Post-diagnostic aspirin[E]				
Men receiving aspirin (%)		486 (26.9)	1,046 (92.5)	
No of prescriptions dispensed		7,524	32,718	

** p-value <0.05.*

***A:** AJCC Staging Manual 5th Ed.*[43]

***B:** Received within one year following diagnosis, (not mutually exclusive)*

***C:** Medication received in the year prior to diagnosis*

***D:** Aspirin exposure in the year prior to diagnosis*

***E:** Receipt of aspirin at any point post-diagnosis.*

Table 4-3: Estimated Hazard Ratios of prostate cancer-specific mortality associated with aspirin exposure

			Prostate cancer-specific mortality		
Aspirin Exposure	**N**	**Person Years**	**No. of deaths (rate)[A]**	**Univariate HR (95%CI)**	**Multivariate HR[B] (95%CI)**
Aspirin unexposed in year prior to diagnosis	1,805	10,060	172 (17.1)	Ref -	Ref -
Aspirin exposed in year prior to diagnosis	1,131	6,070	104 (17.1)	1.01 (0.79, 1.29)	0.88 (0.67, 1.15)
Exposure response:[C] dosing intensity[D]					
Low dosing intensity 0%-86%	564	3,070	61 (19.9)	1.17 (0.87, 1.56)	1.02 (0.74, 1.40)
High dosing intensity 86%-100%	567	3,000	43 (14.3)	0.85 (0.61, 1.18)	0.73 (0.51, 1.05)
P-trend				*0.56*	*0.12*
Exposure response:[C] dose					
Low dose ≤ 75mg	881	4,627	84 (18.2)	1.07 (0.83, 1.39)	0.97 (0.73, 1.30)
High dose > 75mg	250	1,443	20 (13.9)	0.81 (0.51, 1.28)	0.61 (0.37, 0.99)
P-trend				*0.69*	*0.10*
Exposure response:[C] dosing intensity[D] & dose					
Low dosing intensity 0%-86%					
Low dose ≤ 75mg	420	2,256	49 (21.7)	1.28 (0.93, 1.76)	1.13 (0.80, 1.58)
High dose > 75mg	144	814	12 (14.8)	0.86 (0.48, 1.55)	0.71 (0.39, 1.30)
High dosing intensity 86%-100%					
Low dose ≤ 75mg	461	2,371	35 (14.8)	0.88 (0.61, 1.26)	0.81 (0.55, 1.20)
High dose > 75mg	106	629	8 (12.7)	0.74 (0.36, 1.50)	0.50 (0.24, 1.03)

Exposure response: duration[E]					
Aspirin unexposed in 3 years prior to diagnosis	1,003	5,201	74 (14.2)	Ref -	Ref -
Aspirin exposed in 3 years prior to diagnosis					
Aspirin unexposed 0- 2 years pre-diagnosis	226	1,157	14 (13.8)	0.97 (0.56, 1.66)	0.96 (0.55, 1.68)
Aspirin exposed >2 years pre-diagnosis	578	2970	44 (15.3)	1.09 (0.75, 1.58)	1.13 (0.74, 1.71)
P-trend				*0.69*	*0.59*
Exposure response: duration & dosing intensity					
Aspirin exposed 0- 2 years pre-diagnosis					
Low dosing intensity 0%-84%	112	578	9 (15.6)	1.09 (0.55, 2.18)	1.06 (0.55, 2.13)
High dosing intensity 84%-100%	114	579	7 (12.1)	0.85 (0.39, 1.84)	0.85 (0.38, 1.79)
Aspirin exposed >2 years pre-diagnosis					
Low dosing intensity 0%-84%	290	1,455	26 (17.9)	1.26 (0.80, 1.97)	1.31 (0.81, 2.10)
High dosing intensity 84%-100%	288	1,415	18 (12.7)	0.91 (0.54, 1.52)	0.91 (0.52, 1.60)

** p-value < 0.05.*

***A:** Mortality rate (deaths/1000 person years).*

***B:** All multivariate HRs are adjusted for age at diagnosis, tumour grade, tumour size, smoking status at diagnosis, comorbidity score, year of incidence, pre-diagnostic statin exposure, and receipt of radiation (time-varying).*

***C:** Reference group: aspirin unexposed*

***D:** Dosing intensity split on median*

***E:** Cohort with at least 3 years continuous GMS scheme eligibility prior to diagnosis, (N=1,807)*

Table 4-4: Estimated Hazard Ratios of all-cause mortality associated with aspirin exposure

			All-cause mortality		
Aspirin Exposure	**N**	**Person Years**	**No. of deaths (rate)[A]**	**Univariate HR (95%CI)**	**Multivariate HR[B] (95%CI)**
Aspirin unexposed in year prior to diagnosis	1,805	10,060	442 (43.9)	Ref -	Ref -
Aspirin exposed in year prior to diagnosis	1,131	6,070	339 (55.8)	1.28 (1.11, 1.48)*	0.98 (0.84, 1.15)
Exposure response:[C] dosing intensity[D]					
Low dosing intensity 0%-86%	564	3,070	166 (54.1)	1.24 (1.03, 1.48)*	0.93 (0.77. 1.13)
High dosing intensity 86%-100%	567	3,000	173 (57.7)	1.33 (1.11, 1.58)*	1.03 (0.85, 1.25)
P-trend				*<0.01*	*0.85*
Exposure response:[C] dose					
Low dose ≤ 75mg	881	4,627	266 (57.5)	1.33 (1.14, 1.54)*	1.06 (0. 90, 1.25)
High dose > 75mg	250	1,443	73 (50.6)	1.14 (0.89, 1.46)	0.76 (0.59, 0.99)*
P-trend				*0.01*	*0.17*
Exposure response: duration[E]					
Aspirin unexposed in 3 years prior to diagnosis	1,003	5,202	195 (37.5)	Ref -	Ref -
Aspirin exposed in 3 years prior to diagnosis					
Aspirin unexposed 0- 2 years pre-diagnosis	226	1,157	49 (42.4)	1.13 (0.83, 1.55)	0.91 (0.66, 1.26)
Aspirin exposed >2 years pre-diagnosis	578	2870	150 (53.2)	1.40 (1.13, 1.73)*	1.09 (0.86, 1.39)
P-trend				*<0.01*	*0.48*

** p-value < 0.05.*

A: *Mortality rate (deaths/1000 person years).*

B: *All multivariate HRs are adjusted for age at diagnosis, tumour grade, tumour size, smoking status at diagnosis, comorbidity score, year of incidence, pre-diagnostic statin exposure, and receipt of radiation (time-varying).*

C: *Reference group: aspirin unexposed*

D: *Dosing intensity split on median*

E: *Cohort with at least 3 years continuous GMS scheme eligibility prior to diagnosis, (N=1,807)*

4.3.2.3 Interaction Tests & Effect Modification Analyses

No significant interactions between aspirin exposure and receipt of prostate surgery (p-interaction=0.62) or radiation (p-interaction=0.66) were observed; see Table 4-5. Associations between aspirin exposure and prostate cancer mortality across strata of tumour grade were not observed in men with tumours of Gleason score ≤7 (Table 4-6: within strata HR=0.98, 95% CI 0.68, 1.40). However in men with high grade cancer (Gleason score > 7) pre-diagnostic aspirin exposure was associated with a reduced risk of in prostate cancer-specific mortality, though not significantly (Table 4-6: within strata HR=0.68, 95% CI 0.45, 1.05; p-interaction=0.19). In the analysis of effect modification by tumour size, the test for interaction was also non-significant (Table 4-6: p-interaction=0.62).

4.3.2.4 Sensitivity Analyses

In the sensitivity analyses for misclassification of prostate cancer death, the HRs for aspirin exposure were not appreciably different. Univariate and multivariate hazard ratios including prostate cancer deaths classified as in Table 3-4 (Sensitivity Analysis 1) and including prostate cancer deaths classified as secondary or contributory causes of death (Sensitivity Analysis 2) are presented in Table 4-7. The trends observed in the original analysis for increasing dosing intensity and increasing dose were also present in the sensitivity analyses.

Table 4-5: Aspirin exposure and prostate cancer-specific mortality: tests for interaction by receipt of surgery or radiation

		Aspirin Unexposed			Aspirin Exposed		Exposed Vs. Unexposed	
Surgery								
No	**Death/Censored**	133/1,260			85/835			
	Multivariate HR[A] (95% CI)	1.00	Ref -		0.89 (0.66, 1.20)	p = 0.43	0.89 (0.66, 1.20)	p=0.43
Yes	**Death/Censored**	39/373			19/192			
	Multivariate HR[A] (95% CI)	0.97 (0.67, 1.40)		p = 0.85	0.73 (0.44, 1. 21)	p = 0.22	0.76 (0.43, 1.34)	p=0.34
		Multiplicative scale: rHR (95% CI)			Surgery (Yes Vs. No)		**0.86 (0.46, 1.58)**	**p = 0.62**
Radiation								
No	**Death/Censored**	138/991			84/604			
	Multivariate HR[B] (95% CI)	1.00 -		-	0.84 (0.62, 1.13)	p = 0.25	0.84 (0.62, 1.13)	p = 0.25
Yes	**Death/Censored**	34/642			20/423			
	Multivariate HR[B] (95% CI)	0.48	(0.33, 0.71)	p < 0.05	0.46 (0.28, 0.75)	p <0.05	0.96 (0.55, 1.69)	p = 0.89
		Multiplicative scale: rHR (95% CI)			Radiation (Yes Vs. No)		**1.15 (0.62, 2.13)**	**p = 0.66**

A: *Adjusted for age, comorbidity score, tumour size, tumour grade, smoking status at diagnosis, year of incidence, statin exposure and receipt of radiation (time-varying).*

B: *Adjusted for age, comorbidity score tumour size, tumour grade, smoking status at diagnosis, year of incidence and statin exposure.*

Table 4-6: Aspirin exposure and prostate cancer-specific mortality: effect modification by tumour Gleason score and tumour size at diagnosis.

		Aspirin Unexposed			Aspirin Exposed			Exposed Vs. Unexposed	
Gleason Score									
≤7	**Death/Censored**	90/1,232			55/765				
	Multivariate HR[A] (95% CI)	1.00	-	-	0.98 (0.68, 1.40)		p = 0.90	0.98 (0.68, 1.40)	p = 0.90
>7	**Death/Censored**	67/222			36/140				
	Multivariate HR[A] (95% CI)	3.49 (2.53, 4.80)		p < 0.05	2.38 (1.57, 4.80)		p < 0.05	0.68 (0.45, 1.05)	p = 0.08
		Multiplicative scale: rHR (95% CI)			Gleason Score >7 Vs. Gleason Score ≤7			**0.70 (0.41, 1.19)**	**p = 0.19**
Tumour Size									
T1 & T2	**Death/Censored**	145/1,425			85/895				
	Multivariate HR[B] (95% CI)	1.00	-	-	0.92 (0.69, 1.24)		p = 0.58	0.92 (0.69, 1.24)	p = 0.58
T3	**Death/Censored**	27/208			19/132				
	Multivariate HR[B] (95% CI)	1.19	(0.79, 1.80)	p =0.41	0.93 (0.56, 1.54)		p = 0.39	0.78 (0.43, 1.43)	p = 0.42
		Multiplicative scale: rHR (95% CI)			T3 Vs. T1 & T2			**0.85 (0.44, 1.62)**	**p = 0.62**

A: *Adjusted for age at diagnosis, tumour size, smoking status at diagnosis, comorbidity score, year of incidence, pre-diagnostic statin exposure, and receipt of radiation (time-varying).*

B: *Adjusted for age at diagnosis, Gleason Score, smoking status at diagnosis, comorbidity score, year of incidence, pre-diagnostic statin exposure, and receipt of radiation (time-varying).*

Table 4-7: Sensitivity analyses: aspirin exposure and prostate cancer-specific mortality.

			Prostate cancer-specific mortality		
Sensitivity Analysis 1	***N***	**Person Years**	**No. of deaths (rate)[A]**	**Univariate HR (95%CI)**	**Multivariate HR[B] (95%CI)**
Aspirin unexposed	1,805	10,060	177 (17.6)	Ref -	Ref -
Aspirin exposed	1,131	6,070	108 (17.8)	1.02 (0.80-1.29)	0.89 (0.68-1.17)
Exposure response:[C] dosing intensity[D]					
Low dosing intensity 0%-86%	564	3,070	63 (20.5)	1.17 (0.88-1.56)	1.03 (0.75-1.41)
High dosing intensity 86-100%	567	3,000	45 (15.0)	0.86 (0.62-1.19)	0.75 (0.53-1.06)
P-trend				*0.62*	*0.14*
Exposure response:[C] dose					
Low dose ≤ 75mg	881	4,627	88 (19.0)	1.09 (0.84-1.41)	1.00 (0.75-1.32)
High dose > 75mg	250	1,443	20 (13.9)	0.78 (0.49-1.25)	0.60 (0.37-0.97)
P-trend				*0.68*	*0.09*
Sensitivity Analysis 2					
Aspirin unexposed	1,805	10,060	180 (17.9)	Ref -	Ref -
Aspirin exposed	1,131	6,070	114 (18.8)	1.05 (0.83-1.33)	0.92 (0.70-1.20)
Exposure response:[C] dosing intensity[D]					
Low dosing intensity 0-86%	564	3,070	66 (21.5)	1.21 (0.91-1.60)	1.05 (0.77-1.43)
High dosing intensity 86-100%	567	3,000	48 (16.0)	0.90 (0.56-1.24)	0.78 (0.56-1.10)
P-trend				*0.83*	*0.21*
Exposure response:[C] dose					
Low dose ≤ 75mg	881	4,627	91 (19.7)	1.11 (0.86-1.42)	1.01 (0.77-1.34)
High dose > 75mg	250	1,443	23 (15.9)	0.89 (0.58-1.37)	0.65 (0.41-1.03)
P-trend				*0.99*	*0.16*

A: *Mortality rate (deaths/1000 person years).*
B: *Multivariate HR is adjusted for age at diagnosis, tumour grade, tumour size, smoking status at diagnosis, comorbidity score, year of incidence, pre-diagnostic statin exposure, and receipt of radiation (time-varying).*
C*: Reference group: aspirin unexposed*
D: *Dosing intensity by median*

4.3.3 Discussion

In this study, the overall association between any aspirin use prior to diagnosis and prostate cancer-specific mortality was non-significant (HR=0.88, 95% CI 0.67, 1.15) and was similar to that reported for any aspirin use prior to diagnosis in the nested case-control study carried out by Assayag *et al.* in the CPRD (RR=0.93, 95% CI 0.76, 1.15).[297] This association was not observed at two years of follow-up, but was apparent at four and eight years; this suggests, similar to other studies,[131] that it may take a number of years for the influence of aspirin use on cancer mortality to accrue.

Previous studies which have investigated aspirin exposure prior to prostate cancer diagnosis have reported somewhat larger, although still non-significant, associations between aspirin use and prostate cancer-specific mortality. These studies have examined daily aspirin exposure.[131,132,296] In this study, high aspirin dosing intensity, which corresponds to almost daily aspirin use (≥6 days aspirin use per week), was associated with a non-significant 27% lower risk of prostate cancer-specific mortality (HR=0.73 95% CI 0.51, 1.05). This is consistent with a meta-analysis of randomised controlled trials of aspirin in cardiovascular disease by Rothwell *et al.* which reported that daily aspirin use was associated with a non-significant 30% reduced risk of prostate cancer-specific mortality;[131] and two cohort studies by Jacobs *et al.*[132] and Daugherty *et al.*[296] in which daily aspirin use was associated with a 23% lower risk of prostate cancer-specific mortality. See Table 4-1 for further detail of these studies.

Higher doses of aspirin were significantly associated with a reduced risk of both all-cause and prostate cancer-specific mortality. Other observational studies have not examined aspirin dose prescribed, and these findings may have important implications as consensus on the dose, duration and dosing regimen of aspirin associated with reduced cancer mortality has not been reached.[303,304] Rothwell *et al.* in meta-analyses have not determined differential associations between higher and lower doses of aspirin and cancer mortality.[276] They suggest that these findings should be interpreted with caution as the studies of higher aspirin doses were carried out many years prior, and in different populations, to those which examined lower aspirin doses. Within this meta-analyses by Rothwell *et al*, one study, which randomised participants to high (283mg) or low (30mg) aspirin dose,[307] noted a non-significant 29% reduced risk of cancer death in the high dose aspirin arm (OR=0.71, 95% CI 0.44, 1.15). This is somewhat consistent with the findings reported here.

Increasing duration of aspirin exposure has been associated with reduced cancer incidence[285-287] and mortality from cancer[131,132] in some studies. No association between increasing

duration of aspirin exposure prior to diagnosis and prostate cancer-specific mortality was observed in the sub-group analysis of this study. Consistent with results from the full cohort, high aspirin dosing intensity was more strongly associated with reduced mortality from prostate cancer in this analysis. However the smaller cohort of men eligible for this sub-group analysis may have limited the power to detect an association.

Men exposed to aspirin (either pre- or post-diagnosis), diagnosed with localised prostate cancer and treated with radiation or prostatectomy have been reported to have significantly reduced risk of prostate cancer-specific mortality; especially those with high-risk disease i.e. high Gleason Score or larger tumours.[133] Analyses of interaction between aspirin and treatment receipt found no associations of significance with prostate cancer-specific mortality. The results of effect modification analyses by tumour grade at diagnosis suggested greater benefit was associated with aspirin exposure in men with high Gleason score (>7) tumours, compared to low Gleason score (≤7) tumours. The interaction was non-significant. Effect modification of aspirin by tumour size (T1/T2 Vs. T3) was not observed which is in contrast to the significant association reported by Daugherty *et al.* in men with stage III/IV disease.[296] See Table 4-1.

Some limitations of this analysis must also be acknowledged. Treatment cross-over post-diagnosis did occur; 26.9% of aspirin unexposed men received aspirin at some point following prostate cancer diagnosis; however this would be expected to bias results towards the null. Compared to some other studies which have examined associations with mortality in cancer patients, the follow-up was shorter in this study. The analysis of duration response in the cohort with three years of GMS eligibility pre-diagnosis may have been limited by smaller cohort size.

4.4 Study II: Aspirin Use and Mortality in Men with High Grade Prostate Cancer

4.4.1 Introduction

The association between aspirin use and prostate cancer-specific mortality has been reported to be greatest in men with localised prostate cancer[295] in particular those with high-risk tumours, i.e. high Gleason Score and larger tumours.[133] The findings of Study I also suggest men with higher Gleason score (stage I-III) prostate cancer and who are exposed to aspirin may have improved outcomes. Studies which have examined aspirin use and prostate cancer incidence have suggested that aspirin use is associated with a reduced risk of higher grade tumours at diagnosis.[291,292]

A number of studies have reported an association between higher Gleason score tumours and high levels of COX-2 expression.[121,250,308] There is convincing pre-clinical evidence that COX-2 inhibition may have significant anti-cancer effects (Chapter 4, Section 4.1.1). Aspirin inhibition of COX-2 may therefore be of greatest therapeutic relevance in men with prostate tumours which are more poorly differentiated. Considering this, this study has been conducted in a cohort of men with prostate tumours of Gleason score >7.

This study aims firstly, to examine the association between aspirin exposure and prostate cancer-specific mortality in men with high grade (Gleason score >7) prostate cancer of any stage; and secondly, to assess whether this association differs according to prostate cancer tumour stage at diagnosis. The study protocol of this study was published *a priori* in the ENCePP E-register of studies.[16] See Appendix 6.

4.4.2 Methods

4.4.2.1 Study cohort

The NCRI-PCRS database was used to identify the study cohort. Men who met the following criteria were eligible for inclusion in the study: diagnosed with any stage prostate cancer (ICD-O, C61)[145] between 1st January 2001 and 31st December 2006, aged 50-80 years at the time of diagnosis and continuous eligibility for the GMS scheme in the full year preceding diagnosis. Only men with a histologically diagnosed tumour of Gleason score >7 were included.[43] Men who received a prostate cancer diagnosis at death or autopsy and men with a prior invasive tumour other than non-melanoma skin cancer were excluded.

4.4.2.2 Exposure definition

Aspirin exposed men were identified if they had supply of aspirin (for WHO-ATC codes see Appendix 7) available in the year prior to prostate cancer diagnosis. Exposure was stratified by (i) dosing intensity (high/low) split on the median proportion of days with a supply of aspirin available in the year prior to diagnosis;[149] (ii) dose prescribed (low: ≤75mg / high > 75 mg) and (iii) combination of dose and dosing intensity.

4.4.2.3 Outcome Definition

All men were followed from the date of prostate cancer diagnosis to the date of death (prostate cancer-specific: ICD 9 185; ICD 10 C61; or any cause) or end of follow-up (31st December 2010), whichever came first.

4.4.2.4 Statistical Analyses

As in previous survival analyses, Cox proportional hazards models were used to estimate HRs with 95% CIs for prostate cancer-specific mortality associated with aspirin use. Covariates were considered for inclusion in multivariate models based on prior knowledge of clinical and demographic predictors of prostate cancer mortality: age;[212] comorbidity score;[150] smoking status;[213,214] tumour size;[212] diabetes;[74] and exposure to beta-blockers,[148] statins,[225] non-aspirin anti-coagulants,[133,309] non-aspirin NSAIDS[225] and drugs used in BPH.[216] Also considered for inclusion in the model were year of prostate cancer diagnosis (continuous) and treatment received in the year following diagnosis: prostate surgery, radiation, androgen deprivation therapy (time-varying). A backward deletion method, with a 10% maximum change in the effect component of the fully adjusted HR was used to select the final multivariate model.[217] The proportionality of hazard functions was assessed by testing for the interaction between aspirin use and the logarithm of person-time (Wald test for product term).

4.4.2.5 Effect Modification

Analyses were stratified by tumour stage (Stage I-III, IV, unspecified) to assess the potential for modification of the association between aspirin use and prostate cancer mortality according to whether the tumour has progressed to involve lymph nodes or metastases.

4.4.2.6 Sensitivity Analyses

Initiation of aspirin in the six months prior to diagnosis was censored as a sensitivity analysis to guard against bias introduced by new aspirin users receiving aspirin for pain which may be due to the metastatic progression of a yet undetected prostate cancer.

As in the previous survival analyses, sensitivity analyses were conducted to assess the potential misclassification of prostate cancer-specific mortality on death certificates. See Section 3.4.2.8.

4.4.3 Results

4.4.3.1 Cohort Characteristics

The study cohort consisted of 912 men who met the inclusion criteria; N=357 (39.1%) were identified as aspirin exposed in the year prior to diagnosis (Figure 4-3). Similar to the previous study, aspirin exposed men were significantly older than aspirin non-users, (73.0 years vs. 71.1 years, $p<0.0001$) and received more medication in the year prior to diagnosis, (comorbidity score 12.1 vs. 7.5, $p<0.0001$). Aspirin exposed men were also less likely to be current smokers at diagnosis (12.0% vs. 19.8%, $p=0.02$). Cohort characteristics are presented in Table 4-8. Median duration of patient follow-up was 4.6 years.

4.4.3.2 Survival Analysis

Mortality rates and hazard ratio estimates of the association between aspirin use and prostate cancer-specific mortality are presented in Table 4-9. The analyses stratified by dose and dosing intensity are also presented here. The unadjusted HR for prostate cancer-specific mortality associated with aspirin exposure was close to the null (HR=0.99, 95% CI 0.79, 1.24). Similar near-null associations were observed in adjusted analysis (HR=1.06, 95% CI 0.81, 1.37). The adjusted HRs of prostate cancer-specific mortality at two, four and eight years after diagnosis were 1.19 (95% CI 0.69, 2.16); 1.01 (95% CI 0.75, 1.36) and 1.05 (95% CI 0.81, 1.37) respectively. The adjusted Cox model satisfied the proportional hazards assumption, $p=0.74$. Aspirin exposure in this cohort had no association with all-cause mortality (unadjusted HR=1.06, 95% CI 0.89, 1.26; adjusted HR=1.00, 95% CI 0.81, 1.23).

In stratified analyses, a non-significantly increased risk of prostate cancer-specific mortality was associated with higher dosing intensity (HR=1.13, 95% CI 0.82, 1.56) and high aspirin dose (HR=1.22, 95% CI 0.84, 1.77). See Table 4-9. The trends however were non-significant. Men who received higher aspirin doses at high dosing intensity had a statistically significant increased HR of prostate cancer-specific mortality (HR=1.78, 95% CI 1.07, 2.97), however this group was very small (N=41).

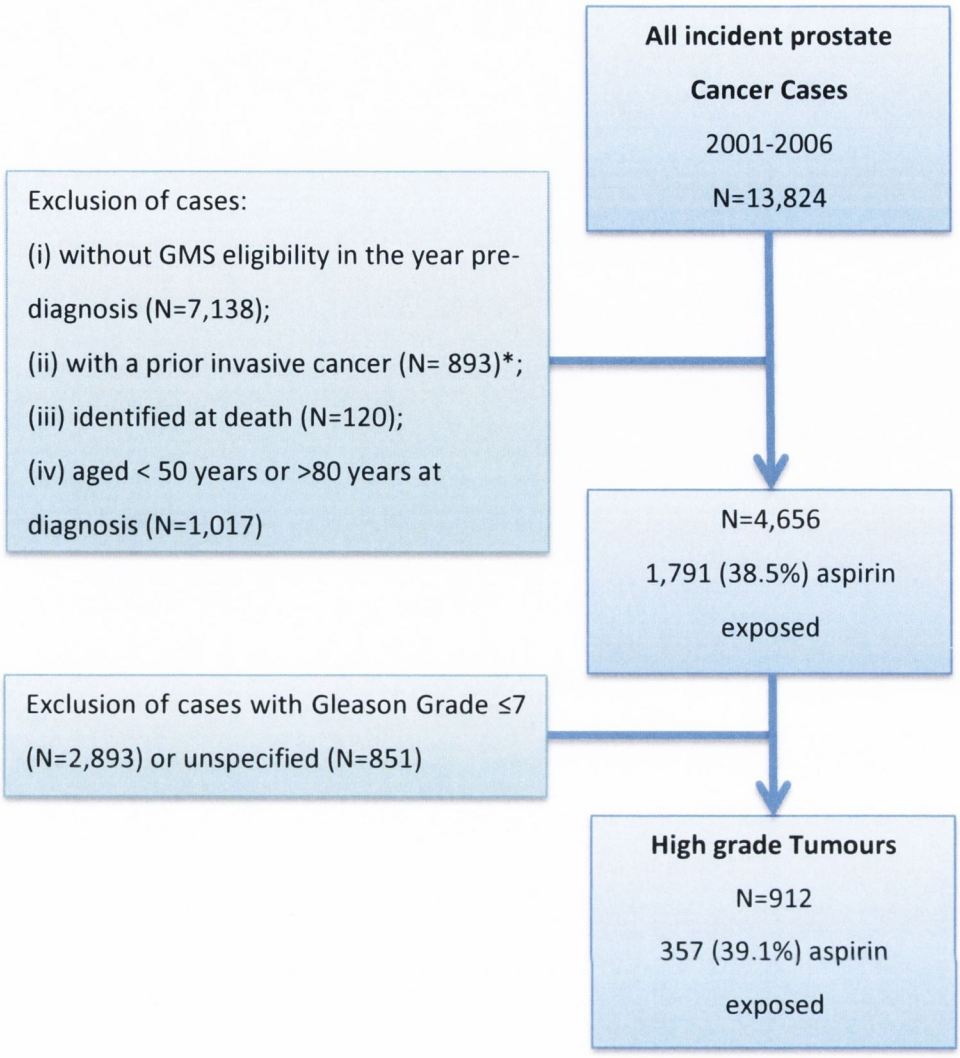

Figure 4-3: Study cohort Selection for aspirin Study II: exclusion criteria

* excluding non-melanoma skin cancers

Table 4-8: Characteristics of aspirin exposed and unexposed men in those with prostate cancer of Gleason score>7

Characteristic		Aspirin Unexposed (N=555)	Aspirin Exposed (N=357)	
Patient details				
Age /years	Mean (SD)	71.1 (6.2)	73.0 (5.3)	*
Comorbidity Score	Mean (SD)	7.5 (6.0)	12.1 (5.9)	*
Smoking Status - (%)	Never	110 (19.8)	43 (12.0)	*
	Former	180 (32.4)	123 (34.5)	
	Current	109 (19.6)	86 (24.1)	
	Unspecified	156 (28.1)	105 (29.4)	
Tumour details				
Stage - (%)[A]	I	2 (0.4)	0 (0)	
	II	237 (42.7)	139 (38.9)	
	III	50 (9.0)	37 (10.4)	
	IV	129 (23.2)	91 (25.5)	
	Unspecified	137 (24.7)	90 (25.2)	
Treatment details				
Treatment - (%)[B]	Surgery	147 (26.5)	114 (31.9)	
	Radiation	161 (29.0)	109 (30.5)	
	ADT	332 (59.8)	223 (62.5)	
	Chemotherapy	23 (4.1)	16 (4.5)	
	No treatment	96 (17.3)	50 (14.0)	
Medication	Beta-blocker	72 (13.0)	141 (39.5)	*
Exposures[C] - (%)	Statin	86 (15.5)	174 (48.7)	*
	Non-aspirin anticoagulant	49 (8.8)	50 (14.0)	*
	Anti-diabetic	28 (5.0)	56 (15.7)	*
	NSAID	235 (42.3)	183 (51.3)	*
	BPH medicines	159 (28.6)	119 (33.3)	
Aspirin exposure details:				
Pre-diagnosis[D]				
No of prescriptions dispensed		- -	10,028	
Dosing intensity	(%) Median (IQR)	- -	86.6% (44.4, 98.4)	
Post-diagnosis[E]				
Men receiving aspirin (%)		144 (26.0)	331 (92.7)	

** p-value <0.05.*

***A:** AJCC Staging Manual 5th Ed.*[43]

***B:** Received within one year following diagnosis.(not mutually exclusive)*

***C:** Medication received in one year prior to diagnosis*

***D:** Aspirin exposure in the year prior to diagnosis*

***E:** Receipt of aspirin at any point post-diagnosis.*

4.4.3.3 Effect Modification Analyses

Stratification of the analysis by tumour stage suggested a non-significantly reduced risk of prostate cancer-specific mortality associated with aspirin use in men with stage I-III tumours (Table 4-10: HR=0.91 95% CI 0.59, 1.40). However men with stage IV prostate cancer had a non-significant increased HR of prostate cancer-specific mortality associated with aspirin exposure (Table 4-10: HR=1.23, 95% CI 0.86, 1.75). The multiplicative interaction for Stage IV versus Stage I-III tumours was non-significant (*p*-interaction=0.26). No association between aspirin use and prostate cancer-specific mortality was observed in men with unspecified tumour stage (within-strata HR=0.96, 95% CI 0.57, 1.75).

As the findings regarding aspirin dose were in conflict with previous results, reported in Study I, post-hoc stratification of the analysis by tumour stage and aspirin dose received was carried out. These results are presented in Table 4-11. Consistent with the findings of Study I (Section 4.3), high dose aspirin was associated with a non-significantly reduced HR of prostate cancer-specific mortality in men with stage I-III prostate cancer (within strata HR=0.61, 95% CI 0.26, 1.42). Conversely, the association between high dose of aspirin and prostate cancer-specific mortality in men with stage IV prostate cancer, was increased and approached statistical significance (HR=1.58, 95% CI 0.98, 2.54).

Table 4-9: Estimated Hazard Ratios of prostate cancer-specific mortality associated with aspirin exposure in the year prior to diagnosis in men with prostate cancer of Gleason score >7

			Prostate cancer-specific mortality				
Aspirin Exposure	**N**	**Person Years**	**No. of deaths (rate)[A]**	**Univariate HR (95%CI)**		**Multivariate HR[B] (95%CI)**	
Aspirin unexposed in year prior to diagnosis	555	2,447	195 (79.7)	Ref	-	Ref	-
Aspirin exposed in year prior to diagnosis	357	1,585	124 (78.3)	0.99	(0.79, 1.24)	1.06	(0.81, 1.37)
Exposure response:[C] dosing intensity[D]							
Low dosing intensity 0%-86%	176	801	60 (74.9)	0.95	(0.71, 1.27)	1.00	(0.73, 1.36)
High dosing intensity 86%-100%	181	784	64 (81.7)	1.03	(0.78, 1.36)	1.13	(0.82, 1.56)
P-trend					*0.94*		*0.49*
Exposure response:[C] dose							
Low dose ≤ 75mg	263	1,204	87 (72.3)	0.91	(0.71, 1.17)	1.00	(0.75, 1.33)
High dose > 75mg	94	381	37 (97.1)	1.25	(0.88, 1.77)	1.22	(0.84, 1.77)
P-trend					*0.58*		*0.41*
Exposure response:[C] dosing intensity[D] & dose							
Low dosing intensity 0%-86%							
Low dose ≤ 75mg	123	579	41 (70.8)	0.90	(0.64, 1.25)	1.03	(0.72, 1.48)
High dose > 75mg	53	222	19 (85.8)	1.10	(0.69, 1.76)	0.94	(0.58, 1.53)
High dosing intensity 86%-100%							
Low dose ≤ 75mg	140	624	46 (73.7)	0.92	(0.67, 1.27)	0.99	(0.70, 1.42)
High dose > 75mg	41	159	18 (112.9)	1.45	(0.90, 2.36)	1.78	(1.07, 2.97)*

** p-value < 0.05.*

***A:** Mortality rate (deaths/1000 person years).*

***B:** All multivariate HRs are adjusted for age at diagnosis, tumour stage (I&II/ III/ IV/ Unspecified), smoking status at diagnosis, comorbidity score, year of incidence, pre-diagnostic exposure to beta-blockers, BPH medicines and statins. **C:** reference group: aspirin unexposed **D**: Dosing intensity by median*

Table 4-10: Aspirin exposure and prostate cancer-specific mortality: effect modification by tumour stage at diagnosis

		Aspirin Unexposed			Aspirin Exposed			Exposed Vs. Unexposed		
Tumour Stage										
I-III	**Death/Censored**	67/222			36/140					
	Multivariate HR[A] (95% CI)	Ref	-		0.91	(0.59, 1.40)	p = 0.67	0.91	(0.59, 1.40)	p = 0.67
IV	**Death/Censored**	89/40			63/28					
	Multivariate HR[A] (95% CI)	5.16	(3.73, 7.14)	p < 0.05	6.34	(4.36, 9.22)	p < 0.05	1.23	(0.86, 1.75)	p = 0.25
		Multiplicative scale: rHR (95% CI)			Stage IV Vs. Stage I-III			**1.35**	**(0.80, 2.28)**	**p = 0.26**

A: *All multivariate HRs are adjusted for age at diagnosis, smoking status at diagnosis, comorbidity score, year of incidence, pre-diagnostic exposure to beta-blockers, BPH medicines and statins*

Table 4-11: Estimated Hazard Ratios of prostate-cancer specific mortality associated with aspirin use at low and high dose, stratified by tumour stage

	Aspirin Unexposed	Aspirin Exposed Low Dose	Aspirin Exposed High Dose	Low Dose exposed Vs. Unexposed	High Dose User Vs. Unexposed
Tumour Stage					
I-III Death/Censored	67/222	30/107	6/33		
Multivariate HR[A] (95% CI)	Ref - -	1.01 (0.64, 1.59) p = 0.97	0.61 (0.26, 1.42) p = 0.25	1.01 (0.64, 1.59) p = 0.97	0.61 (0.26,1.42) p = 0.25
IV Death/Censored	89/40	41/17	23/10		
Multivariate HR[A] (95% CI)	5.17 (3.77, 7.16) p < 0.05	5.60 (3.67, 8.55) p < 0.05	8.17 (4.97,13.43) p < 0.05	1.08 (0.72, 1.63) p = 0.70	1.58 (0.98,2.54) p = 0.06
	Multiplicative scale: rHR (95% CI)		Stage IV Vs. Stage I-III	**1.07 (0.60, 1.91) p = 0.81**	**2.59 (0.99,6.77) p = 0.05**

A: *All multivariate HRs are adjusted for age at diagnosis, smoking status at diagnosis, comorbidity score, year of incidence, pre-diagnostic exposure to beta-blockers, BPH medicines and statins.*

Table 4-12: Sensitivity analyses: aspirin exposure and prostate cancer-specific mortality: aspirin exposure censored in the 6 months prior to diagnosis

			Prostate cancer-specific mortality		
Aspirin Use	**N**	**Person Years**	**No. of deaths (rate)[A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI)**
Aspirin unexposed in year prior to diagnosis	598	2,631	210 (79.8)	Ref -	Ref -
Aspirin exposed in 12-6 months pre- diagnosis	314	1,401	109 (77.8)	0.99 (0.78, 1.24)	1.07 (0.82, 1.40)
Exposure response:[C] dosing intensity[D]					
Low dosing intensity 0%-90% [C]	160	730	58 (79.5)	1.02 (0.76, 1.36)	1.07 (0.78, 1.47)
High dosing intensity 90%-100%	154	670	51 (76.0)	0.95 (0.70, 1.30)	1.07 (0.76, 1.50)
P-trend				*0.81*	*0.67*
Exposure response:[C] dose					
Low dose ≤ 75mg	226	1,049	73 (69.6)	0.88 (0.67, 1.15)	0.98 (0.72, 1.33)
High dose > 75mg	88	351	36 (102.5)	1.31 (0.92, 1.87)	1.27 (0.87, 1.85)
P-trend				*0.51*	*0.33*
Exposure response:[C] dosing intensity[D] & dose					
Low dosing intensity 0%-90%					
Low dose ≤ 75mg	105	503	37 (73.6)	0.94 (0.66, 1.33)	1.08 (0.74, 1.58)
High dose > 75mg	55	227	21 (92.7)	1.18 (0.76, 1.86)	1.06 (0.66, 1.69)
High dosing intensity 90%-100%					
Low dose ≤ 75mg	121	546	36 (65.9)	0.82 (0.58, 1.17)	0.92 (0.62, 1.34)
High dose > 75mg	33	125	15 (120.4)	1.55 (0.92, 2.62)	1.80 (1.04, 3.12)*

** p-value < 0.05.*

***A:** Mortality rate (deaths/1000 person years).*

***B:** All multivariate HRs are adjusted for age at diagnosis, tumour stage (I&II/ III/ IV/ Unspecified), smoking status at diagnosis, comorbidity score, year of incidence, pre-diagnostic exposure to beta-blockers, BPH medicines and statins. **C:** reference group: aspirin unexposed **D**: Dosing intensity for the year prior to diagnosis, stratified by median*

4.4.3.4 SENSITIVITY ANALYSES

Results of sensitivity analysis which censored aspirin use in the six months preceding diagnosis are presented in Table 4-12. The characteristics of aspirin exposed (N=314) and unexposed (N=598) men were similar to that of the original analysis. The association between aspirin exposure and prostate cancer-specific mortality in this analysis was similar to that of the original analysis and do not suggest the presence of protopathic bias.

Sensitivity analyses considering other causes of prostate cancer death did not alter point estimates appreciably. Univariate and multivariate hazard ratios including prostate cancer deaths classified as in Table 3-4 (Sensitivity Analysis 1) and including prostate cancer deaths classified as secondary or contributory causes of death (Sensitivity Analysis 2) are presented in Table 4-13.

Table 4-13: Sensitivity analyses: aspirin exposure and prostate cancer-specific mortality in men with prostate cancer of Gleason score >7.

			Prostate cancer-specific mortality		
Sensitivity Analysis 1	**N**	**Person Years**	**No. of deaths (rate)[A]**	**Univariate HR (95%CI)**	**Multivariate HR[B] (95%CI)**
Aspirin unexposed	555	2,447	201 (82.2)	Ref -	Ref -
Aspirin exposed	357	1,585	124 (78.3)	0.96 (0.77, 1.20)	1.03 (0.79, 1.34)
Sensitivity Analysis 2					
Aspirin unexposed	555	2,447	201 (82.2)	Ref -	Ref -
Aspirin exposed	357	1,585	133 (83.9)	1.03 (0.83, 1.28)	1.06 (0.82, 1.37)

A: *Mortality rate (deaths/1000 person years).*
B: *All multivariate HRs are adjusted for age at diagnosis, tumour stage (I&II/ III/ IV/ Unspecified), smoking status at diagnosis, comorbidity score, year of incidence, pre-diagnostic exposure to beta-blockers, BPH medicines and statins.*

4.4.4 Discussion

An association between pre-diagnostic aspirin use and prostate cancer-specific mortality was not observed in men with high-grade prostate cancer of any stage. High aspirin dosing intensity and higher doses of aspirin were associated with non-significant increases in risk of prostate cancer-specific death. This association was not appreciably attenuated in sensitivity analyses where aspirin initiated in the six months prior to diagnosis was censored. These observations differ from previous findings in this population, which observed that men with stage I-III prostate cancer who received aspirin at high dosing intensity had reduced risk of prostate cancer-specific mortality similar to other studies;[131,132] and men who received higher doses of aspirin (>75mg) had a statistically significant reduced risk of mortality from prostate cancer (HR=0.61, 95% CI 0.37, 0.99). Aspirin exposure was not associated with all-cause mortality, which is consistent with previous findings.

On stratification of the analysis by tumour stage a non-significant association with reduced mortality in men with tumours confined to the prostate was observed. Aspirin use was however associated with non-significant increased risks of prostate cancer-specific mortality in men with stage IV disease. This is in conflict with a recent study presented by Daugherty *et al.* which observed a significantly reduced risk of prostate cancer-specific mortality in men receiving daily aspirin diagnosed with stage III/IV prostate cancer.[296] These findings also differ from the study by Grytli *et al.* in a Norwegian cohort (N=3,561) with high-risk prostate cancer, 53% of patients had prostate cancer with Gleason score >7; a significant reduction of approximately 20% in risk of prostate cancer-specific mortality associated with aspirin exposure pre- and post-diagnosis was reported.[298] However the immortal time bias introduced by the methodology used in that study has already been discussed.[299]

Protopathic bias is the bias which may occur due to an exposure starting, stopping or otherwise changing based on factors associated with the baseline manifestation of the disease.[1] This differs from confounding by indication where the exposure may be associated with the disease outcome. Confounding by indication is also known as "channelling", where patients may be selected into an exposure group based on disease severity.[1] In this study it is possible that protopathic bias may be incurred as prescription of higher doses of aspirin in men prior to their date of diagnosis may be for analgesia in patients who may have a yet undiagnosed advanced prostate cancer. This potential bias has been addressed by censoring aspirin prescriptions in the six months preceding diagnosis. However this did not affect point estimates significantly. It is possible that a longer censoring period may be required however

due to the small number of patients in this cohort and the relatively short pre-diagnostic period examined (one year), it was not appropriate to run multiple analyses in this cohort.

Some limitations of this analysis must also be acknowledged. The sample size was small in comparison to prior studies, as it was limited by the number of patients who met the inclusion criteria. Because a definitive diagnosis of high-grade cancer was an inclusion criterion, this study is restricted to men who were fit for a prostate biopsy which may reduce the external validity of the study. Some treatment cross-over post-diagnosis occurred, 26.0% of non-users received aspirin during follow-up. This would normally be expected to bias results towards the null.

4.5 Overall Discussion

The overall association between aspirin exposure prior to diagnosis and prostate cancer-specific mortality was non-significant in Study I which examined the association in men with localised (stage I-III) prostate cancer. A non-significant 27% reduction in risk of prostate cancer death, in men with high aspirin dosing intensity was observed; which is consistent with prior studies investigating daily aspirin use.[131,132,296] The association with reduced risk of prostate cancer mortality was significant in men receiving higher aspirin doses (>75mg); this is a novel finding, and will require replication in larger studies. There was also the suggestion of effect modification in patients with high grade tumours, which may be due to elevated COX-2 expression in these tumours. This was somewhat consistent with the findings of the study carried out in the CaPSURE cohort, examining aspirin exposure in men with localised prostate cancer treated with radiation or prostatectomy although the aspirin exposure definitions differed.[133]

In Study II, the possibility that aspirin exposure was associated with greater survival benefit in men with high grade tumours was examined further. In this cohort of men with high-grade prostate cancer, of any stage, the overall association was not significant. Poorer survival was observed in men with stage IV disease compared to men with stage I-III disease. Men with localised disease appear to have a modest survival benefit associated with aspirin exposure prior to their prostate cancer diagnosis; it appears that men with metastatic prostate cancer of Gleason score >7 at diagnosis do not have a survival benefit. This may be because their disease has advanced beyond the prostate, progressed to lymph nodes or metastasised, despite aspirin exposure prior to diagnosis, possibly indicating a tumour that is refractory to aspirin.

The proposed mechanisms of action of aspirin in cancer must be considered when interpreting the biological plausibility of these results. Aspirin, at therapeutic concentrations, has been shown to inhibit prostate epithelial cell growth and it has been suggested that it plays a role in inhibiting the transition between benign and cancerous states in the prostate.[277] Given the role of inflammation in the development of prostate cancer,[27] some men who are exposed to aspirin may derive a benefit prior to their prostate cancer diagnosis. Aspirin potentially alters tumour development, such that the tumour does not advance to lymph nodes or metastasise. This hypothesis would support a number of observed epidemiological associations between aspirin use and prostate cancer: reduced prostate cancer incidence,[62,70] less advanced

prostate tumours at diagnosis,[284,285,288,292] and reduced mortality from cancer was more marked in prostate cancer cases where the tumour had not metastasised.[295]

The findings of the studies above, with respect to localised cancer in particular are broadly in agreement with this hypothesis, and daily use of aspirin prior to diagnosis,[131,132,296,297] has been associated with reduced risk of prostate cancer mortality. However aspirin use following diagnosis has not been associated with reduced mortality[297,301] (See also Appendix 4); nor has it been associated with reduced risk of developing metastases in men with localised disease at diagnosis.[297,301] In Study II an association between aspirin use and prostate cancer-specific mortality was not observed in men with stage IV disease. Considering both the COX-1 and COX-2 mediated activity of aspirin there are a number of potential reasons for this.

Firstly as an anti-platelet agent, aspirin is prescribed at low doses; it undergoes rapid metabolism and is unlikely to reach pharmacological targets other than COX-1 in platelets.[275] The anti-thrombotic warfarin has been associated with reduced incidence of prostate cancer[65,215] and lower histological grade and stage of prostate cancer at diagnosis.[64] However in a meta-analysis of patients randomised to warfarin compared to placebo there was no difference in mortality from prostate or any other adenocarcinoma.[131] Anti-coagulants have, however, been associated with greater freedom from biochemical failure, in men with localised prostate cancer treated with radiation.[309] Interference with aggregation of platelets through COX-1 inhibition by aspirin may however be of little benefit where micro-metastases have already disseminated.[310] Therefore, it is plausible that aspirin may not be associated with reduced mortality in patients with advanced prostate cancer.

With respect to COX-2 inhibition as a potential mechanism of anti-cancer activity, a small randomised controlled trial of the selective COX-2 inhibitor celecoxib in men with localised prostate cancer prior to prostatectomy has reported reduced angiogenesis and increased apoptosis in prostate tissue.[127] However, randomised studies which have examined the selective COX-2 inhibitor celecoxib as adjuvant treatment for prostate cancer have not reported it to be beneficial as treatment.[311,312] This may imply that the anti-inflammatory mechanism of aspirin acts pre-diagnostically and that the COX-2 pathway may not be particularly significant in tumours which are well developed. Additionally there may be other biological factors associated with the tumour i.e. it may adapt to overcome COX-2 inhibition, which may explain the lack of an observed association in men with stage IV disease.

Lipoxygenase (LOX) enzymes, introduced in Chapter 1, Section 1.2.4.3 are a family of enzymes which have similar substrates to COX enzymes, eicosanoids and long-chain fatty acids.[24] Some LOX enzymes have been found to be elevated in prostate tumour cells;[313] correlated with mutation of the tumour suppressor gene p53[314] and with increasing Gleason grade in prostate tissues;[315] the enzyme 15-LOX-1 has also been reported to up regulate and activate the IGF-1 receptor.[316] As the LOX substrates are similar to COX enzymes, it is important to acknowledge that the inhibition of COX-2 by indomethacin has been shown to increase the production of LOX metabolites in prostate cancer cells.[317] This may be harmful as LOX metabolites have been reported to up regulate EGF-dependent cell proliferation; enhance MAPK signalling[313] activate NF-κB;[318] mediate tumour angiogenesis and progression;[319] and have been associated with increased prostate cancer cell survival.[320] Inhibition of 5-LOX causes prostate cancer cell apoptosis.[321] Thus dual inhibition of COX-2 and LOX enzymes may be a more beneficial means of treating prostate cancer.[28] However not all LOX enzymes are detrimental in prostate cancer; 15-LOX-2 is expressed in greater amounts in normal prostate tissue than cancerous tissue and may have an opposing role to 15-LOX-1 in prostate carcinogenesis.[313] This is an area requiring further study in prostate cancer.

Reported observations regarding the association between aspirin and NSAIDs and prostate cancer incidence have varied somewhat.[322] Similar findings for aspirin and NSAIDs were observed in some observational studies, and are suggestive of reduced risk of prostate cancer.[285,290,291] Recent studies from the US[286] and Finland[70] have reported reduced prostate cancer incidence for aspirin users but not users of other NSAIDs. There may be some reasons for this related to the differential pharmacology and prescribing practices. NSAIDs inhibit COX-1 reversibly, whereas aspirin inhibits COX-1 irreversibly. Adherence to aspirin therapy is likely to be greater when it is indicated for prevention of cardiovascular disease, whereas NSAID use may be more intermittent as the indication is for mild-moderate pain, and may be prescribed *pro re nata* or "as needed". Another caveat is that investigation of NSAID use and cancer incidence is potentially associated with protopathic bias, as NSAIDs may be prescribed prior to prostate cancer diagnosis to treat symptoms of an undiagnosed cancer. This bias may attenuate any association to be observed between NSAID use and reduced cancer incidence. Associations between NSAID use and prostate cancer-specific mortality have not been examined in a large observational study.

Prior studies have examined the association between aspirin use and prostate cancer mortality in patients who were originally enrolled in randomised controlled trials,[131,276,296]

research cohorts i.e. CaPSURE,[133] and the Health Professionals Follow-up Study.[301] Aspirin exposure in trials is randomised, which negates biases introduced in observational research. In the observational studies to date, aspirin use is self-reported,[133,296,301] with the exception of those in the CPRD where exposure is determined based on prescriptions issued by GPs. The chief strength of the studies in this chapter is the detailed patient level data and most importantly the accurate aspirin prescribing data. Low-dose aspirin is only available on prescription in the Republic of Ireland, as licensing is based on the need for medical evaluation of the patient;[125] therefore misclassification of aspirin use due to over the counter purchases is likely to be minimal.

It should be acknowledged that dispensing of aspirin does not infer treatment compliance and some misclassification of exposure may occur in patients who fill a prescription for aspirin but do not take all of their prescribed doses. This would be likely to bias the observed associations towards the null. Similar to the studies of digoxin, longer periods of pre-diagnostic exposure may have been beneficial in elucidating a relationship between duration of pre-diagnostic aspirin exposure and prostate cancer-specific mortality.

4.6 Conclusion

Pre-clinical studies have identified a number of potential mechanisms of action of aspirin, and similar pharmacological agents, in prostate cancer cell lines and mouse models. Observational studies and meta-analyses have suggested that daily dosing of aspirin is associated with reduced mortality from cancers including prostate cancer. The first study presented in this chapter is consistent with a number of these prior studies which showed similar non-significant reductions in prostate cancer-specific mortality in men exposed to aspirin.[131,132,296,297] The second study suggested that men with stage IV disease, exposed to aspirin, do not have improved outcomes. This may suggest that aspirin exposure is only associated with reduced prostate cancer-specific mortality in disease which has not spread beyond the prostate, and has some consistency with studies which have observed no association between aspirin use following diagnosis and mortality.[297,301] These findings together have implications for the design of future randomised studies of aspirin in men with, or at risk of, prostate cancer.

Chapter 5 CONCLUSION

This chapter summarises the main findings of the thesis and what this research has added to the field of prostate cancer pharmacoepidemiology. It outlines some further potential areas for study, and discusses the potential for future pharmacoepidemiology research in prostate cancer.

5.1 Prostate Cancer: Advances in Research

Prostate cancer continues to challenge clinicians and researchers; few factors apart from age, family history and race are strongly associated with risk of the disease.[4,77] The prevalence of men living with prostate cancer is going to continue to increase in the coming decades due to increasing life expectancy.[51] There are a variety of curative treatment choices available to men with localised disease, however due to the associated side-effects a conservative management approach is sometimes the preferred choice. Men with advanced disease at diagnosis, and men with early stage disease who progress to biochemical failure following treatment or who develop metastatic disease have, to date, had fewer therapeutic options.

There are several emerging treatments for castrate resistant prostate cancer, these include another anti-androgen enzalutamide, which has higher affinity for the androgen receptor than bicalutamide and has been shown to improve overall survival by a median of 5 months compared to placebo.[323] The US Food and Drug Administration (FDA) has also authorised sipuleucel-T to treat advanced castrate resistant prostate cancer.[324] This is a novel autologous active cellular immunotherapy; the patient's peripheral mononuclear blood cells, including dendritic cells, are collected and activated in vitro, before re-injecting them into the patient to target prostate cancer cells. Compared to placebo patients randomised to sipuleucel-T had an improvement in survival (median overall survival 4.1 months, similar to abiraterone, licenced for the same indication).[324] These advances are to be welcomed; however there is scope, through cancer pharmacoepidemiology, to examine existing medicines as potential anti-cancer agents.

Cancer pharmacoepidemiology investigates associations between medications and cancer risk and mortality. These studies may identify other molecular pathways that can be targeted to provide clinically meaningful improvements in disease outcomes for men with prostate cancer. Pre-clinical researchers have proposed many hypotheses about the anti-cancer mechanisms of existing medicines; however until these hypotheses are tested in humans, their progress towards use in cancer treatment is limited. Few pharmaceutical companies will invest money in randomised trials of these medicines as their patents have expired; therefore, cancer pharmacoepidemiology enables these hypotheses to be examined in a wider population.

5.2 Digoxin in Prostate Cancer

There has been convincing pre-clinical evidence that digoxin or other cardiac glycosides may have anti-cancer potential; however the exact mechanism for this anti-cancer effect in prostate cancer has not been elucidated. The inhibition of HIF-1α has been suggested to be a very promising mechanism of anti-cancer action. This would be a novel method of treating prostate cancer,[46] as it acts by inhibiting the tumour's adaptation to hypoxia through induction of angiogenesis and reprogramming energy metabolism.

Digoxin is prescribed at relatively low prevalence in a population with existing cardiovascular morbidity i.e. heart failure or atrial fibrillation. Therefore the study of prostate cancer outcomes and digoxin exposure in this cohort was a challenging research question. In Chapter 3, Study I (Section 3.3) examined the association between digoxin exposure prior to diagnosis and prostate cancer stage and grade at diagnosis. Despite Platz *et al.* reporting that digoxin is associated with reduced prostate cancer incidence,[61] no significant association between prostate cancer stage, or prostate cancer Gleason score and pre-diagnostic digoxin exposure was observed. This study was limited by missing data; many digoxin patients did not have their cancers completely staged, or Gleason score determined, therefore it was difficult to draw conclusions from these results. The digoxin exposure period identified prior to diagnosis was one year, compared to the study by Platz *et al.* which examined more than ten years of self-reported digoxin use.[61]

In Chapter 3, Study II examined the association between digoxin use at the time of diagnosis and prostate cancer-specific mortality; this study was similarly limited by incomplete data for digoxin patients. The use of a propensity score to match digoxin exposed men to unexposed men, did balance the patient characteristics. However in the full cohort or the propensity score matched cohort, no association was observed between digoxin and prostate cancer-specific mortality.

5.2.1 Future Research into Digoxin as an Anti-Cancer Agent

As already discussed, digoxin is being examined in clinical trials of recurrent prostate cancer (NCT01162135);[210] the outcome measure in this study is PSA doubling time. However as digoxin, at concentrations close to therapeutic plasma concentrations, has been shown to reduce expression of PSA in a pre-clinical study,[186] the use of this measure in determining a response is questionable. Digoxin is also being examined in a window of opportunity study in women with operable breast cancer to examine the influence of digoxin on molecular markers

of response (NCT01763931). The results of this study may establish whether digoxin levels *in vivo* in humans have a meaningful effect on tumours, and whether this is mediated through HIF-1α inhibition or another mechanism.

The inhibition of HIF-1α, proposed as the promising anti-cancer mechanism of digoxin has not been proven as a stand-alone therapeutic target for prostate or other cancer.[242] In time, HIF-1α inhibition may have therapeutic use in the treatment of cancer in synergy with another anti-cancer agent. However the most significant issue in testing the hypotheses proposed by laboratory researchers regarding the use of cardiac glycosides in cancer is that of tolerable dose. Firstly the drug concentrations tested in cell cultures far exceed the plasma levels tolerable in humans; and secondly, mice used for *in vivo* tumour models have far higher tolerance for cardiac glycosides than humans, who are sensitive to their cardio-toxic side effects.[237,238]

Other cardiac glycosides, and the related bufadienolide compounds, continue to be examined in preclinical and clinical studies for their anti-cancer potential in prostate and other cancers.[325] The proposed anti-cancer mechanisms of action of these other cardenolide and bufadienolide compounds have some similarities to those proposed for digoxin i.e. disruption of intracellular calcium homeostasis, HIF-1α inhibition, however a plethora of other mechanisms have also been proposed, and differ according to the cancer type.[152] Less is understood about the safety profile of these other compounds in humans and it remains to be seen whether these compounds will progress through the drug development cycle to be used as anti-cancer agents. It is unlikely that digoxin will become a suitable therapy for patients with prostate cancer. Despite promising results in pre-clinical studies, the risks of toxicity in humans will undoubtedly be too great to recommend its use and furthermore no study to date has shown therapeutic benefit in men with the disease.

5.3 Aspirin in Prostate Cancer

A number of mechanisms have been proposed by which aspirin has anti-cancer activity. In the preclinical setting there is considerable evidence that platelets support the development of distant tumour metastases;[49] it has been suggested that aspirin may reduce tumour dissemination and metastasis formation through inhibition of COX-1 mediated platelet function.[271] In clinical studies, COX-2 expression in prostate cancer tissue has been associated with tumours which have poorer prognosis; and inhibition of COX-2 in prostate cancer has shown promising effects in preclinical studies.[26] These have been described in Chapter 4, Section 4.1.

The consistency of the associations observed in Study I (Chapter 4, Section 4.3) with prior studies examining any[297] and daily[131,132,296] aspirin use in conjunction with the stronger association observed in men who received higher doses of aspirin, would be suggestive of a causal association between aspirin use and reduced mortality from prostate cancer. The observed association between higher aspirin dose and reduced risk of prostate cancer-specific mortality should perhaps be interpreted with some caution as there may be some residual confounding. In Study II (Chapter 4, Section 4.4) the suggestion that aspirin use does not appear to be associated with a reduced risk of prostate cancer-specific mortality in men with stage IV disease of Gleason score >7 is an interesting finding. However given that no associations have been observed between aspirin use post-diagnosis and the development of metastases or prostate cancer-specific mortality[296,299] it is plausible that aspirin may only mediate an anti-cancer effect in disease which has not metastasised. This may be because aspirin exposure prior to diagnosis reduces the risk of presenting with advanced cancer, as reported in meta-analyses of randomised controlled trials and observational studies.[284]

Despite the results of the aspirin Study I suggesting an association between aspirin use and prostate cancer-specific mortality in men with non-metastatic prostate cancer, it cannot be elucidated from these results whether this is due to the COX-1 mediated anti-platelet effect of aspirin; the COX-2 anti-inflammatory effect of aspirin; or one of the other mechanisms of action of aspirin which have been referred to earlier (Chapter 4; Section 4.1). It would be advantageous therefore for pharmacoepidemiology to integrate further with molecular epidemiology to establish patient cohorts and tumour bio-banks to determine what patient or tumour factors i.e. anti-platelet effects or COX-2 inhibition, are associated with this observed reduction in risk of prostate cancer-specific mortality in men who receive aspirin.

5.3.1 Future Research into Aspirin as an Anti-Cancer Agent

Aspirin has potential for further research in prostate cancer. The quality of the prescription claims data in the NCRI-PCRS database means that collaboration with other researchers internationally may be feasible. This would enable a larger meta-analysis of individual or aggregated patient data to further investigate these associations in the observational setting. Based on the results of pre-clinical studies, the findings of this work and others, clinical studies may be best directed towards cohorts of patients who are at risk of prostate cancer as it does not appear that aspirin use following diagnosis is associated with reduced risk of prostate cancer-specific mortality. In particular research should focus on determining what molecular or pathological tumour characteristics are predictive of a therapeutic response to aspirin.

5.3.1.1 Chemo-Preventative Potential of Aspirin in Cancer

The role of aspirin in cancer prevention has been the subject of much discussion.[275] Aspirin exposure has been most significantly associated with reduced incidence of colorectal cancer[326] and improved outcomes in colorectal cancer patients.[131,132,327] A randomised trial of either aspirin (600mg) or placebo in patients with Lynch syndrome found that patients who were randomised to aspirin had a reduced risk of developing colorectal cancer.[328] Prior to the meta-analyses of randomised studies examining aspirin use and cancer incidence and mortality,[131,276,326] there was not sufficient information to appropriately consider the risk-benefit balance of aspirin in chemo-prevention.[329] A recent review of the potential use of aspirin as a chemo-preventative agent for colorectal cancer has suggested that the potential combined benefits of aspirin for long term prevention of chronic disease be reconsidered against the bleeding risks.[327] Considering that the risk-benefit profile of aspirin as primary prevention of cardiovascular disease is not favourable,[130] if aspirin was introduced as a chemo-preventative agent in cancer, the most suitable group would potentially be patients at increased risk of cancer. This may include those with Lynch syndrome, in whom aspirin has been shown to significantly reduce the risk of colorectal cancer.[328]

Aspirin use has not been strongly associated with reduced risk of prostate cancer.[62,284] However, daily aspirin use prior to diagnosis has been associated with non-significantly reduced risk of prostate cancer specific mortality in prostate cancer patients in the study presented here (Chapter 4 Section 4.3) and others.[131,132,296] There is, therefore, some rationale for investigating these associations further to determine which sub-groups of men may respond to aspirin. It would also be of interest to investigate whether men diagnosed with pre-cancerous diseases of the prostate such as PIA or PIN may benefit from aspirin use.

Currently these pre-cancerous conditions are not treated,[36] even though they are recognised as pre-cancerous states in animal models of prostate cancer.[4] Investigation of the molecular and pathological characteristics of prostate tumours in men who have received aspirin routinely, prior to their diagnosis, would also provide a better understanding regarding the mechanisms of action through which aspirin may influence tumour development.

5.3.1.2 Post-diagnostic Exposure

The studies presented in this thesis did not examine exposure following prostate cancer diagnosis. Many reviewers and other researchers have called for investigation of exposures following diagnosis and the association with mortality to examine the clinical potential for repositioning medicines in the treatment of cancer.[304] In prostate cancer alone three studies have specifically examined the association between aspirin use following prostate cancer diagnosis and prostate cancer mortality and the methodologies used have differed considerably.[297,301] These are discussed in Chapter 4, Section 4.2.3.2. The overall conclusion to be drawn from these studies is that there does not appear to be a causal association between aspirin use following diagnosis and prostate cancer-specific mortality. However these studies have highlighted some of the challenges in examining medication use following prostate cancer diagnosis, in particular time-varying confounding.

Due to the changes in post-diagnostic use of aspirin documented in these studies, the cancer pharmacoepidemiology research group is examining aspirin prescribing following diagnosis in patients with breast, prostate and colorectal cancers using the linked NCRI-PCRS database. In particular, this study will focus on identifying changes in aspirin prescribing associated with disease progression. Given that potentially confounded estimates of the association between aspirin use following diagnosis and prostate cancer specific mortality have been presented, there is a need to bring these altered patterns of aspirin prescribing in cancer patients to the attention of researchers in this area.

There are statistical methods such as inverse probability weighting or marginal structural modelling which may be used to address time-varying confounding.[330] In this type of analysis, propensity for receipt of treatment i.e. aspirin, is determined conditional on other covariates at different time-points following diagnosis. The results from these analyses are dependent on the correct specification of these propensity scores, which require accurate information on factors which may influence aspirin prescribing. These factors could be related to cancer progression, or alternatively they could be related to other morbidities which could prompt aspirin prescribing, i.e. myocardial infarction. At present the NCRI-PCRS database does not

have sufficient information to carry out this type of analysis. In fact few databases of routinely gathered data have sufficiently detailed data for this type of analysis.

5.4 Potential for Future Study

5.4.1 Building on Existing Resources

This pharmacoepidemiology research has been made possible by the linkage of the NCRI and PCRS databases. Continuation of this type of research will require further investment in data collection and linkage of these data sources as well as the incorporation of other datasets. In time, longer duration of medication exposure and patient follow-up will enhance this resource. The NCRI also links cancer patient records to records from the Hospital In-Patient Enquiry (HIPE) Scheme; a database managed by the Economic and Social Research Institute in association with the HSE. This is a database of acute hospital discharge data (including diagnoses, medical and surgical procedures) of all in-patient episodes provided by acute public hospitals in Ireland. For prostate cancer the capture of this data may not be complete because many treatment procedures are carried out in the out-patient setting. Therefore, this data was not used in these studies. This additional linked resource may be of value in future research projects, particularly of other cancers.

It is also intended that the longitudinal prescription claims of GMS patients who are not diagnosed with cancer will be linked to be used for research in combination with the linked NCRI-PCRS database. It will then be possible to carry out studies in the GMS cohort to examine the association between medicines use and cancer incidence.

5.4.2 Enhancing Inter-disciplinary Collaboration

This research has not been able to elucidate the reasons that prostate cancer patients exposed to digoxin do not appear benefit from it, or through what mechanisms prostate cancer patients may respond to aspirin. Therefore a more in-depth examination of the relationship between pharmacological mechanisms of action of these drugs and disease characteristics is required. There is a need to build on the routinely collected data sources and establish prospective cohorts of cancer patients. The incorporation of molecular, pathological and clinical information into existing data sources would generate databases with huge research potential, including data on genetic mutations, enzyme or receptor expression and biomarkers. Ideally these resources would include accurate follow-up of treatments received which are not routinely collected by the NCRI i.e. chemotherapy type, disease progression (biochemical failure) or recurrence. This type of resource can provide the platform for inter-disciplinary collaborative research across many disciplines; this is demonstrated by the Trans-disciplinary Prostate Cancer Partnership (ToPCaP)[331], which integrates molecular pathology

and epidemiology data sources. This collaboration includes over 60 prostate cancer researchers from 10 institutions in Europe and the United States.

These large collaborations are required as cancer research begins to focus on less common mutations or tumour characteristics. For example Liao *et al.* have identified that colorectal cancer patients with a mutation of PIK3CA, and who received aspirin following their cancer diagnosis had significantly reduced risk of colorectal cancer death.[332] This illustrates the need for interdisciplinary research incorporating pharmacoepidemiology with other research areas, to obtain more detailed patient and tumour information and identify the patient groups who respond to various therapies.

5.5 Conclusion

The discipline of pharmacoepidemiology has capitalised on the availability of large volumes of data capturing information on medication usage and patient health outcomes. The studies undertaken here have been based on existing pre-clinical and pharmacoepidemiological evidence. The promising pre-clinical evidence regarding digoxin does not appear to translate to meaningful improvements in outcomes for prostate cancer patients, owing perhaps to the differences in digoxin concentration used *in vitro* and tolerated in humans. The findings regarding aspirin and mortality are consistent with the findings of pre-clinical studies regarding aspirin potentially impeding tumour development, and those studies which have demonstrated reduced risks of mortality in men with localised disease.

Pharmacoepidemiology affords the cancer research community the opportunity to examine on a population-level whether the pharmacology of existing medicines may have a role in the development, prevention or treatment of cancer. Pharmacoepidemiologists have developed skills in the evaluation of pre-clinical data, the analysis of large linked databases and knowledge of the drugs and therapeutic areas which they study. These multi-skilled researchers are required for the development of epidemiology into the twenty-first century and beyond.[18] However in order to increase the value and impact of this research, pharmacoepidemiology in Ireland will require interdisciplinary collaboration with expert pre-clinical researchers and clinicians. This will assist in advancing our understanding of cancer, improving therapies and ultimately improving patient outcomes.

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APPENDICES

APPENDIX 1: A COHORT STUDY OF DIGOXIN EXPOSURE AND MORTALITY IN MEN WITH PROSTATE CANCER

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SUMMARY

Objectives:

- To examine the association between digoxin exposure and mortality in men with prostate cancer using linked Irish national cancer registry and pharmacy claims data.
- Digoxin users were matched to non-users using a propensity score to identify men with similar cardiovascular comorbidity.

Patients and Methods:

- Prostate cancer cases were identified from the database and digoxin exposure at prostate cancer diagnosis was identified from prescription claims.
- Adjusted hazard ratios (HR) and 95% confidence intervals (CI) were estimated for the association between digoxin exposure and all-cause and prostate cancer-specific mortality.
- Analyses were repeated in the propensity score matched cohort.
- Effect modification of treatment with radiation or androgen deprivation therapy by digoxin exposure was also assessed.

Results:

- 5,732 men with a prostate cancer diagnosis (2001-2006) were identified (digoxin exposed, N=391). Median follow-up 4.3 years.
- Digoxin exposure was associated with a small non-significant increase in prostate cancer-specific mortality in this full cohort (HR=1.13, 95%CI 0.91, 1.42) and the propensity score matched cohort (HR=1.17, 95%CI 0.88, 1.57).
- Adjusted HRs for all-cause mortality were increased for digoxin exposed men (HR=1.24, 95%CI 1.07, 1.43).
- Interactions with treatments received were not significant.

Conclusions:

- These results suggest digoxin exposure is not associated with reduced prostate cancer-specific mortality.
- Further investigation of other cardiac glycosides which have shown anti-cancer potential may be warranted.

KEYWORDS:

Prostate neoplasms; propensity score; cardiac glycoside; hypoxia-inducible factor 1-α; mortality; digoxin.

Introduction

Preclinical studies have shown that the cardiac glycoside, digoxin, inhibits hypoxia inducible factor 1α (HIF-1α) protein synthesis and the expression of HIF-1 target genes in prostate cancer cells.[1] Overexpression of HIF-1α in prostate cancer is associated with larger tumour size,[2] increased angiogenesis,[3] treatment resistance and poorer prognosis.[2, 4] The down regulation of HIF-1α signalling by digoxin in prostate cancer models has been shown to inhibit tumour growth[1] and tumour vascularisation.[5] Digoxin and other cardiac glycosides have also been shown to reduce the development of metastases in prostate[6] and breast tumour models;[7] and to sensitise cancer cells to radiation.[8] These preclinical results suggest that digoxin exposure may be associated with reduced mortality in men with prostate cancer.

To date, observational research has focused on associations between digoxin exposure and cancer incidence. In a recent observational study, regular digoxin exposure was associated with a 23% reduction in the risk of prostate cancer.[9] The results from this study indicated the presence of an exposure-response effect, with longer duration of digoxin use (≥ 10 years) associated with greater reductions in risk.[9] Inhibition of HIF-1α by digoxin was proposed as one of the potential mechanisms for this risk reduction. Conversely, digoxin exposure has been associated with an increased risk of oestrogen dependent cancers.[10-14] It has been suggested that this increased risk may be due to phyto-oestrogenic properties of digoxin and its ability to bind with oestrogen receptors.[15] Targeting oestrogen receptors in prostate cancer has been explored as a treatment for the disease and may represent an alternative potential mechanism by which digoxin could influence prostate cancer outcomes.[15, 16]

There is little information available on whether digoxin exposure is associated with longer survival in men with prostate cancer. The aims of this study were to investigate: (i) associations between digoxin exposure at diagnosis and mortality in men with prostate cancer; (ii) whether associations between digoxin exposure and mortality are modified by receipt of radiation therapy or androgen deprivation therapy (ADT).

Patients and Methods

Setting & Data Sources

Patient records from the National Cancer Registry Ireland (NCRI), linked to Ireland's Health Services Executive (HSE) – Primary Care Reimbursement Service (PCRS) pharmacy claims database, were used to conduct this study. The NCRI uses active registration methods to collect detailed information on all incident cancers in the population usually resident in Ireland. Trained, hospital based tumour registration officers collect information on patient

characteristics, tumour details, treatment received and death from multiple sources including pathology laboratories, radiology departments, oncology departments, hospital administrative systems, individual medical records and death certificates. The HSE-PCRS general medical services (GMS) scheme provides state-funded universal healthcare, including medicines, to approximately one third (1.4 million) of the Irish population.[17] Eligibility for the GMS scheme is assessed through means test and age. The GMS database records full details of all prescription drugs dispensed from community pharmacies to eligible patients. Drugs are coded according to the WHO Anatomical Therapeutic Chemical Classification (ATC) system.[18] The use, for research, of anonymised data held by the NCRI is covered by the Health (Provision of Information) Act 1997.

Study Cohort

Men were eligible for inclusion in the study if they had a diagnosis of prostate cancer (ICD-O, C61.9)[19] of any stage between 1st January 2001 and 31st December 2006, and eligibility for the GMS scheme from at least one year prior to diagnosis. Men with prior invasive tumours other than non-melanoma skin cancer or diagnosed with prostate cancer at the time of death were excluded.

Exposure Definition

Men exposed to digoxin at the time of prostate cancer diagnosis were identified from prescription claims for digoxin (ATC: C01AA05) in the 90 days prior to diagnosis. The date, dose and number of days' supply on each prescription were recorded. Digoxin exposure in the 90 days prior to diagnosis was stratified by dosing intensity, defined as the proportion of days in the 90 days prior to diagnosis that a man has a supply of digoxin available, divided into tertiles (low, intermediate, high).

Outcome Definitions

Deaths from prostate cancer (ICD9: 185; ICD10: C61), from other causes, and associated dates of death were identified from the NCRI database. Patients were followed from date of diagnosis until the first of death or December 31st 2009.

Covariate Definitions:

The NCRI database was used to identify the following tumour details: tumour stage[20] (I, II, III, IV, Unspecified) and tumour grade[20] (grade I, Gleason score <5; grade II, Gleason score 5-7; grade III & IV, Gleason score >7; unspecified). Treatment received in the year following diagnosis (surgery, radiation, ADT, chemotherapy); age at diagnosis (years, continuous) and

smoking status at diagnosis (current, never, former, unspecified) were also identified from this database.

Prescription dispensing data from the GMS database was used to identify the use of other co-prescribed medication (exposed, unexposed) prior to diagnosis (Table A1). The number of distinct medication classes (5 character ATC code) dispensed in the year prior to diagnosis was used as a measure of comorbidity (number of medication classes, continuous). This medication based comorbidity score is a validated predictor of healthcare usage and mortality in an older adult population. [21]

STATISTICAL ANALYSIS:

The frequency and proportion of digoxin exposed and unexposed men were tabulated by clinical and demographic variables. Unadjusted all-cause and prostate cancer-specific mortality rates were calculated for digoxin exposed and unexposed men. Adjusted cumulative probabilities for prostate cancer-specific and all-cause mortality were estimated for digoxin exposed and unexposed men.[22] Univariate and multivariate hazard ratios (HR) and 95% confidence intervals (CI) for associations between digoxin exposure and (i) all-cause mortality (ii) prostate-cancer specific mortality were estimated using Cox proportional hazards models (SAS, PROC PHREG). Covariates were assessed for inclusion in the multivariate model based on prior knowledge of potential predictors of prostate cancer mortality (age;[23] comorbidity score;[21] smoking status;[24, 25] tumour stage;[23] tumour grade;[23] diabetes;[26] and exposure to aspirin,[27] beta-blockers,[28, 29] warfarin,[30] statins,[31] Non-Steroidal Anti-Inflammatory Drugs (NSAIDS)[31] or drugs used for the treatment of Benign Prostatic Hypertrophy (BPH)[32]). The year of incidence, and treatments received in the year following diagnosis (surgery, radiation, ADT; time dependent covariates) were also assessed for inclusion. Backwards elimination of variables in a stepwise manner up to a 10% maximum cumulative change in the effect component of the fully adjusted HR, was used to select the final multivariate model.[33]

Exposure response analyses were conducted by tertiles of digoxin dosing intensity (low, intermediate, high). The presence of effect modification by radiation therapy or ADT received in the year following diagnosis was assessed. Measures of interaction were estimated on a multiplicative scale (ratio of hazard ratios, rHR) with 95% CIs. SAS Version 9.2 was used for all analyses (SAS Institute, Cary NC). Results were considered statistically significant at a two-sided α-level of 0.05.

Matched Analysis

The high cardiovascular comorbidity associated with indications for digoxin use may confound associations between digoxin exposure and prostate cancer outcomes through differential effects on the selection and use of prostate cancer treatments.[34-37] Secondary analyses of all-cause and prostate cancer specific mortality were carried out using propensity score trimmed and matched cohorts.[38] A propensity score model was developed to predict digoxin exposure at the time of prostate cancer diagnosis as follows: Covariates were assessed for inclusion in the propensity score model based on prior knowledge of demographic covariates associated with cardiovascular comorbidity (age, comorbidity score) and exposure to cardiovascular medications commonly co-prescribed with digoxin (Table A2).[39] Logistic regression models (SAS®, PROC LOGISTIC) were used to estimate propensity scores for digoxin exposure using these covariates. Main effects, interaction terms and quadratic or cubic terms were included as appropriate.[40] Covariate balance within propensity score quintiles was assessed by standardised differences (*d*), with a $d<0.1$ being the desired limit.[41] The multivariate propensity score model which achieved the optimal balance of matched covariates between digoxin exposed and unexposed men was selected. Men with a propensity score outside the 1st to 99th percentile for digoxin exposed men were excluded[42] (trimmed cohort) and the propensity score was re-estimated in this population.[40] Digoxin exposed and unexposed men were then matched (1:1) within a calliper of 0.2 standard deviations of the propensity score logit[41, 43] using greedy matching without replacement.[44] Covariate balance between digoxin exposed and unexposed men in the matched cohort was assessed by standardised differences ($d<0.1$).

Sensitivity analysis

Sensitivity analyses were conducted to assess the possibility that prostate cancer specific mortality was misclassified on death certificates. Firstly mortality from prostate cancer was defined using ICD mortality site codes for ill-defined cancer sites, secondary cancer sites, cancers of uncertain or unknown behaviour (see table A3.1).[45] Secondly an analysis was carried out in which deaths where prostate cancer was identified as a secondary or contributory cause of death on the death certificate were defined as prostate cancer deaths.

Results

Cohort Characteristics

A flow diagram outlining the study cohort selection is presented in Figure 1. The characteristics of digoxin exposed and unexposed men in the full cohort and the propensity score matched cohort are presented in Table 1. In the full cohort digoxin exposed men (n=391) were older and had a higher comorbidity score than unexposed men (n=5,341). Digoxin exposed men were also more likely to have stage IV disease and less likely to have received radiation. Men in the low, intermediate and high dosing intensity tertiles had mean post-diagnostic digoxin exposures in the year post-diagnosis of 53.8%, 70.8% and 80.6% respectively. The median follow-up was 4.3 years.

In the trimmed cohort, differences between exposed and unexposed men were reduced, but remained significant for some covariates, including age and comorbidity score. In the propensity score matched cohort, acceptable balance for matched covariates was achieved between digoxin exposed (n=387) and unexposed men (n=387). Tumour stage, tumour grade and treatment received in the year following diagnosis were also comparable between the matched groups, although digoxin exposed men were marginally less likely to have been treated with radiation (13.2% versus 17.1 %, d=0.11).

Survival analysis

Adjusted cumulative probability plots and hazard ratios for all-cause and prostate cancer-specific mortality in the full cohort are presented in Figure 2 and Table 2 respectively. In the full cohort digoxin use was associated with a 24% increase in the risk of all-cause mortality (multivariate HR=1.24, 95%CI 1.07, 1.43) and a non-significant 13% increase in the risk of prostate cancer-specific mortality (HR=1.13, 95%CI 0.91, 1.42). Adjusted estimates were not appreciably different in the propensity score trimmed (Table 2: all-cause HR=1.23, 95%CI 1.07, 1.43; prostate cancer-specific HR=1.12, 95%CI 0.90, 1.41) or matched populations (Table 2: all-cause HR=1.20, 95%CI 1.00, 1.49; prostate cancer-specific HR=1.17, 95%CI 0.88, 1.57). Adjusted cumulative probability plots indicate that associations between digoxin exposure and prostate cancer specific mortality did not vary considerably over time.

In multivariate exposure response analyses (Table 2) no trend was observed for associations between prostate cancer-specific mortality and increasing dosing intensity (*P-trend*=0.59). Analyses of interaction between digoxin use and the receipt of radiation with respect to prostate cancer-specific mortality (Table 3, *P-interaction*=0.14), or ADT (Table 4, *P-interaction*=0.35) were also non-significant. Within-strata of men who received radiation and

ADT adjusted HRs suggested the possibility of increased prostate cancer-specific mortality for digoxin exposed men compared to unexposed men, (Table 3, HR=1.77, 95% CI 0.95, 3.30and Table 4, HR=1.22, 95%CI 0.93, 1.59 respectively). However the risk estimates did not reach formal statistical significance. These results were unchanged in sensitivity analyses for misclassification of prostate cancer-specific cause of death. Table A3.2. & Table A3.3.

DISCUSSION

Preclinical evidence has suggested a possible role for digoxin in the treatment of prostate cancer. In this study of 5,732 men with prostate cancer, digoxin use was not associated with a reduction in prostate cancer-specific mortality. These results were unchanged in matched analyses of men with similar cardiovascular comorbidities, suggesting that the lack of observed effect is not confounded by associations between high cardiovascular comorbidity and less aggressive treatment of prostate cancer in digoxin treated men. Additionally, no trend was observed for increasing digoxin dosing intensity in exposure response analyses. Digoxin dose dispensed (low, ≤125 mcg; high, > 125 mcg) was not associated with any trend in all-cause or prostate cancer-specific mortality either (results not presented).

In a recent observational study of men with metastatic prostate cancer treated with docetaxel, digoxin exposure was associated with an increased risk of all-cause mortality (HR=1.43, 95%CI 1.01, 2.03).[46] The authors concluded that this increased risk was due to higher levels of cardiovascular comorbidity in the digoxin exposed group. Similarly, the results presented here also show an increased risk of all-cause mortality among digoxin exposed men; this is most likely also due to increased cardiovascular comorbidity in this group. In the propensity score matched cohort associations with all-cause mortality were reduced although these approached significance after adjustment.

Several preclinical studies have indicated a role for digoxin in prostate cancer through the inhibition of HIF-1α. It has, however, been suggested that digoxin plasma levels achievable in humans may not be sufficient to effectively inhibit HIF-1α and prostate cancer progression.[47, 48] In pre-clinical studies, digoxin has been shown to inhibit HIF-1α in prostate cancer cell lines at concentrations of 100 nM and prostate cancer cell proliferation at concentrations of 23-255 nM.[9] These digoxin concentrations are, however, considerably higher than the therapeutic plasma concentrations normally tolerated in humans, 1.6 ± 1.0 nM.[49] The possibility that typical levels of digoxin exposure in humans may not adequately inhibit HIF-1α may explain why digoxin exposure was not associated with reduced prostate cancer-specific mortality in this study. It has been suggested that prolonged exposure to

digoxin, even at normal therapeutic concentrations, may successfully inhibit HIF-1α in humans.[1] There was, however, no evidence in this study of a dose-response trend with increasing digoxin dosing intensity. Additionally it should be noted that the clinical benefits of HIF-1α inhibition as a therapeutic target in prostate cancer, have yet to be demonstrated in a randomised setting.[50]

Elevated HIF-1α expression in prostate tumours has been associated with increased resistance to radiation[51] and it has been suggested that digoxin may have synergistic activity in combination with radiation therapy.[52] Analyses of interaction between digoxin use and receipt of radiation in the year after prostate cancer diagnosis did not indicate that digoxin use was associated with additional clinical benefit in men receiving radiation therapy. There was, instead, the suggestion that digoxin use in men receiving radiation therapy may be associated with increased prostate cancer-specific mortality. The reasons for this are unclear. It should, however, be noted that the number of men, receiving both digoxin and radiation in this subgroup analysis was small and these results will require confirmation in larger studies.

Digoxin has also been reported to have phyto-oestrogenic properties.[53] Increased endogenous oestrogen levels, and alterations in the testosterone-oestrogen ratio have been weakly associated with prostate cancer risk.[54] Oestrogens have also been used in the treatment of prostate cancer.[55] More recently, it has been shown in prostate cancer that oestrogen signalling is mediated through two oestrogen receptor subtypes, ERα and ERβ, with opposing effects.[56, 57] ERα signalling in prostate cancer has been associated with increased tumour cell proliferation, ERβ signalling has been reported to have anti-proliferative effects that balance the proliferative action of androgens in prostatic tissue;[57] ERβ can also be associated with a more aggressive prostate cancer phenotype.[4] Concerns have been raised about the proliferative effects of agents with ER-α agonist activity in prostate cancer;[54, 56, 57] However, the exact ER-subtype that digoxin is proposed to act on has not been identified. The results presented here do not out rule the possibility that digoxin exposure may be associated with an increased risk of prostate cancer-specific mortality.

The strengths of this study include the cohort design, high quality outcome data and the availability of detailed digoxin prescription histories. The study also has some limitations. High levels of comorbidity associated with digoxin use may have limited the ability to detect small or longer term benefits from digoxin exposure. Subgroup analyses, stratified by treatment receipt, were limited by small numbers and the results from these should be interpreted with caution. Digoxin use was based upon prescriptions dispensed and non-compliance with

received treatment will have resulted in exposure misclassification. Additionally, digoxin exposure groups were defined at diagnosis and post-diagnostic treatment crossover will also have resulted in exposure misclassification; such misclassifications will usually bias results towards the null. In the recent study by Platz et al, digoxin use for longer than ten years was associated with a significantly reduced incidence of prostate cancer.[9] Prescription histories of this duration were not available for analysis in this study.

In conclusion, the results from this analysis do not suggest that digoxin use is associated with a reduction in prostate cancer-specific mortality.

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Table 1: Characteristics of digoxin exposed and unexposed men

Characteristic		Full cohort			Propensity score matched cohort		
		Unexposed (N=5341)	Exposed (N=391)		Unexposed (N=387)	Exposed (N=387)	
Patient details							
Age /years	Mean (SD)	73.1 (7.9)	77.5 (7.2)	**	77.8 (7.0)	77.5 (7.2)	
Comorbidity Score	Mean (SD)	8.9 (6.2)	13.2 (6.6)	**	13.0 (6.3)	13.2 (6.5)	
Smoking Status - (%)	Never	1712 (32.1)	134 (34.3)		143 (37.0)	131 (33.9)	
	Former	1020 (19.1)	85 (21.7)		93 (24.0)	85 (22.0)	
	Current	853 (16.0)	55 (14.1)		47 (12.1)	55 (14.2)	
	Unspecified	1756 (32.9)	117 (29.9)		104 (26.9)	116 (30.0)	
Tumour details							
Stage - (%) [a]	I	166 (3.1)	12 (3.1)	*	19 (4.9)	12 (3.1)	
	II	2589 (48.5)	147 (37.6)		151 (39.0)	145 (37.5)	
	III	410 (7.7)	22 (5.6)		17 (4.4)	22 (5.7)	
	IV	761 (14.2)	84 (21.5)		86 (22.2)	82 (21.2)	
	Unspecified	1415 (26.5)	126 (32.2)		114 (29.5)	126 (32.6)	
Grade - (%)	I	334 (6.3)	30 (7.7)	*	23 (5.9)	30 (7.8)	
	II	2769 (51.8)	155 (39.6)		167 (43.2)	152 (39.3)	
	III / IV	1061 (19.9)	70 (17.9)		75 (19.4)	70 (18.1)	
	Unspecified	1177 (22.0)	136 (34.8)		122 (31.5)	135 (34.9)	
Treatment details							
Treatment - (%) [b]	Surgery / RP	1339/ (25.1)/ 181 (3.4)	98/5 (25.1) /1.3)		87/ 3 (22.5)/ (0.8)	97/5 (25.1)/ (1.3)	
	Radiotherapy	1509 (28.3)	52 (13.3)	*	66 (17.1)	51 (13.2)	~
	ADT	2617 (49.0)	198 (50.6)		202 (52.2)	195 (50.4)	
	Chemotherapy	111 (2.1)	6 (1.5)		7 (1.8)	6 (1.6)	
Medication Exposures - (%)	Aspirin	1986 (37.2)	194 (49.6)	*	197 (50.9)	194 (50.1)	
	Beta-blocker	1158 (21.7)	97 (24.8)		122 (31.5)	95 (24.5)	~
	Statin	1293 (24.2)	95 (24.3)		91 (23.5)	95 (24.5)	
	Warfarin	214 (4.0)	150 (38.4)	*	91 (23.5)	148 (38.2)	~
	Anti-diabetic	417 (7.8)	48 (12.3)	*	32 (8.3)	48 (12.4)	~
	NSAID	1833 (34.3)	139 (35.5)		151 (39.0)	136 (35.1)	
	BPH medicines	1393 (26.1)	111 (28.4)		125 (32.3)	109 (28.2)	
Digoxin exposure details (90 days pre-diagnosis)							
No of prescriptions dispensed		- -	1030		- -	1011	
Dosing intensity - (%)	Median (IQR)	- -	84.6 (75.6, 100)		- -	84.5 (75.6, 100)	
Digoxin exposure details (1 year post-diagnosis)							
No of Prescriptions dispensed		567	3374		99	3336	
Dosing intensity - (%)	Median (IQR)	0.01 (0.0, 0.0)	69.6 (36.4, 99.2)		1.8 (0.0, 0.0)	69.5 (36.4, 99.2)	

*** p-value for t-test <0.05; * p-value for Chi-squared test <0.05*

~ Standardised differences between exposed and unexposed groups >0.10

***RP:** Radical Prostatectomy*

***ADT:** Androgen Deprivation Therapy*

***a)** AJCC Staging Manual 5th Ed[20]*

***b)** Received within one year following diagnosis – (treatments are not mutually exclusive; the most common combinations were: N=792 (13.8%) received ADT and radiation; N=203 (8.2%) received ADT and surgery)*

Table 2: Univariate and multivariate hazard ratios for digoxin exposure and mortality

			All-cause mortality			Prostate cancer-specific mortality		
Digoxin use	**N**	**Person Years**	**No. of deaths (rate)[a]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [b]**	**No. of deaths (rate)[a]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [b]**
Digoxin unexposed	5,341	22,774	2096 (92.0)	Ref -	Ref -	995 (43.7)	Ref -	Ref -
Digoxin exposed	391	1,277	253 (198.1)	2.11 (1.86, 2.41)	1.24 (1.07, 1.43)	103 (80.7)	1.77 (1.45, 2.17)	1.13 (0.91, 1.42)
Exposure response: dosing intensity								
Digoxin exposed								
Dosing intensity 0%-85%	117	319	89 (279.2)	2.94 (2.38, 3.63)	1.59 (1.27, 1.97)	33 (103.5)	2.22 (1.57, 3.14)	1.18 (0.83, 1.68)
Dosing intensity 86%-99%	120	413	78 (188.9)	2.02 (1.61, 2.54)	1.33 (1.05, 1.67)	33 (79.6)	1.78 (1.26, 2.52)	1.39 (0.97, 1.98)
Dosing intensity 100%	154	544	86 (157.9)	1.69 (1.36, 2.10)	0.93 (0.74, 1.18)	37 (67.9)	1.50 (1.08, 2.08)	0.93 (0.65, 1.32)
Propensity score trimmed cohort analysis								
Digoxin unexposed	3,940	15,938	1,780 (111.7)	Ref -	Ref -	833 (52.3)	Ref -	Ref -
Digoxin exposed	389	1,272	252 (198.1)	1.75 (1.53, 2.00)	1.23 (1.07, 1.43)	102 (80.2)	1.49 (1.21, 1.82)	1.12 (0.90, 1.41)
Propensity score matched cohort analysis								
Digoxin unexposed	387	1,339	234 (174.8)	Ref -	Ref -	105 (78.4)	Ref -	Ref -
Digoxin exposed	387	1,269	250 (197.1)	1.13 (0.94, 1.35)	1.20 (1.00, 1.45)	101 (79.6)	1.02 (0.77, 1.34)	1.17 (0.88, 1.57)

a) *Deaths per 1000 person years*

b) All multivariate hazard ratios are adjusted for *age (years, continuous), comorbidity score (number of medication classes, continuous) tumour stage (I, II, III, IV unspecified), tumour grade (I, II, III/IV, unspecified), smoking status at diagnosis, year of incidence, warfarin exposure and statin exposure.*

Table 3: Digoxin use & prostate cancer-specific mortality – Effect modification by receipt of radiation therapy in the year following diagnosis

Radiation		Digoxin Unexposed		Digoxin Exposed		Exposed Vs Unexposed	
No	**Death/Censored**	845/2987		92/247			
	Multivariate HR (95%CI)	1.00 -	-	1.08 (0.86, 1.37)	p = 0.51	1.08 (0.86, 1.37)	p = 0.51
Yes	**Death/Censored**	150/1359		11/41			
	Multivariate HR (95%CI)	0.95 (0.79,1.15)	p = 0.62	1.69 (0.92 3.10)	p = 0.09	1.77 (0.95, 3.30)	p = 0.07
				Multiplicative scale: rHR (95%CI) yes v no		**1.64 (0.85, 3.14)**	**p = 0.14**

Adjusted for age (years, continuous), comorbidity score (number of medication classes, continuous) tumour stage (I, II, III, IV unspecified), tumour grade (I, II, III/IV, unspecified), smoking status at diagnosis, year of incidence, warfarin exposure and statin exposure

Table 4: Digoxin use & prostate cancer specific mortality – Effect modification by receipt of androgen deprivation therapy in the year following diagnosis

Androgen Deprivation Therapy		Digoxin Unexposed		Digoxin Exposed		Exposed Vs Unexposed	
No	**Death/Censored**	375/2358		35/158			
	Multivariate HR (95%CI)	1.00 -	-	1.00 (0.69, 1.43)	p = 0.98	1.00 (0.69, 1.43)	p = 0.98
Yes	**Death/Censored**	620/1988		68/130			
	Multivariate HR (95%CI)	1.06 (0.92, 1.21)	p = 0.46	1.29 (0.97, 1.70)	p = 0.08	1.22 (0.93, 1.59)	p = 0.14
				Multiplicative scale: rHR (95%CI) yes v no		**1.23 (0.80, 1.89)**	**p = 0.35**

Adjusted for age (years, continuous), comorbidity score (number of medication classes, continuous) tumour stage (I, II, III, IV unspecified), tumour grade (I, II, III/IV, unspecified), smoking status at diagnosis, year of incidence, warfarin exposure and statin exposure

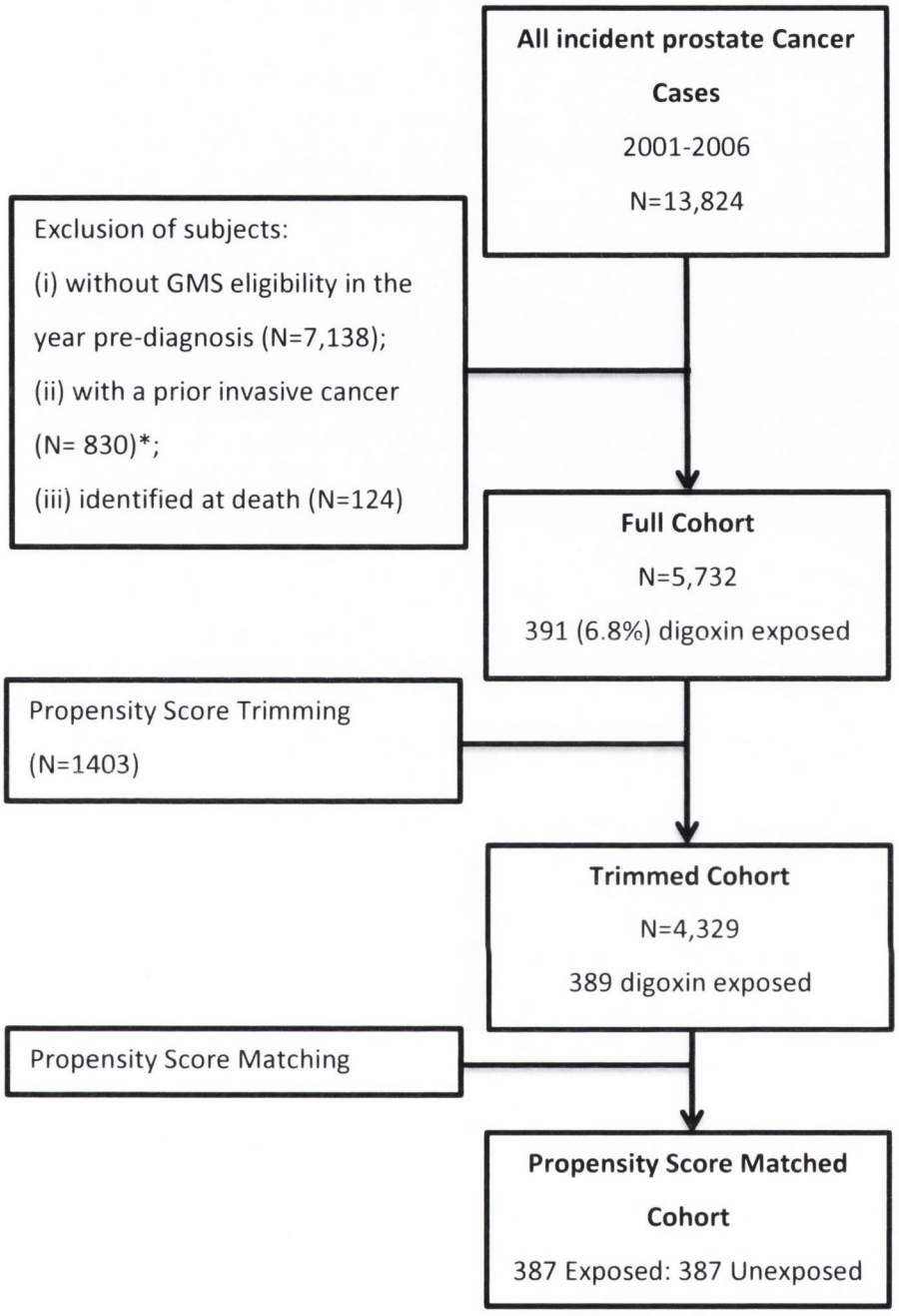

Figure 1: Flow chart for study cohorts, showing inclusion and exclusion criteria.

* other than non-melanoma skin cancer

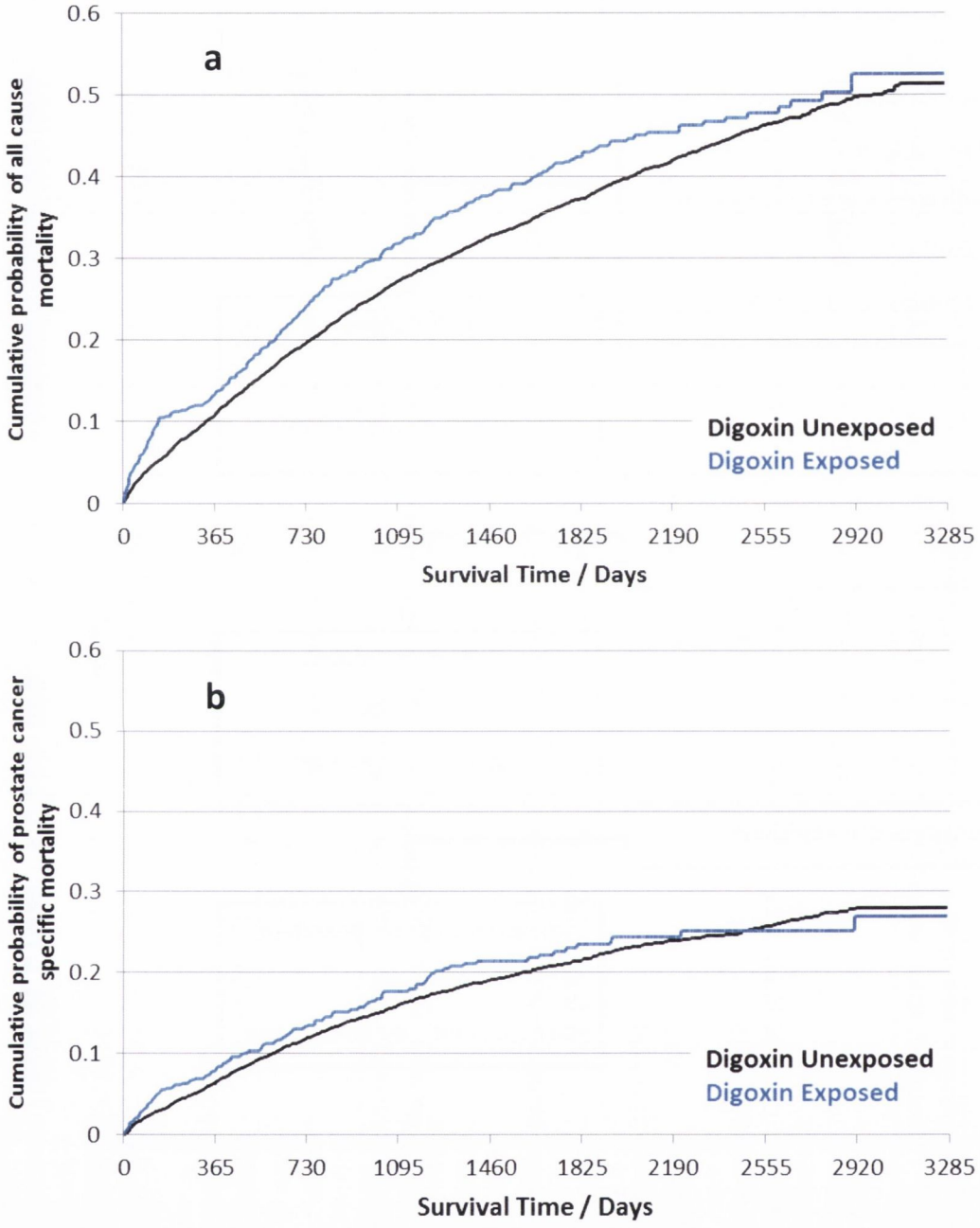

FIGURE 2: Adjusted cumulative probability curves of (a) all-cause mortality and (b) prostate cancer-specific mortality.

APPENDIX 1: MEDICATION EXPOSURES ASSESSED

Table A1: WHO-ATC codes used to identify medication exposures in the 180 days prior to diagnosis

Medication Group	WHO-ATC Codes
Anti-diabetic medication	A10
Biguanides	A10BA; A10BD01; A10BD02; A10BD03; A10BD05; A10BD07
Aspirin	B01AC06; M01BA03; N02BA01; N02BA51; N02BA71
Other anti-thrombotic agents (excluding aspirin)	B01A, (excluding B01AC06)
Warfarin	B10AA03
Digoxin	C01AA05
Antiarrhythmic agents	C01B
Antiarrhythmic agents Class I a	C01BA
Antiarrhythmic agents Class I c	C01BC
Class III antiarrhythmic agents	C01BD
Cardiac stimulants	C01C
Nitrates	C01DA
Other Cardiac agents	C01E
Low-ceiling diuretic	C03B
High-ceiling diuretic	C03C
Aldosterone antagonists	C03DA
Peripheral Vasodilators	C04
Beta-blocker	C07
Calcium Channel Blocker Vascular	C08C
Calcium Channel Blocker Cardiac	C08D
Verapamil	C08DA01; C08DA51
Angiotensin converting enzyme inhibitors	C09A; C09B
Angiotensin II receptor blockers	C09C; C09D
Statin	C10AA; C10B
Benign Prostatic Hypertrophy Medication	G04C

APPENDIX 2: COVARIATES INCLUDED IN PROPENSITY SCORE MODEL

Table A2: Covariates Included In Propensity Score Model

Demographic Variables

Age at diagnosis (years)

Age squared

Age cubed

Comorbidity score (Distinct medication classes, 5 character ATC)

Comorbidity score squared

Comorbidity score cubed

Medication Exposures

Aspirin

Other anti-thrombotic agents (excluding aspirin, including warfarin)

Cardiac stimulants

Class III antiarrhythmic agents

Nitrates

High-ceiling diuretic

Aldosterone antagonists

Verapamil

Angiotensin converting enzyme inhibitors

Angiotensin II receptor blockers

Statins

Interactions

Aspirin*other antithrombotic

Aspirin*Statins

Aspirin*High-Ceiling Diuretics

Aspirin*Nitrates

Other Antithrombotic*Verapamil

Other Antithrombotic *High-Ceiling Diuretics

Other Antithrombotic *Aldosterone Antagonist

APPENDIX 3: SENSITIVITY ANALYSIS OF PROSTATE CANCER DEATH

Table A3.1: Potential other cancer sites which prostate cancer death may be misclassified:[45]

Cancer Site	ICD 9 Code	ICD 10 Code
Malignant neoplasm of prostate	185	C61
Malignant neoplasm of other male genital organs, site unspecified	187.9	C63.9
Malignant neoplasm of pelvis	195.3	C41.4
Secondary malignant neoplasm	196-198	C76-C80
Malignant neoplasm without specification of site	199	C80.9
Benign neoplasm of prostate	222.2	D29.1
Benign neoplasm of male genital organs, site unspecified	222.9	D29.9
Neoplasm of uncertain behaviour of prostate	236.5	D40.0
Neoplasm of uncertain behaviour of other and unspecified male genital organs	236.6	D40.9
Neoplasm of uncertain behaviour, site unspecified	238.9	D48.9
Neoplasm of unspecified nature of other genitourinary organs	239.5	D40.7, D41
Neoplasm of unspecified nature, site unspecified	239.9	D48.9

Table A3.2: Sensitivity Analysis: Univariate and multivariate hazard ratios for digoxin exposure and mortality, including prostate cancer deaths classified as in Table A3.1

			Prostate cancer-specific mortality			
Digoxin Use	N	Person Years	No. of deaths (rate)[a]		Univariate HR (95%CI)	Multivariate HR[b] (95%CI)
Full cohort						
Digoxin unexposed	5431	22,774	1018	(44.7)	Ref -	Ref -
Digoxin exposed	391	1,277	106	(83.0)	1.78 (1.46, 2.18)*	1.14 (0.91, 1.42)
Propensity Score Trimmed Cohort						
Digoxin unexposed	3,940	15,938	852	(53.5)	Ref -	Ref -
Digoxin exposed	389	1,272	105	(82.5)	1.49 (1.22, 1.83)*	1.13 (0.90, 1.41)
Propensity Score Matched Cohort						
Digoxin unexposed	387	1,339	109	(81.4)	Ref -	Ref -
Digoxin exposed	387	1,269	104	(82.0)	1.01 (0.77, 1.32)	1.14 (0.86, 1.51)

***a**) Deaths per 1000 person years*

***b**) All multivariate hazard ratios are adjusted for age (years, continuous), comorbidity score (number of medication classes, continuous) tumour stage (I, II, III, IV unspecified), tumour grade (I, II, III/IV, unspecified), smoking status at diagnosis, year of incidence, warfarin exposure and statin exposure.*

Table A3.3: Sensitivity Analysis: Univariate and multivariate hazard ratios for digoxin exposure and mortality, including prostate cancer deaths classified as secondary or contributory causes of death

			Prostate cancer-specific mortality			
Digoxin Use	**N**	**Person Years**	**No. of deaths (rate)[a]**		**Univariate HR (95%CI)**	**Multivariate HR[b] (95%CI)**
Full cohort						
Digoxin unexposed	5431	22,774	1068	(46.9)	Ref -	Ref -
Digoxin exposed	391	1,277	115	(90.1)	1.83 (1.51, 2.22)*	1.19 (0.96, 1.46)
Propensity Score Trimmed Cohort						
Digoxin unexposed	3,940	15,938	899	(56.4)	Ref -	Ref -
Digoxin exposed	389	1,272	114	(89.6)	1.53 (1.26, 1.86)*	1.17 (0.95, 1.45)
Propensity Score Matched Cohort						
Digoxin unexposed	387	1,339	113	(84.4)	Ref -	Ref -
Digoxin exposed	387	1,269	113	(89.1)	1.06 (0.81, 1.37)	1.24 (0.94, 1.63)

***a**) Deaths per 1000 person years*

***b**) All multivariate hazard ratios are adjusted for age (years, continuous), comorbidity score (number of medication classes, continuous) tumour stage (I, II, III, IV unspecified), tumour grade (I, II, III/IV, unspecified), smoking status at diagnosis, year of incidence, warfarin exposure and statin exposure.*

APPENDIX 2: A COHORT STUDY INVESTIGATING ASPIRIN USE AND SURVIVAL IN MEN WITH PROSTATE CANCER

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ABSTRACT:

BACKGROUND: Aspirin use has been associated with reduced mortality from cancer including prostate cancer in some studies. A number of anti-cancer mechanisms of aspirin have been proposed including the inhibition of the cyclooxygenase enzymes, through which aspirin mediates both anti-platelet and anti-inflammatory activity. This cohort study examines associations between pre-diagnostic aspirin use (overall and by dose and dosing intensity) and mortality in men with localised prostate cancer.

PATIENTS AND METHODS: Men with stage I-III prostate cancer were identified from Irish National Cancer Registry records which have been linked to national prescribing data from the Irish General Medical Services scheme. Aspirin use in the year preceding prostate cancer diagnosis was identified from this linked prescription claims data. Adjusted hazard ratios (HR) and 95% confidence intervals (CI) were estimated for associations between aspirin use and all-cause and prostate cancer-specific mortality. Associations between prescribed dose and dosing intensity were examined. The presence of effect modification by type of treatment received and tumour characteristics was also assessed.

RESULTS: 2,936 men with a diagnosis of stage I-III prostate cancer (2001-2006) were identified (aspirin users, *N*=1,131). Median patient follow-up was 5.5 years. In adjusted analyses aspirin use was associated with a small, but non-significant, reduced risk of prostate cancer-specific mortality (HR=0.88, 95%CI 0.67-1.15). In dose-response analyses, stronger associations with prostate cancer-specific mortality were observed in men with higher aspirin dosing intensity (HR=0.73, 95%CI 0.51-1.05) and in men receiving >75mg of aspirin (HR=0.61, 95%CI 0.37, 0.99). Analyses of effect modification by treatment type or tumour characteristics were non-significant.

CONCLUSIONS: Consistent with prior studies, aspirin use was associated with a non-significant reduced risk of prostate cancer-specific mortality in men with localised prostate cancer. Men receiving higher doses of aspirin had statistically significant reduced risk of prostate cancer-specific mortality. These findings regarding aspirin dose require further investigation.

KEYWORDS: Prostate neoplasm; mortality; aspirin; pharmacoepidemiology

Introduction

Pre-clinical studies have suggested a number of possible anti-cancer mechanisms for aspirin, a cyclooxygenase 1/2 (COX-1/2) inhibitor, in prostate cancer. These include the inhibition of prostate tumour growth and metastasis.[1,2] Aspirin use prior to a prostate cancer diagnosis has been associated with a lower risk of advanced disease at diagnosis; studies have also suggested that aspirin use is associated with reduced mortality from prostate cancer.[3-6] These studies have also suggested the possibility of greater benefit in men with larger or higher grade tumours.[6] However, the magnitude of association between aspirin use and prostate cancer mortality has varied considerably between studies, with some reporting no association.[7] It is also unclear what influence the dose, frequency and timing of aspirin use may have on prostate cancer outcomes.[8]

The aims of this study were to investigate, in men with incident localised (stage I-III) prostate cancer: (i) associations between aspirin use prior to diagnosis, and mortality; (ii) the influence of dose, frequency and duration of aspirin use on mortality, and (iii) whether tumour characteristics, such as tumour size or Gleason score, modify associations between aspirin use and mortality.

Methods

Design, Setting and Data Sources:

We conducted a cohort study using patient records from the National Cancer Registry Ireland (NCRI), linked to pharmacy claims data from Ireland's General Medical Services (GMS) scheme. Detailed information on all incident cancers in the Irish population is recorded by NCRI hospital-based tumour registration officers. Follow-up is achieved by linking death certificates to cancer registrations. The GMS scheme delivers state-funded healthcare, including prescription medicines, to approximately one third (1.4 million) of the Irish population.[9] GMS scheme eligibility is assessed primarily through means test and age. The GMS database records claims for all prescription drugs, classified by WHO-ATC code, dispensed from community pharmacies to GMS eligible patients. Low-dose aspirin is only available on prescription in the Republic of Ireland; this is similar to other European countries. Although higher doses are available over the counter, this is only for short term indications, and at increased cost to the patient. The use of anonymised NCRI data for research purposes is covered by the Health (Provision of Information) Act 1997.

Cohort

Men who met the following criteria were eligible for inclusion in the study cohort: diagnosed with pathological (or in the absence of pathological information, clinical) stage I-III[10] prostate cancer (ICD-O, C61)[11] between 01/01/2001 and 31/12/2006; aged 50-80 years at diagnosis; and with GMS scheme eligibility continuously for at least one year immediately prior to diagnosis. We excluded men if they had a prior invasive cancer other than non-melanoma skin cancer, or if their prostate cancer diagnosis was made at the time of death.

Exposure Definition

We identified prescriptions for aspirin dispensed to men in the study cohort from the GMS database using WHO-ATC codes (Appendix 1). Men were defined as aspirin users if they had a supply of aspirin available in the year prior to prostate cancer diagnosis. We identified the date, dose and number of days' supply on each prescription. These were used to stratify pre-diagnostic aspirin use by: (i) dosing intensity, defined as the proportion of days with a supply of aspirin available in the year prior to diagnosis and split on the median (high; low); and (ii) dose prescribed (low-dose: all prescriptions for ≤75mg; high-dose: at least one prescription for >75 mg).

Outcome Definitions

We used information from death certificates to identify the date and cause of death. Cause of death was classified as (i) prostate cancer-specific deaths (ICD-9 185; ICD-10 C61) and (ii) deaths from all-causes. All men accrued follow-up time from the date of diagnosis to the first of death or the end of follow-up (31st December 2010).

Study Covariates

The following patient demographics and tumour characteristics were identified from the NCRI database: patient age (years); smoking status (current/former/non-smoker/unspecified); tumour grade (Gleason Score <5/5-7/>7, unspecified);[10] tumour stage (I/II/III)[10] and tumour size (T1/T2/T3).[10] NCRI data was also used to identify the date and type of treatments received in the year post-diagnosis (yes/no): prostate surgery (radical-prostatectomy/other-prostatectomy), radiation or androgen deprivation therapy (ADT).

A medication-based comorbidity score was calculated as the sum of distinct medication classes (defined as the first five ATC code characters) received in the year prior to diagnosis. This comorbidity score is based on a previously validated method in an elderly population.[12]

Prescription claims data was also used to identify exposure to other medication in the year prior to prostate cancer diagnosis (yes/no; Appendix 1) including anti-diabetic drugs which were used to identify men with diabetes.[13]

STATISTICAL ANALYSES:

Cohort characteristics were tabulated and univariate analyses were used to assess differences between aspirin users and non-users. We used Cox regression models to estimate hazard ratios (HR) with 95% confidence intervals (CI) for associations between aspirin use and prostate cancer-specific mortality and all-cause mortality. A backward deletion method was used to select covariates in the multivariate model; with a 10% maximum change in the effect component of the fully adjusted HR used to select the final multivariate model. Covariates strongly associated with prostate cancer outcomes in prior studies were fixed in the multivariate model (age at diagnosis, tumour size, tumour grade).[14] Based on prior knowledge of clinical and demographic predictors of prostate cancer mortality, the following additional covariates were then considered for inclusion: comorbidity score;[12] smoking status;[15] diabetes;[13] and exposure to statins,[16] non-aspirin anti-coagulants,[6] non-aspirin NSAIDs,[16] beta-blockers,[17] and medication for the treatment of benign prostatic hypertrophy (BPH).[18] The year of prostate cancer diagnosis (continuous) and treatment received in the year following diagnosis (time varying) were also assessed for inclusion. In addition to estimating HRs for the entire follow-up time available, we estimated HRs at two, four and eight years of follow-up, to assess variation in the HR over time.[3] The proportionality of hazard functions was assessed by testing for the interaction between aspirin use and the logarithm of person-time (Wald test for product term): all hazards were proportional (P=0.75).

Exposure-response analyses for associations between aspirin use and mortality were conducted by strata of aspirin dosing intensity, dose and a combination of dosing intensity and dose. Associations between duration of aspirin use and mortality were also examined in an analysis of the subgroup of men with at least three years of GMS eligibility prior to diagnosis. Duration of pre-diagnostic aspirin use was categorized by the length of time from first aspirin exposure in the three years prior to diagnosis (0-2 years; >2 years). This analysis was stratified by median dosing intensity from time of first aspirin prescription to date of diagnosis.

Prior studies have reported stronger associations between aspirin use and prostate cancer-specific mortality in men with larger tumours, tumours of higher Gleason score, and tumours treated with prostatectomy or radiation.[6] Therefore, we assessed effect modification of associations between aspirin use and prostate cancer-specific mortality by tumour grade and tumour size at diagnosis; interactions between aspirin use and treatment receipt (prostate surgery, radiation) were also examined. All interactions were assessed on a multiplicative scale (maximum likelihood ratio). We also carried out sensitivity analyses to assess the possibility that observed associations could be explained by misclassification of deaths from prostate cancer on death certificates. These are described in Appendix 3. The following post-hoc subgroup analysis was conducted: we assessed effect modification of associations between aspirin use and prostate cancer-specific mortality by comorbidity score. All analyses were performed using SAS® v9.2 (SAS Institute, Cary, NC). Significance at $P<0.05$ is assumed.

Results

Cohort Characteristics

The flow diagram (Figure 1) outlines the cohort selection for the study; 2,936 men met the inclusion criteria for the study cohort. Of these, 38.5% were identified as aspirin users in the year prior to diagnosis. The cohort characteristics are presented in Table 1. Aspirin users were significantly older at diagnosis and had higher comorbidity scores than non-users. The median duration of patient follow-up was 5.5 years.

Survival Analysis

Aspirin use was associated with a small, non-significantly reduced risk of prostate cancer-specific mortality in multivariate analysis (Table 2: HR=0.88, 95%CI 0.67-1.15). See Appendix 2 for full multivariate model. Adjusted HRs for the association between aspirin use and prostate cancer-specific mortality at two, four and eight years follow-up were 1.02 (95%CI 0.61-1.69); 0.90 (95%CI 0.64-1.27); and 0.88 (95%CI 0.67-1.17) respectively. In sensitivity analyses for misclassification of prostate cancer death, the HRs for prostate cancer-specific mortality were not appreciably different, see Appendix 3.

In analyses stratified by dose and dosing intensity, men with high aspirin dosing intensity appeared to have a lower risk of prostate cancer-specific mortality compared to men with low dosing intensity, although trends were not significant. In analyses stratified by aspirin dose, use of higher aspirin doses (>75mg) was associated with a statistically significant reduction in

the risk of prostate cancer-specific mortality compared to no aspirin (Table 2: HR=0.61, 95%CI 0.37-0.99, P=0.04). We observed stronger associations between higher aspirin dosing intensity and lower risk of prostate cancer-specific mortality in men receiving both high and low doses of aspirin (Table 2). In the analysis considering deaths from all causes, there was no significant association between any aspirin use and all-cause mortality (Appendix 4: HR=0.98, 95%CI 0.84-1.15). No additional benefit for men with longer duration of aspirin use (>2 years) compared to shorter duration of use (0-2 years) (Table 2; P-trend=0.59) was observed in the analysis examining duration of pre-diagnostic aspirin use and prostate cancer-specific mortality. However, this analysis was limited by smaller numbers, and the duration of pre-diagnostic exposure examined was shorter than that reported in other studies.[3,4]

In effect modification analyses (Appendix 5), we observed no significant interactions between aspirin use and receipt of prostate surgery (P-interaction=0.62) or radiation (P-interaction=0.66). Associations between aspirin use and prostate cancer-specific mortality appeared stronger in men with high grade tumours (Gleason score >7, HR=0.68, 95%CI 0.45-1.05; Gleason score ≤7, HR=0.98, 95%CI 0.68-1.40), however tests for interaction did not reach significance (P-interaction=0.19). In the analysis of effect modification by tumour size, the test for interaction was also non-significant (P-interaction=0.62). There was no evidence of effect modification by comorbidity score (P=0.51).

Discussion

We observed a small, non-significant association between any aspirin use prior to diagnosis and a lower risk of prostate cancer-specific mortality in this cohort study of 2,936 men with stage I-III prostate cancer. This association was not observed at two years of follow-up, but was apparent at four and eight years; suggesting that, similar to other studies,[3] it may take a number of years for any influence of aspirin use on cancer mortality to accrue.

Previous studies investigating use of aspirin prior to prostate cancer diagnosis have reported somewhat larger, although still non-significant, associations between aspirin use and prostate cancer-specific mortality.[3,4,19] In a meta-analysis of randomised controlled trials of aspirin in cardiovascular disease, Rothwell et al reported that daily aspirin use was associated with a non-significant 30% lower risk of prostate cancer-specific mortality (0-5 years' follow-up HR=0.70, 95%CI 0.29-1.73).[3] Similarly, aspirin use was associated with a 23% lower risk of prostate cancer-specific mortality in two separate cohort studies by Jacobs et al (RR=0.77,

95%CI 0.53-1.12)[4] and Daugherty et al (HR=0.77, 95%CI 0.48-1.25).[19] These studies have, however, only included men with daily aspirin use, in contrast to our study which included men with any aspirin exposure. When we stratified our analysis by aspirin dosing intensity, we observed a non-significant 27% lower risk of prostate cancer-specific mortality (HR=0.73, 95%CI 0.51-1.05) among men with high pre-diagnostic dosing intensity (equivalent to aspirin use ≥6 days/week); which is consistent with these prior findings.[3,4,19]

Studies examining associations between aspirin use following a prostate cancer diagnosis and prostate cancer-specific mortality have not reported consistent results. Choe et al studied self-reported aspirin use, at or following diagnosis, and prostate cancer-specific mortality in men who received prostatectomy or radiation treatment. In this study any aspirin use was associated with a reduced risk of death (HR=0.43, 95%CI 0.21-0.87).[6] Dhillon et al also examined self-reported aspirin use following diagnosis, but did not find daily aspirin use to be associated with a lower risk of prostate cancer-specific mortality (HR=1.08, 95%CI 0.76-1.54).[7] Finally, in a recent study by Assayag et al, aspirin use initiated after a prostate cancer diagnosis was associated with an increased risk of prostate cancer mortality (RR=1.69, 95%CI 1.43-2.00).[20] There were differences between these three studies that may explain the lack of consistency, including; patient characteristics, treatments received, and the length of lag time used for entry of the post-diagnostic aspirin use into analyses. Additionally, pre-diagnostic aspirin use was only accounted for in the studies by Dhillon and Assayag.

The availability of detailed information about the dose of aspirin dispensed enabled us to examine associations between aspirin dose and prostate cancer mortality; this has not been possible in previous studies. In these analyses men who had received higher doses of aspirin (>75mg) had the lowest risk of prostate cancer-specific mortality, and this was statistically significant. The number of men receiving higher doses of aspirin was, however, small (N=250), and these results require confirmation in larger cohorts. In our study, no significant association with prostate cancer-specific mortality was observed for low dose aspirin (≤75mg) although there was the suggestion of a lower risk of death in men taking low-dose aspirin at high intensity.

Aspirin use has been reported to be associated with greater reductions in risk of prostate cancer-specific mortality in men with high-risk disease.[6,19] The results of effect modification analyses by tumour characteristics at diagnosis in our study did suggest greater benefit for aspirin use in men with high Gleason score (>7) tumours, compared to low Gleason score tumours; although this was non-significant. Further study is warranted to determine the

molecular and tumour characteristics that may be associated with aspirin's effect in prostate cancer.

The strengths of this study include the detailed patient level data and most importantly the longitudinal prescription refill data. Low-dose aspirin is a prescription only medication in Ireland, therefore capture of aspirin use by prescription refill data is expected to be accurate. Some limitations must also be acknowledged. As exposure is based on prescription refill data, any non-compliance will have resulted in misclassification of exposure; treatment cross-over post-diagnosis did occur; 26.6% of non-users received aspirin at some point following prostate cancer diagnosis, and some aspirin users received as few as one prescription. However these effects would normally be expected to bias results towards the null. We did not have information on the presence of comorbid conditions other than prostate cancer; however, we did adjust for comorbidity using information on prescribed medications. Also, the study cohort comprised of men with prostate cancer eligible for the GMS scheme only; therefore, they are older and more socially deprived than the general population; this may influence the generalizability of the results. Finally, the median follow-up time of 5.5 years was short and further studies with longer follow-up are necessary.

In conclusion, we observed a modest, but non-significant, reduced risk of prostate cancer-specific mortality in men who were prescribed aspirin prior to diagnosis. Results stratified by dose and dosing intensity were consistent with a lower risk of prostate cancer-specific mortality in men with frequent aspirin use and use of higher aspirin doses. These findings have implications for the design of future randomised studies of aspirin in men with, or at risk of, prostate cancer.

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DISCLOSURE

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TABLES

TABLE 1: Characteristics of aspirin users and non-users in study cohort (*n*=2,936)

Characteristic		Aspirin Non-user (*N*=1,805)	Aspirin User (*N*=1,131)	
Patient details				
Age /years	Mean (SD)	69.5 (6.8)	71.5 (5.7)	*
Comorbidity Score	Mean (SD)	6.9 (5.7)	11.1 (5.6)	*
Smoking Status - (%)	Never	556 (30.8)	374 (33.1)	*
	Former	331 (18.3)	230 (20.3)	
	Current	325 (18.0)	161 (14.2)	
	Unspecified	593 (32.9)	366 (32.4)	
Tumour details				
Stage - (%) [a]	I	92 (5.1)	62 (5.5)	
	II	1478 (81.9)	918 (81.2)	
	III	235 (13.0)	151 (13.4)	
Tumour Size- (%) [A]	T1	484 (26.8)	313 (27.7)	
	T2	1,086 (60.2)	667 (59.0)	
	T3	235 (13.0)	151 (13.4)	
Grade - (%)	Gleason Score <5	129 (7.1)	77 (6.8)	
	Gleason Score 5-7	1193 (66.1)	743 (65.7)	
	Gleason Score >7	289 (16.0)	176 (15.6)	
	Unspecified	194 (10.7)	135 (11.9)	
Treatment details				
Treatment - (%) [B]	Surgery	412 (22.8)	211 (18.7)	*
	Radical Prostatectomy	133 (7.4)	41 (3.6)	*
	Other Prostatectomy	279 (15.5)	170 (15.0)	
	Radiation	678 (37.6)	443 (39.2)	
	ADT	781 (43.3)	544 (48.1)	*
Medication Exposures [C] - (%)				
	Beta-blocker	222 (12.3)	433 (38.3)	*
	Statin	271 (15.0)	622 (55.0)	*
	Non-aspirin anticoagulant	186 (10.3)	149 (13.2)	*
	Anti-diabetic	94 (5.2)	163 (14.4)	*
	NSAID	738 (40.9)	521 (46.1)	*
	BPH medicines	429 (23.8)	282 (24.9)	
Aspirin exposure details (1 year pre-diagnosis)				
No of prescriptions dispensed		- -	10,762	
Dosing intensity - (%)	Median (IQR)	- -	86.0% (48.5, 98.4)	

**P-value <0.05;*

***A:** AJCC Staging Manual 5th Ed[19].*

***B:** Received within one year following diagnosis, (not mutually exclusive)*

***C:** Medication received in the year prior to diagnosis*

TABLE 2: Estimated Hazard Ratios For Association Between Aspirin Use and Prostate Cancer-Specific Mortality

Aspirin Use	*N*	Person Years	Prostate cancer-specific mortality		
			No. of deaths (rate)[A]	Univariate HR (95%CI)	Multivariate HR (95%CI)
Pre-diagnostic aspirin use[B]					
Aspirin non-user in year prior to diagnosis	1,805	10,060	172 (17.1)	Ref -	Ref -
Aspirin user in year prior to diagnosis	1,131	6,070	104 (17.1)	1.01 (0.79-1.29)	0.88 (0.67-1.15)
Exposure response: dosing intensity[C]					
Low dosing intensity 0%-86%	564	3,070	61 (19.9)	1.17 (0.87-1.56)	1.02 (0.74-1.40)
High dosing intensity 86%-100%	567	3,000	43 (14.3)	0.85 (0.61-1.18)	0.73 (0.51-1.05)
P-trend				*0.56*	*0.12*
Exposure response: dose					
Low dose ≤ 75mg	881	4,627	84 (18.2)	1.07 (0.83-1.39)	0.97 (0.73-1.30)
High dose > 75mg	250	1,443	20 (13.9)	0.81 (0.51-1.28)	0.61 (0.37-0.99)*
P-trend				*0.69*	*0.10*
Exposure response: dosing intensity[C] & dose					
Low dosing intensity 0%-86%					
Low dose ≤ 75mg	420	2,256	49 (21.7)	1.28 (0.93-1.76)	1.13 (0.80-1.58)
High dose > 75mg	144	814	12 (14.8)	0.86 (0.48-1.55)	0.71 (0.39-1.30)
High dosing intensity 86%-100%					
Low dose ≤ 75mg	461	2,371	35 (14.8)	0.88 (0.61-1.26)	0.81 (0.55-1.20)
High dose > 75mg	106	629	8 (12.7)	0.74 (0.36-1.50)	0.50 (0.24-1.03)
Exposure response: duration[D]					
Aspirin non-user in 3 years prior to diagnosis	1,003	5,202	74 (14.2)	Ref -	Ref -
Aspirin user in 3 years prior to diagnosis					
Aspirin user 0- 2 years pre-diagnosis	226	1,157	14 (13.8)	0.97 (0.56-1.66)	0.96 (0.55-1.68)
Aspirin user >2 years pre-diagnosis	578	2870	44 (15.3)	1.09 (0.75-1.58)	1.13 (0.74-1.71)
P-trend				*0.69*	*0.59*
Exposure response: duration & dosing intensity					
Aspirin user 0- 2 years pre-diagnosis					
Low dosing intensity 0%-84%	112	578	9 (15.6)	1.09 (0.55-2.18)	1.06 (0.55-2.13)
High dosing intensity 84%-100%	114	579	7 (12.1)	0.85 (0.39-1.84)	0.85 (0.38-1.79)
Aspirin user >2 years pre-diagnosis					
Low dosing intensity 0%-84%	290	1,455	26 (17.9)	1.26 (0.80-1.97)	1.31 (0.81-2.10)
High dosing intensity 84%-100%	288	1,415	18 (12.7)	0.91 (0.54-1.52)	0.91 (0.52-1.60)

** P-value < 0.05.*

***A:** Mortality rate (deaths/1000 person years).*

***B:** All multivariate HRs are adjusted for age at diagnosis, tumour grade, tumour size, smoking status at diagnosis, comorbidity score, year of incidence, pre-diagnostic statin exposure, and receipt of radiation (time-varying).*

***C**: Dosing intensity by median*

***D:** Cohort with at least 3 years continuous GMS scheme eligibility prior to diagnosis, (N=1,807)*

FIGURES

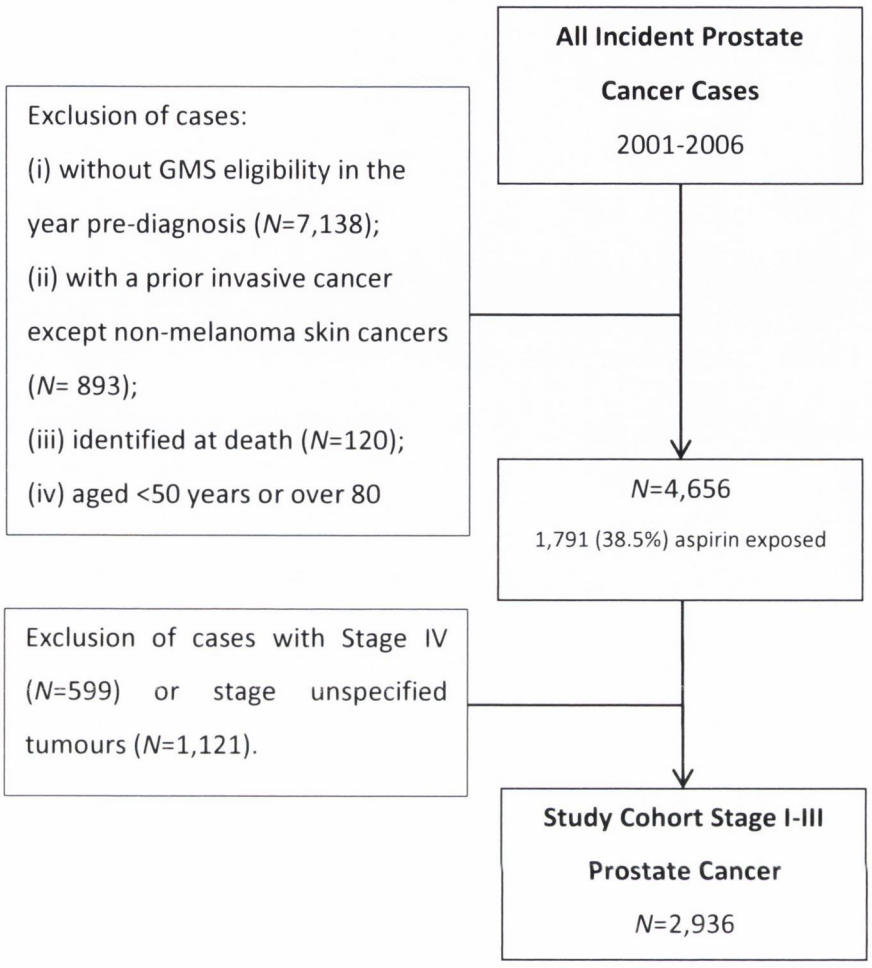

FIGURE 1: Cohort Selection for Study

APPENDIX 1

WHO ATC DRUG CODES FOR MEDICATION EXPOSURES

Drug Exposure	WHO ATC Code[1]
Aspirin & Combinations	B01AC06, M01BA03, N02BA01, N02BA51, N02BA71
Anti-diabetic medication	A10
Statins	C10AA
Non-aspirin anti-coagulants	B01A, excluding B01AC06
Non-aspirin NSAIDS	M01A
Benign Prostatic Hypertrophy	G04C

1. WHO. ATC/DDD Index 2012. In. Oslo: WHO collaborating centre for drug statisitcs methodology 2012.

APPENDIX 2

The hazard ratios for covariates included in the final multivariate model are presented in Table S2.1 below.

TABLE S2.1: Final Multivariate model examining Aspirin Use and Prostate Cancer-Specific Mortality

Covariate		Adjusted HR	(95% CI)
Aspirin Exposure		0.88	(0.67-1.15)
Age /years	Continuous	1.06	(1.04-1.09)
Comorbidity Score	Continuous	1.03	(1.01-1.05)
Year of incidence	Continuous	0.90	(0.82-0.96)
Smoking Status	Current	1.00	Ref
	Former	0.64	(0.44-0.92)
	Never	0.56	(0.40-0.78)
	Unspecified	0.57	(0.41-0.81)
Tumour Size	T1	1.00	Ref
	T2	1.54	(1.13-2.12)
	T3	1.54	(1.02-2.32)
Grade - (%)	Gleason Score <5	1.00	Ref
	Gleason Score 5-7	1.60	(0.84-3.05)
	Gleason Score >7	4.72	(2.45-9.09)
	Unspecified	1.85	(0.89-3.82)
Radiation	Time-varying	0.53	(0.39-0.73)
Statin Exposure		0.73	(0.53-1.00)

* *P*-value < 0.05

APPENDIX 3

Due to the possibility of prostate cancer death on death certificates being misclassified as death from other or unspecified cancers, sensitivity analysis I was carried out using the causes of death listed in Table S3.1. Results are presented in Table S3.2. In Sensitivity Analysis 1 no difference was seen in the unadjusted HR for aspirin use. The adjusted HR was similar to the original analysis (Multivariate HR 0.89, 95%CI 0.68-1.17) as were the trends observed. See Table S3.2. Sensitivity Analysis 2 considered prostate cancer causes of death which were reported as secondary or contributory causes of death as the primary cause of death. The adjusted HR was modestly elevated in this sensitivity analysis, and the adjusted HR was slightly closer to the null (Multivariate HR 0.92, 95%CI 0.70-1.20). See Table S3.2.

TABLE S3.1: ICD-10 codes for alternative definitions of prostate cancer death in sensitivity analysis 1[1]

Cancer Site	ICD 9 Code	ICD 10 Code
Malignant neoplasm of prostate	185	C61
Malignant neoplasm of other male genital organs, site unspecified	187.9	C63.9
Malignant neoplasm of pelvis	195.3	C41.4
Secondary malignant neoplasm	196-198	C76-C80
Malignant neoplasm without specification of site	199	C80.9
Benign neoplasm of prostate	222.2	D29.1
Benign neoplasm of male genital organs, site unspecified	222.9	D29.9
Neoplasm of uncertain behaviour of prostate	236.5	D40.0
Neoplasm of uncertain behaviour of other and unspecified male genital organs	236.6	D40.9
Neoplasm of uncertain behaviour, site unspecified	238.9	D48.9
Neoplasm of unspecified nature of other genitourinary organs	239.5	D40.7, D41
Neoplasm of unspecified nature, site unspecified	239.9	D48.9

1. Trends in Cancer Survival in Scotland 1971-1995. In. Edinburgh: Scottish Cancer Intelligence Unit 2000; Appendix 7.

TABLE S3.2: Sensitivity Analyses: Estimated Hazard Ratios For Association Between Aspirin Use and Prostate Cancer-Specific Mortality

			Prostate cancer-specific mortality		
Sensitivity Analysis 1	***N***	**Person Years**	**No. of deaths (rate)[A]**	**Univariate HR (95%CI)**	**Multivariate HR[B] (95%CI)**
Aspirin non-user	1,805	10,060	177 (17.6)	Ref -	Ref -
Aspirin user pre-diagnosis	1,131	6,070	108 (17.8)	1.02 (0.80-1.29)	0.89 (0.68-1.17)
Exposure response: dosing intensity[C]					
Low dosing intensity 0%-86%	564	3,070	63 (20.5)	1.17 (0.88-1.56)	1.03 (0.75-1.41)
High dosing intensity 86%-100%	567	3,000	45 (15.0)	0.86 (0.62-1.19)	0.75 (0.53-1.06)
P-trend				*0.62*	*0.14*
Exposure response: dose					
Low dose ≤ 75mg	881	4,627	88 (19.0)	1.09 (0.84-1.41)	1.00 (0.75-1.32)
High dose > 75mg	250	1,443	20 (13.9)	0.78 (0.49-1.25)	0.60 (0.37-0.97)
P-trend				*0.68*	*0.09*
Sensitivity Analysis 2					
Aspirin non-user	1,805	10,060	180 (17.9)	Ref -	Ref -
Aspirin user pre-diagnosis	1,131	6,070	114 (18.8)	1.05 (0.83-1.33)	0.92 (0.70-1.20)
Exposure response: dosing intensity[C]					
Low dosing intensity 0%-86%	564	3,070	66 (21.5)	1.21 (0.91-1.60)	1.05 (0.77-1.43)
High dosing intensity 86%-100%	567	3,000	48 (16.0)	0.90 (0.56-1.24)	0.78 (0.56-1.10)
P-trend				*0.83*	*0.21*
Exposure response: dose					
Low dose ≤ 75mg	881	4,627	91 (19.7)	1.11 (0.86-1.42)	1.01 (0.77-1.34)
High dose > 75mg	250	1,443	23 (15.9)	0.89 (0.58-1.37)	0.65 (0.41-1.03)
P-trend				*0.99*	*0.16*

A: *Mortality rate (deaths/1000 person years).*
B: *Multivariate HR is adjusted for age at diagnosis, tumour grade, tumour size, smoking status at diagnosis, comorbidity score, year of incidence, pre-diagnostic statin exposure, and receipt of radiation (time-varying).*
C*: Dosing intensity by median*

APPENDIX 4

TABLE S.4: Estimated Hazard Ratios For Association Between Aspirin Use and All-Cause Mortality

Aspirin Use	*N*	Person Years	All-cause mortality		
			No. of deaths (rate)[A]	Univariate HR (95%CI)	Multivariate HR (95%CI)
Pre-diagnostic aspirin use[B]					
Aspirin non-user in year prior to diagnosis	1,805	10,060	442 (43.9)	Ref -	Ref -
Aspirin user in year prior to diagnosis	1,131	6,070	339 (55.8)	1.28 (1.11-1.48)*	0.98 (0.84-1.15)
Exposure response: dosing intensity[C]					
Low dosing intensity 0%-86%	564	3,070	166 (54.1)	1.24 (1.03-1.48)*	0.93 (0.77-1.13)
High dosing intensity 86%-100%	567	3,000	173 (57.7)	1.33 (1.11-1.58)*	1.03 (0.85-1.25)
P-trend				*<0.001*	*0.846*
Exposure response: dose					
Low dose ≤ 75mg	881	4,627	266 (57.5)	1.33 (1.14-1.54)*	1.06 (0. 90-1.25)
High dose > 75mg	250	1,443	73 (50.6)	1.14 (0.89-1.46)	0.76 (0.59-0.99)*
P-trend				*0.008*	*0.171*
Exposure response: dosing intensity[C] **& dose**					
Low dosing intensity 0%-86%					
Low dose ≤ 75mg	420	2,256	124 (55.0)	1.26 (1.03-1.54)*	0.99 (0.80-1.22)
High dose > 75mg	144	814	42 (51.6)	1.17 (0.85-1.61)	0.79 (0.57-1.10)
High dosing intensity 86%-100%					
Low dose ≤ 75mg	461	2,371	142 (59.9)	1.39 (1.15-1.68)*	1.14 (0.93-1.39)
High dose > 75mg	106	629	31 (49.3)	1.10 (0.77-1.59)	0.72 (0.49-1.05)
Exposure response: duration[D]					
Aspirin non-user in 3 years prior to diagnosis	1,003	5,202	195 (37.5)	Ref -	Ref -
Aspirin user in 3 years prior to diagnosis					
Aspirin user 0- 2 years pre-diagnosis	226	1,157	49 (42.4)	1.13 (0.83-1.55)	0.91 (0.66-1.26)
Aspirin user >2 years pre-diagnosis	578	2870	150 (53.2)	1.40 (1.13-1.73)*	1.09 (0.86-1.39)
P-trend				*0.002*	*0.475*
Exposure response: duration & dosing intensity					
Aspirin user 0- 2 years pre-diagnosis					
Low dosing intensity 0%-84%	112	578	21 (36.3)	0.97 (0.62-1.52)	0.81 (0.51-1.28)
High dosing intensity 84%-100%	114	579	28 (48.4)	1.29 (0.87-1.92)	1.01 (0.67-1.52)
Aspirin user >2 years pre-diagnosis					
Low dosing intensity 0%-84%	290	1,455	77 (52.9)	1.41 (1.09-1.84)*	1.11 (0.84-1,48)
High dosing intensity 84%-100%	288	1,415	73 (51.6)	1.38 (1.06-1.81)*	1.08 (0.80-1.44)

* *P*-value < 0.05.

A: Mortality rate (deaths/1000 person years).

B: All multivariate HRs are adjusted for age at diagnosis, tumour grade, tumour size, smoking status at diagnosis, comorbidity score, year of incidence, pre-diagnostic statin exposure, and receipt of radiation (time-varying).

C: Dosing intensity by median

D: Cohort with at least 3 years continuous GMS scheme eligibility prior to diagnosis, (*N*=1,807)

APPENDIX 5

Supplementary results of effect modification analyses by prostate cancer treatment received (Table 5.1) and tumour characteristics (Table 5.2)

TABLE S5.1: Aspirin Use and Prostate Cancer-Specific Mortality – Effect Modification by Prostate Cancer Treatment

	Aspirin Non-user	Aspirin User	User Vs. Non-user
Surgery			
No **Death/Censored**	133/1,260	85/835	
Multivariate HR[A] (95% CI)	Ref -	0.89 (0.66-1.20) p = 0.43	0.89 (0.66-1.20) *P* = 0.43
Yes **Death/Censored**	39/373	19/192	
Multivariate HR[A] (95% CI)	0.97 (0.67-1.40) p = 0.85	0.73 (0.44-1. 21) p = 0.22	0.76 (0.43-1.34) *P* = 0.34
	Multiplicative scale: rHR (95% CI) Surgery (Yes Vs. No)		**0.86 (0.46-1.58) *P* = 0.62**
Radiation			
No **Death/Censored**	138/991	84/604	
Multivariate HR[B] (95% CI)	Ref -	0.84 (0.62-1.13) p = 0.25	0.84 (0.62-1.13) *P* = 0.25
Yes **Death/Censored**	34/642	20/423	
Multivariate HR[B] (95% CI)	0.48 (0.33-0.71) p < 0.05	0.46 (0.28-0.75) p <0.05	0.96 (0.55-1.69) *P* = 0.89
	Multiplicative scale: rHR (95% CI) Radiation (Yes Vs. No)		**1.15 (0.62-2.13) *P* = 0.66**

A: *Adjusted for age, comorbidity score, tumour size, tumour grade, smoking status at diagnosis, year of incidence, statin exposure and receipt of radiation (time-varying).* ***B:*** *Adjusted for age, comorbidity score tumour size, tumour grade, smoking status at diagnosis, year of incidence and statin exposure.*

TABLE S5.2: Aspirin Use and Prostate Cancer-Specific Mortality by Tumour Characteristics.

	Aspirin Non-user	Aspirin User	User Vs. Non-user
Gleason Score			
≤7 Death/Censored	90/1,232	55/765	
Multivariate HR[A] (95% CI)	Ref -	0.98 (0.68-1.40) p = 0.90	0.98 (0.68-1.40) *P* = 0.90
>7 Death/Censored	67/222	36/140	
Multivariate HR[A] (95% CI)	3.49 (2.53,4.80) p < 0.05	2.38 (1.57, 4.80) p < 0.05	0.68 (0.45-1.05) *P* = 0.08
	Multiplicative scale: rHR (95% CI)	Gleason score : >7 Vs. ≤7	**0.70 (0.41-1.19) *P* = 0.19**
Tumour Size			
T1 & T2 Death/Censored	145/1,425	85/895	
Multivariate HR[B] (95% CI)	Ref - -	0.92 (0.69-1.24) p = 0.58	0.92 (0.69-1.24) *P* = 0.58
T3 Death/Censored	27/208	19/132	
Multivariate HR[B] (95% CI)	1.19 (0.79-1.80) p =0.41	0.93 (0.56-1.54) p = 0.39	0.78 (0.43-1.43) *P* = 0.42
	Multiplicative scale: rHR (95% CI)	T3 Vs. T1 &T2	**0.85 (0.44-1.62) *P* = 0.62**

A: *Adjusted for age at diagnosis, tumour size, smoking status at diagnosis, comorbidity score, year of incidence, pre-diagnostic statin exposure, and receipt of radiation (time-varying).* ***B:*** *Adjusted for age at diagnosis, tumour grade, smoking status at diagnosis, comorbidity score, year of incidence, pre-diagnostic statin exposure, and receipt of radiation (time-varying).*

APPENDIX 3: PROSTATE SPECIFIC ANTIGEN TESTING IS ASSOCIATED WITH MEN'S PHYSICAL, AND PSYCHOLOGICAL HEALTH AND HEALTHCARE UTILISATION, IN A NATIONALLY REPRESENTATIVE SAMPLE.

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Under review

ABSTRACT

BACKGROUND: Prostate cancer incidence has risen in recent years due to Prostate Specific Antigen (PSA) testing in primary care.

OBJECTIVES: To investigate associations between PSA testing and the physical and psychological health and healthcare utilisation of men in a population where PSA testing is widespread

METHODS: A cross-sectional study was carried out in a population-representative sample of men ≥50 years enrolled in The Irish Longitudinal Study on Ageing (TILDA). Men were classified as ever/never having received a PSA test. Multivariate logistic regression (Odds Ratios (OR) and 95% Confidence Intervals (CI) was used to determine associations between PSA testing, and men's psychological and physical health and healthcare utilisation.

RESULTS: The analysis included 3,628 men, 68.2% of whom had a PSA test. In adjusted analysis, sub-threshold depression (OR=0.79, 95%CI 0.64-0.97), anxiety (OR=0.79, 95%CI 0.57-1.09), frailty (OR=0.61, 95%CI 0.31-1.05) and eligibility for free primary care (OR=0.63, 95%CI 0.52-0.77) were inversely associated with testing. PSA testing was positively associated with more chronic illnesses (OR=1.11, 95%CI 1.05-1.19), more primary care visits (OR=1.03, 95%CI 1.01-1.05) and preventative health practices including cholesterol testing and influenza vaccination (OR=1.35, 95%CI 1.13-1.60).

CONCLUSIONS: Men's psychological and physical health and healthcare utilisation are associated with PSA testing in primary care. The negative association between poorer psychological health and PSA testing in primary care may impact on informed decision making and requires further investigation. These findings may have wider implications for other cancer screening.

KEY WORDS: Prostate Specific Antigen, PSA, men's health; health service; General practice, Depression

Introduction

Prostate cancer (PCa) incidence has increased in the last two decades, due to increasing prostate specific antigen (PSA) testing and subsequent prostate biopsy (1). PSA testing in Ireland is high, with the majority of tests being performed in general practice (2). This opportunistic testing has led to increased PCa incidence, younger age at diagnosis and a shift towards more localized disease (3). Increased PCa detection has important consequences for men's quality-of-life (4), consequently, guidelines and recommendations on PSA emphasise the importance of informed decision making (5,6). It is therefore important to understand factors associated with PSA testing to facilitate informed decision making.

Psychological health negatively impact breast, cervical and colorectal cancer screening (7-9). However, its impact on cancer screening in men (9) and PSA testing has received little attention and results have been conflicting, due to small sample sizes and different measures used (10-13). In addition, a small number of studies have recently reported that markers of healthcare utilisation influenced whether men have PSA tests and other cancer screening (11,13,14).

Our objective was to investigate, at the population level, associations between PSA testing and men's psychological and physical health and their health services utilisation.

Methods

Setting

Ireland has a mixed public-private healthcare system. Approximately one-third of the population are eligible for the state-funded General Medical Services (GMS) Scheme, as determined by means-test and age (15), which entitles them to free General Practitioner (GP) and hospital visits and prescriptions at a cost of €0.50 per item. GPs are reimbursed for GMS patients by the Health Services Executive. Approximately half the population have private health insurance (PHI). However, most insurance plans do not cover GP visits, and patients pay €50-60 per visit.

Study Population

This study population consisted of males aged ≥50 years participating in wave 1 (2009-2011) of The Irish Longitudinal Study on Aging (TILDA) (16). TILDA is a study of the health, lifestyle and financial situation of a population-representative sample of people aged ≥50 years

involving Computer Aided Personal Interview (CAPI) in participants' homes, a Self-Completion Questionnaires (SCQ) and comprehensive health assessment (HA) in one of two health centres. Where travel to health centres was unfeasible (~10% of participants), nurses performed the HA in participant's homes (Appendix 1).

OUTCOME VARIABLE

The main outcome variable WAS ever having had a PSA test. Men were included if they gave a definitive answer to the CAPI question asking had they ever had "a PSA blood test to screen for PCa". Men who responded "don't know" or declined to answer were excluded (n=116).

COVARIATES

Healthcare utilisation

Self-reported Healthcare utilisation variables recorded were: number of GP visits in the previous year; eligibility for GMS (17) (yes/no); cholesterol testing (ever/never); influenza vaccination (ever/never); number of regular medicines (prescription/other) including chronic cardio-preventative medication, statins, and aspirin (yes/no) classified using WHO ATC Classification.

Psychological assessments

Three scales were used to measure psychological health; depression was assessed using the Centre for Epidemiologic Studies Depression (CES-D) scale (0-7 not depressed; 8-15 sub-threshold; ≥16 case-level depression (18)); anxiety was assessed using the Hospital Anxiety and Depression Scale (HADS-A): (0-7 not anxious; 8-10 borderline; ≥11 case-level anxiety (19)); global cognitive function was assessed using the Mini Mental State Examination (MMSE; 26-30 normal cognitive function; 20-25 mild cognitive impairment; <20 moderate cognitive impairment (20)). Mild and moderate cognitive impairment groups were combined because of the small number of men in the latter group. Participants for whom data was unavailable were classified as "unspecified" for these categories (16).

Physical health status

Men's overall physical health was measured by summing the number of self-reported chronic illnesses from: heart attack, heart failure, angina; stroke; diabetes; hypertension; high cholesterol; lung disease; asthma; cataracts; cancer; Parkinson's disease; peptic ulcer; arthritis; osteoporosis or hip fracture. Men taking medications in the WHO-ATC category G04C

were classified as treated for Benign Prostatic Hypertrophy (BPH). A previous cancer diagnosis (yes/no) was identified separately. A frailty score was derived within TILDA from five measurements; self-reported weight-loss of ≥4.5kg in the year pre-interview; weakness based on grip-strength; self-reported exhaustion; gait speed; and low physical activity. Other variables associated with frailty were: self-reported arthritis, joint replacement and osteoporosis (yes/no), hip or wrist fracture (ever/never). Subjective health status variables investigated included overall self-rated health, and self-rated emotional or mental health (excellent/very good, good, fair/poor).

Socio-demographic characteristics

Sociodemographic characteristics including age, marital status, work status, smoking status, highest educational level achieved and PHI status were recorded at CAPI.

STATISTICAL ANALYSIS

Univariate analyses (chi-square test, Wilcoxson rank-sum) were used to identify associations between covariates and ever having had a PSA test. Logistic regression was used to build a multivariate model of predictors of PSA testing. Analysis was conducted in two stages. Firstly, a core model was developed from socio-demographic, healthcare utilisation and health status variables previously associated with PSA testing (age, marital status, education, employment, smoking status, number of GP visits) and covariates with a p-value <0.1 in univariate analyses. Collinearity was addressed by including one of two potentially correlated variables (e.g. number of chronic illnesses, but not number of medicines). Covariates retained in the core model were: number of chronic illnesses, influenza vaccine, prior cancer diagnosis, treated for BPH, and GMS eligibility. In stage two, psychological and physical health measures were added separately to the core model, to assess their independent association with PSA testing.

Sensitivity analysis was performed to examine effects on multivariate risk estimates of PSA testing of excluding men who had a previous PCa diagnosis (n=93). Individual comorbidities were assessed for association with PSA testing in the core model, as an additional analysis.

TILDA data V 1-7-3 and STATA V 12 were used for analyses. Significance at $p<0.05$ was assumed.

Results

Study Population Characteristics

Median age of men was 63 years (IQR 56-71, N=3,628) and more than two-thirds (68.2%) reported ever having a PSA test (Table 1). Of these men, 84.2% returned the SCQ, and HA data was available for 72.3%. (Figure 1).

Stage 1: Core model

The core multivariate model is presented in Table 2. Ever having a cholesterol test was the factor most strongly associated with PSA testing in univariate analysis (OR=17.0 95%CI 12.9-22.4). Therefore, to assess other independent associations with PSA testing, this was removed from the model. In multivariate analyses, physical health (more chronic conditions (OR=1.11 per unit increase in conditions, 95%CI 1.05-1.19); previous cancer diagnosis (OR=2.74, 95%CI 1.74-4.30); BPH treatment (OR=2.66, 95%CI 1.65-4.27)), healthcare utilisation (increased number of GP visits (OR=1.01, 95%CI 1.01-1.05; having an influenza vaccination (OR=1.35, 95%CI 1.13-1.60)); and sociodemographic variables (higher educational attainment and being married/cohabiting compared to other marital status) were associated with increased likelihood of having PSA tests. Men were significantly less likely to have had PSA tests if they were: current smokers (OR=0.56, 95%CI 0.45-0.69), GMS eligible (OR=0.63, 95%CI 0.52-0.77) or were not employed (OR=0.67, 95%CI 0.53-0.85) (Table 2).

Stage 2: Associations between psychological and physical health and PSA testing

One-fifth of these men had depression, of whom 15% and 7% had sub-threshold and case-level depression, respectively. Prevalence of borderline and case-level anxiety were 16.3% and 5.4%, respectively. Men with sub-threshold depression were significantly less likely to have had PSA tests (adjusted OR=0.97, 95%CI 0.62-0.97). Men with case-level anxiety had reduced likelihood of PSA testing in unadjusted analyses compared to non-anxious men, but not significantly post-adjustment (OR=0.79, 95%CI 0.57-1.09). Lower self-rated emotional or mental health was associated with reduced likelihood of PSA testing in univariate analysis, but was no longer significant in adjusted analyses (Table 3).

Patients with a degree of cognitive impairment were significantly less likely to have had PSA tests. Those with mild-moderate cognitive impairment were less likely to have had PSA tests, compared to those with unimpaired cognition, though non-significantly (OR=0.79, 95%CI 0.58-1.08).

Frailty was associated with reduced likelihood of PSA testing, this was significant for men who were pre-frail (adjusted OR=0.68, 95%CI 0.56-0.83). Individual frailty measures associated with non-testing were low grip strength (OR=0.84, 95%CI 0.69-1.02), low gait speed (OR=0.61, 95%CI 0.43-0.86) and low levels of physical activity (OR=0.66, 95%CI 0.50-0.87) (Appendix 3). Men who reported heart attack/heart failure/angina (OR=0.62, 95%CI 0.47-0.80), stroke (OR=0.55, 95%CI 0.32-0.95) and lung disease (OR=0.64, 95%CI 0.43-0.95) were significantly less likely to have had PSA tests in adjusted analyses (Appendix 4).

Exclusion of men who had a PCa diagnosis (N=93) did not affect associations between any covariates and ever having a PSA test, except previous cancer diagnosis (Appendix 5).

Discussion

Men with lower self-reported physical and psychological health including depression, anxiety, cognitive impairment and frailty were less likely to have had a PSA test, while men with excellent or very good self-reported health were more likely to have had PSA tests in this nationally representative sample of men aged ≥50 years, after adjusting for socio-demographic factors. Increased healthcare utilisation was also associated with increased likelihood of PSA testing, however, men eligible for free healthcare were less likely to have been tested. We applied three hypotheses of health behaviour to explain these observations (21,22).

Firstly, there is evidence of a 'healthy user effect' (21) whereby men taking preventative medication, including statins and influenza vaccinations were more likely to have had PSA tests. The healthy user effect is a multidimensional concept incorporating 'health-seeking' tendencies, i.e. healthier patients request or accept more screening tests and have increased adherence to medications, but it also incorporates 'health status' i.e. the ability of patients, physically and cognitively to attend primary care and to get prescriptions filled (21). Fleming proposed four hypotheses to explain the role of comorbidities on cancer stage at diagnosis (22), two of which we have applied to elaborate on the role of health status on the likelihood of having PSA tests.

Multi-morbidity results in polypharmacy and increased health services utilisation (23). We found that, despite adjusting for number of GP visits, men with more chronic illnesses were more likely to be tested, suggesting that some PSA tests can be ascribed to the 'surveillance hypothesis' i.e. men with coexisting conditions have more frequent contact with the healthcare system facilitating early diagnosis (14,22). However, while not the central focus of

this paper, but in agreement with other studies, we found that the association between comorbidity and PSA testing depended on the coexisting disease (24). In this cohort, likelihood of testing was increased in men with angina, high cholesterol, cataracts and hypertension ('surveillance hypothesis'), but was negatively associated while frailty, cardiac diseases and stroke suggesting that poorer physical health, distracts GPs from undertaking, or encouraging men to have PSA tests, the 'competing demand hypothesis' (22).

Negative associations between poorer psychological health and likelihood of PSA testing is further evidence of the 'competing demand hypothesis' and has received little attention. Men with sub-threshold depression were significantly less likely to have PSA tests than men who were not depressed, suggesting that somatic symptoms associated with depression are more pertinent during healthcare visits or that GPs may be less likely to initiate discussions about PSA testing and PCa with depressed men for fear of exacerbating their condition. Our findings concur with previous work which observed lower rates of breast, cervical and colorectal cancer screening among people with depression (7,8,25), despite increased usage of primary care services (8). However, we found that case-level depression was not associated with PSA testing. This may be due to various reasons; men with case-level depression may be receiving management for depression and thus may be more likely to be PSA tested, consistent with the 'surveillance hypothesis', the number of men with case-level depression may be too small to detect significant effects, or the effect of case-level depression on PSA testing may be no longer significant when other aspects of psychological health e.g. anxiety was included in the model. In support of the latter hypothesis, Kotwal et al observed that men with depressive symptoms were less likely to have PSA tests, however, this effect was medicated by levels of perceived stress (13). While stress was not measured in this cohort, we found that case-level anxiety was associated with reduced likelihood of PSA testing in univariate but not adjusted analysis. Anxiety effects PSA testing in different ways; it propels men to have PSA tests if men have anxiety about PCa or deters men from having PSA tests if they are anxious about screening (12,13). Furthermore, associations between anxiety and PSA testing depend on the number of GP visits (14). Men with increased cognitive impairment were also significantly less likely to have had PSA tests, which again may be explained by the 'competing demands' hypothesis. This is the first time associations between psychological health and PSA testing has been observed in men in Ireland and our findings add to the growing body of literature on the effect of psychological health on preventative health and cancer screening.

GMS eligibility is associated with more frequent GP visits (17), however, despite adjustment for socio-demographic, health, and healthcare factors including number of GP visits, GMS eligibility was negatively associated with PSA testing, consistent with income-related inequality in uptake of PSA testing observed in Ireland and elsewhere (26). This highlights that in mixed public-private systems, free healthcare services does not produce equity in uptake of primary care services and may in part explain the higher PCa incidence in higher socioeconomic groups (www.ncri.ie)

Socio-demographic factors were strong predictors of PSA testing and our findings are broadly in agreement with others (13,14,26). Married men were more likely to have PSA tests possibly because their wives engage in breast and cervical cancer screening (Drummond et al unpublished data). Odds of testing were greatly reduced in current smokers, which concurs with previous studies (27). Smoking-related illnesses may be prioritised by GPs, the 'competing demands hypothesis' and/or smokers may avoid engagement with health services because they anticipate unwanted advice to quit smoking (28).

This study has several strengths. It is a large sample, representative of the population (16), with data on a wide range of variables. Standardised measures of depression, anxiety and cognitive function were used, although stress was not measured. We acknowledge several limitations; data on PSA testing was self-reported, which is subject to recall bias (29). However, the estimated prevalence of PSA testing in this population is high and comparable to that expected based on extrapolations from numbers of PSA tests analysed nationally (30). Sensitive information may have been withheld e.g. use of anti-depressants; or chronic conditions misclassified and the strength of some associations with PSA testing may have been limited due to small numbers in sub-groups. Finally, the influence of GPs i.e. the 'provider effect' on whether men were PSA tested was not measured.

In conclusion, this study provides insight into the characteristics of men who have, and have not had PSA tests in primary care. Men in poorer psychological and physical health, smokers and those eligible for free GP services were less likely to have had PSA tests while men in good overall health and those engaging in health-seeking behaviours were more likely to have been tested. These findings should be considered by physicians and policy makers in the development of public health strategies to enable men make informed decisions about PSA testing and may apply to other cancer screening services.

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TABLE 1: Characteristics of the study population according to PSA testing (ever/never)

Population Characteristics			Ever received PSA test		Never received PSA test		
			N= 2473	(68.2%)	N=1153	(31.8%)	p-value
Socio-demographic Characteristics							
Age at interview	years	Median, (IQR)	64	(57, 71)	59	(54, 69)	<0.001
Marital Status	Married	N (%)	1916	(77.4)	795	(69.0)	<0.001
	Single	N (%)	248	(10.0)	178	(15.4)	
	Sep/Divorced	N (%)	112	(4.5)	89	(7.7)	
	Widowed	N (%)	199	(8.0)	91	(7.9)	
Education	Primary	N (%)	771	(31.2)	425	(36.9)	<0.001
	Secondary	N (%)	952	(38.5)	463	(40.2)	
	Third Level	N (%)	752	(30.4)	265	(23.0)	
Employment	Employed	N (%)	1007	(40.7)	508	(44.1)	<0.001
	Retired	N (%)	1225	(49.5)	406	(35.2)	
	Other	N (%)	243	(9.8)	239	(20.7)	
Smoking Status	Never	N (%)	919	(37.2)	374	(32.4)	<0.001
	Past	N (%)	1215	(49.1)	471	(40.8)	
	Current	N (%)	340	(13.7)	308	(26.7)	
Private Health Insurance	At time of CAPI	N (%)	1618	(65.4)	514	(44.6)	<0.001
Health-Care Utilisation							
No. of GP visits	year pre-CAPI	Median (IQR)	3	(1, 5)	2	(0, 4)	<0.001
Cholesterol test	Ever	N (%)	2408	(97.4)	791	(68.9)	<0.001
Influenza Vaccine	Ever	N (%)	1352	(54.6)	468	(40.6)	<0.001
No. of medicines	Self-report	Median (IQR)	2	(0, 4)	1	(0, 3)	<0.001
	BPH-Medicine	N (%)	166	(4.7)	21	(1.8)	<0.002
	Aspirin	N (%)	673	(27.2)	216	(18.7)	<0.001
	Statin	N (%)	869	(35.1)	277	(24.0)	<0.001
GMS eligibility	At time of CAPI	N (%)	1072	(43.3)	544	(47.2)	0.029
Physical, Mental and Emotional Health							
Self-rated health relative to others of the same age	Ex. / V. good	N (%)	1398	(56.5)	601	(52.3)	0.012
	Good	N (%)	690	(27.9)	376	(32.7)	
	Fair / Poor	N (%)	386	(15.6)	173	(15.0)	
Chronic illnesses		Median (IQR)	2	(1, 3)	1	(0,2)	<0.001
Cancer diagnosis	Ever	N (%)	179	(7.2)	24	(2.1)	<0.001
	Prostate cancer	N (%)	93	(3.8)	0	(0)	<0.001
Frailty	Not frail	N (%)	1265	(51.1)	486	(42.2)	<0.001
	Pre-frail	N (%)	481	(19.4)	244	(21.2)	
	Frail	N (%)	57	(2.3)	23	(2.0)	
	Unrecorded	N (%)	672	(27.5)	400	(34.7)	
Self-rated Emotional or Mental Health	Ex. / V. good	N (%)	1604	(64.8)	689	(59.8)	0.007
	Good	N (%)	676	(27.3)	347	(30.1)	
	Fair / Poor	N (%)	195	(7.9)	117	(10.2)	
Depression Score	CESD	Median (IQR)	3	(0, 6)	3	(1, 8)	0.0013
Depression	No	N (%)	1954	(79.9)	847	(74.6)	0.002
	Sub-threshold	N (%)	344	(14.1)	200	(17.6)	
	Case-Level	N (%)	151	(6.2)	88	(7.8)	
Anxiety Score	HADS-A (SCQ)	Median (IQR)	4	(2, 7)	5	(2, 7)	0.1674
Anxiety Categorical	Not anxious	N (%)	1679	(67.8)	695	(60.2)	0.001
	Borderline	N (%)	277	(11.2)	115	(10.0)	
	Case-Level	N (%)	118	(4.8)	75	(6.5)	
	Unclassified	N (%)	401	(16.2)	268	(23.2)	
Cognition	MMSE score	Median (IQR)	29	(27, 30)	29	(28, 30)	
MMSE Categorical	Normal	N (%)	1677	(67.8)	699	(60.6)	<0.001
	Mild-moderate Impairment	N (%)	157	(6.3)	83	(7.2)	
	Unrecorded	N (%)	641	(25.9)	371	(32.2)	

Ex. / V. Good: Excellent / Very good. p-values for comparisons between tested and untested men.

TABLE 2: Stage 1 - Associations between socio-demographic characteristics, healthcare utilisation and aspects of physical health and ever having had a PSA test. Variables for which multivariate ORs are presented are those contained within the core model

Variables associated with PSA testing		Univariate Analysis			Multivariate Analysis		
		OR	95% CI	p-value	OR	95% CI	p-value
Socio-demographic factors							
Age at interview	(years)	1.03	1.02-1.04	<0.001	1.02	1.00- 1.03	0.012
Marital Status	Married	1.00	Ref		1.00	Ref	
	Single	0.58	0.47-0.72	<0.001	0.69	0.55-0.87	0.001
	Sep/Div	0.52	0.39-0.70	<0.001	0.68	0.50-0.92	0.014
	Widowed	0.91	0.70-1.18	0.466	0.70	0.53-0.94	0.017
Education	Primary	1.00	Ref		1.00	Ref	
	Secondary	1.13	0.96-1.33	0.131	1.31	1.09-1.57	0.004
	Third Level	1.56	1.30-1.88	<0.001	1.49	1.22-1.83	<0.001
Employment	Employed	1.00	Ref		1.00	Ref	
	Retired	1.52	1.30-1.78	<0.001	1.23	0.99-1.53	0.056
	Other	0.51	0.42-0.63	<0.001	0.67	0.53-0.85	0.001
Smoking Status	Never	1.00	Ref		1.00	Ref	
	Past	1.05	0.89-1.23	0.553	0.96	0.81-1.14	0.634
	Current	0.45	0.37-0.55	<0.001	0.56	0.45-0.69	<0.001
Private Health Insurance		2.35	2.04-2.71	<0.001			
Healthcare utilisation factors							
No of GP visits	(Continuous)	1.04	1.02-1.06	<0.001	1.03	1.01-1.05	0.001
Influenza Vaccine	Ever	1.76	1.53-2.03	<0.001	1.35	1.13-1.60	0.001
GMS Scheme Eligible		0.86	0.74-0.98	0.029	0.63	0.52-0.77	<0.001
No of medicines		1.12	1.08-1.15	<0.001			
Cholesterol test		17.00	12.9-22.4	<0.001			
Physical Health							
Chronic illnesses	(Continuous)	1.25	1.19-1.32	<0.001	1.11	1.05-1.19	0.001
Prior Cancer diagnosis		3.66	2.38-5.64	<0.001	2.74	1.74-4.30	<0.001
Treated BPH		3.87	2.45-6.14	<0.001	2.66	1.65-4.27	<0.001

Sep/Div: Separated / Divorced; Multivariate OR is adjusted for age (continuous), marital status (married/ single/separated or divorced/ widowed), education level attained (primary/ secondary/ third level), employment status (employed/retired/ other), smoking status (never/ past/ current), number of GP visits in the past year (continuous), receipt of influenza vaccine (ever/never), number of chronic illness reported (continuous).

TABLE 3: Stage 2- Assessment of the association between PSA testing (yes/no) and physical, mental and emotional health covariates, multivariate ORs are adjusted for the core model (Table 2).

Physical, Mental and Emotional Health		Univariate Analysis			Multivariate Analysis		
		OR	95% CI	p-value	OR	95% CI	p-value
Self-rated Health	Ex / V. good	1.00	Ref		1.00	Ref	
	Good	0.79	0.67-0.92	0.003	0.78	0.66-0.93	0.005
	Fair / Poor	0.96	0.78-1.18	0.688	0.88	0.69-1.38	0.320
Self-rated	Ex / V. good	1.00	Ref		1.00	Ref	
Emotional or	Good	0.84	0.72-0.98	0.026	0.91	0.77-1.08	0.258
Mental Health	Fair / Poor	0.72	0.56-0.92	0.008	0.82	0.62-1.08	0.158
Depression	CES-D	0.98	0.97-0.99	0.002	0.99	0.98-1.00	0.126
Depression	No	1.00	Ref		1.00	Ref	
	Sub-threshold	0.75	0.62-0.90	0.003	0.79	0.64-0.97	0.025
	Case-Level	0.74	0.56-0.98	0.035	0.85	0.62-1.15	0.293
Anxiety	Continuous	0.98	0.96-1.00	0.078	1.00	0.97-1.02	0.791
Anxiety Categorical	Not anxious	1.00	Ref		Ref	1.00	
	Borderline	0.99	0.79-1.26	0.980	1.02	0.79-1.30	0.906
	Case-Level	0.65	0.48-0.88	0.003	0.79	0.57-1.09	0.159
	Unclassified	0.62	0.52-0.74	<0.001	0.71	0.59-0.87	0.001
Cognition: MMSE score	Continuous	1.04	1.01-1.08	0.023	1.05	1.01-1.10	0.024
MMSE score for	Unimpaired	1.00	Ref		1.00	Ref	
cognitive	Mild-moderate	0.79	0.60-1.04	0.096	0.79	0.58-1.08	0.134
impairment	Unrecorded	0.72	0.62-0.84	<0.001	0.84	0.71-1.00	0.049
Frailty	Not frail	1.00	Ref		1.00	Ref	
	Pre-frail	0.76	0.62-0.91	0.003	0.68	0.56-0.83	<0.001
	Frail	0.95	0.58-1.56	0.846	0.61	0.35-1.05	0.072
	Unrecorded	0.65	0.55-0.76	<0.001	0.72	0.60-0.85	<0.001
Arthritis		1.59	1.32-1.91	<0.001	1.23	0.99-1.52	0.058
Aspirin		1.62	1.36-1.92	<0.001	1.18	0.96-1.44	0.113
Statin		1.71	1.46-2.00	<0.001	1.28	1.06-1.54	0.009

Multivariate OR is adjusted for age (continuous), marital status (married/ single/separated or divorced/ widowed), education level attained (primary/ secondary/ third level), employment status (employed/retired/ other), smoking status (never/ past/ current), number of GP visits in the past year (continuous), receipt of influenza vaccine (ever/never), number of chronic illness reported (continuous).

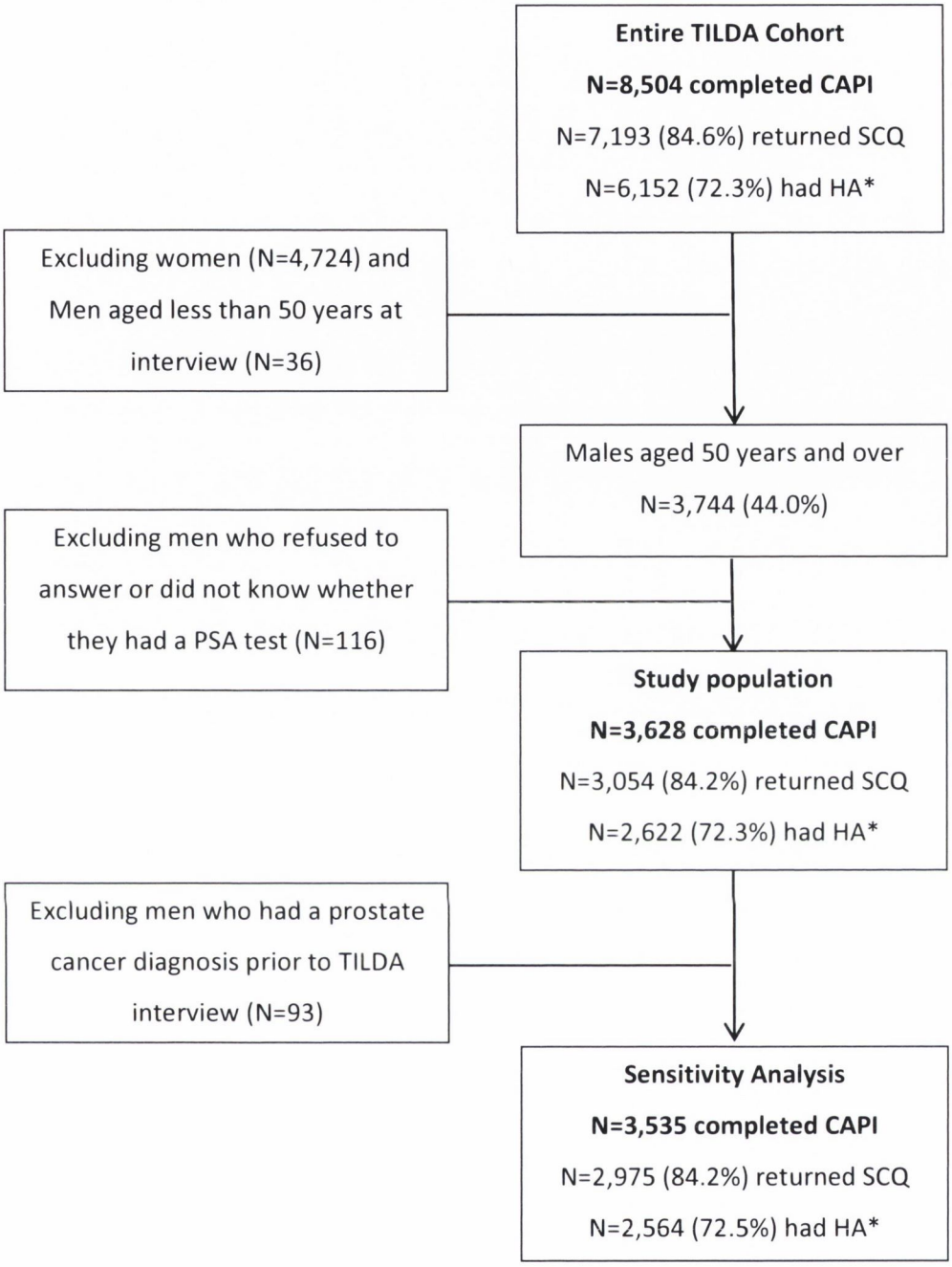

FIGURE 1: Flow diagram of the study population identified from the TILDA study

CAPI: Computer aided personal interview carried out in an individual's home

SCQ: Self-Completion Questionnaire

HA: Health Assessment

*62% attended a health centre for a comprehensive health assessment, with approximately 10% having a shorter health assessment in their home with a research nurse

APPENDIX 1: Detailed description of dataset covariates

TABLE A1: List of covariates, the component of the TILDA study the information was captured, the number and per cent of the study population for whom there was complete data for univariate and multivariate analyses.

Dataset Covariates	Details	Variable type	Capture	Univariate		Multivariate	
Socio-demographic Characteristics				N	%	N	%
Age at interview	(years)	Continuous	CAPI	3,624	99.9%	3,624	99.9%
Marital Status		Categorical	CAPI	3,628	100%	3,624	99.9%
Education		Categorical	CAPI	3,628	100%	3,624	99.9%
Employment		Categorical	CAPI	3,628	100%	3,624	99.9%
Smoking Status		Categorical	CAPI	3,627	100%	3,624	99.9%
Health-Care Utilisation							
Number of GP visits	year pre-CAPI	Continuous	CAPI	3,628	100%	3,624	99.9%
Cholesterol test	Ever/Never	Categorical	CAPI	3,620	99.8%	n/a	n/a
Influenza Vaccine	Ever/Never	Categorical	CAPI	3,628	100%	3,624	99.9%
Chronic illnesses~	Sum (from list)	Continuous	CAPI	3,628	100%	3,624	99.9%
Cancer diagnosis	Ever/Never	Categorical	CAPI	3,628	100%	3,624	99.9%
Number of medicines – WHO ATC code	Self-report	Continuous	CAPI	3,595	99.1%	n/a	n/a
Aspirin – (WHO ATC: B01AC06; M01BA03, N02BA01, N02BA51, N02BA71)	Self-report at CAPI	Categorical	Generated	3,628	100%	3,628	100%
Statin – (WHO ATC: C10AA)	Self-report at CAPI	Categorical	Generated	3,628	100%	3,628	100%
GMS Scheme Eligibility	At time of CAPI	Categorical	CAPI	3,628	100%	3,624	99.9%
Private Health Insurance	At time of CAPI	Categorical	CAPI	3,628	100%	n/a	n/a
Mental and Emotional Health							
Self-rated Emotional or Mental Health	Likert	Categorical	CAPI	3,628	100%	3,624	99.9%
Depression Score	8 item CESD	Continuous	HA	2,610	71.9%	2,608	71.9%
Depression	CESD	Categorical	CAPI	3,584	98.8%	3,581	98.7%
Anxiety Score	HADS-A	Continuous	SCQ	2,959	81.4%	2,956	81.5%

Anxiety Categorical	from HADS-A	Categorical	Generated*	3,628	100%	3,624	99.9%
MMSE Score			HA	2,616	72.1%	2,614	72.1%
MMSE Categorical	from MMSE	Categorical	Generated*	3,628	100%	3,624	99.9%
Physical Health							
Self-rated health relative to others of the same age	Likert	Categorical	CAPI	3,624	99.9%	3,620	99.8%
Frailty score categories#		Categorical	HA	2,556	70.5%	n/a	n/a
Frailty		Categorical	Generated*	3,628	100%	3,624	99.9%
Weight Loss unintended of 4.5 kg or more	in past year	Categorical	CAPI	3,619	99.8%	3,615	99.6%
Low Grip Strength		Categorical	HA	2,614	72.1%	2,612	72.0%
Self-report exhaustion		Categorical	CAPI	3,626	99.9%	3,621	99.8%
Gait Speed		Categorical	HA	2,590	71.4%	2,207	60.8%
Low Activity (IPAQ <383 kcal for men)	8 item IPAQ	Categorical	CAPI	2,589	71.4%	2,587	71.3%
Fracture hip or wrist		Categorical	CAPI	3,628	100%	3,529	97.3%
Fall in past year		Categorical	CAPI	3,628	100%	3,622	99.8%
Joint replacement		Categorical	CAPI	3,627	100%	3,623	99.8%

*Generated categorical variables for anxiety, MMSE, Frailty were recorded where there were observations missing to give a complete dataset

~ Chronic illnesses: sum from self-reported: heart attack or heart failure or angina; stroke; diabetes; self-reported hypertension; self-reported high cholesterol; lung disease; asthma; cataracts; cancer; Parkinson's disease; peptic ulcer; arthritis; osteoporosis or hip fracture

Frailty score categories: derived from five measurements (i) self-reported weight-loss, of 4.5kg (10 lb.) or more in the year pre-interview (CAPI); (ii) weakness based on grip-strength (home assessment or health centre); (iii) self-reported exhaustion (CAPI); (iv) gait speed (home assessment or health centre); (v) low physical activity (International Physical Activity Questionnaire shortened form, within CAPI).

APPENDIX 2: Question from Computer Aided Personal Interview (CAPI) related to health screening.

"**INTRO:** Have you ever had any of the following medical tests or procedures?

PH701: A flu shot?

PH702: A blood test for cholesterol?

PH710: An examination of your prostate to screen for cancer?

PH711: A PSA blood test to screen for cancer?

NOTE: PSA blood test is a test to screen for prostate cancer"

Question PH710 regarding examination of prostate to screen for cancer.

TABLE A2: Cross tabulation of men who answered yes to having had a prostate exam and PSA test.

	Ever had a PSA blood test (PH711)							
	Yes		**No**		**Don't Know / Refused**		**Total**	
Ever had prostate exam (PH710)	N	(% total)	N	(% total)	N	(% total)		
Yes	1922	(51.3)	204	(5.4)	58	(1.5)	**2184**	**(58.3)**
No	545	(14.6)	943	(25.2)	40	(1.1)	**1528**	**(40.8)**
Don't Know / Refused	8	(0.2)	6	(0.1)	18	(0.5)	**32**	**(0.8)**
Total	**2475**	**(66.1)**	**1153**	**(30.8)**	**116**	**(3.1)**	**3744**	**(100.0)**

Chi-squared (6DF) = 1600, $p<0.001$

APPENDIX 3: Break-down of covariates which make up the frailty score and their association with PSA testing

TABLE A3: Frailty covariates and their association with PSA testing, tabulation, univariate odds ratio and multivariate odds ratio from the adjusted model

Frailty Covariates	Ever received PSA test		Never received PSA test			Unadjusted Analysis			Adjusted Multivariate		
	N	(%)	N	(%)	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Weight Loss	155	(67.1)	76	(32.9)	0.691	0.94	0.71-1.25	0.691	0.93	0.67-1.27	0.629
Low Grip Strength	754	(70.8)	311	(29.2)	0.579	1.05	0.88-1.25	0.579	0.84	0.69-1.02	0.085
Self-report exhaustion	186	(63.3)	108	(36.7)	0.057	0.79	0.61-1.01	0.057	0.83	0.63-1.10	0.192
Gait Speed	154	(68.4)	71	(31.6)	0.504	0.90	0.67-1.21	0.505	0.61	0.43-0.86	0.005
Low Activity	190	(64.6)	104	(35.4)	0.025	0.74	0.58-0.96	0.025	0.66	0.50-0.87	0.003
Fracture hip or wrist	291	(68.5)	134	(31.5)	0.883	1.02	0.82-1.26	0.883	1.07	0.85-1.35	0.581
Fall in past year	453	(68.5)	208	(31.5)	0.840	1.02	0.85-1.22	0.840	0.94	0.77-1.14	0.534
Joint replacement	203	(77.5)	59	(22.5)	0.001	1.66	1.23-2.23	<0.001	1.33	0.97-1.83	0.080

Multivariate OR is adjusted for age (continuous), marital status (married/ single/separated or divorced/ widowed), education level attained (primary/ secondary/ third level), employment status (employed/retired/ other), smoking status (never/ past/ current), number of GP visits in the past year (continuous), receipt of influenza vaccine (ever/never), number of chronic illness reported (continuous).

APPENDIX 4: Post-hoc analysis of the individual chronic illnesses and their association with PSA testing

TABLE A4: Chronic illnesses and their association with PSA testing, tabulation, univariate odds ratio and multivariate odds ratio adjusted for the covariates of the core model

Self-reported chronic Illness	PSA test					Unadjusted Analysis			Multivariate Analysis		
	Ever		Never								
	N	(%)	N	(%)	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Heart attack / Heart Failure / Angina	298	(70.0)	128	(30.0)	0.413	1.1	0.88-1.37	0.413	0.62	0.47-0.80	<0.001
Angina	188	(75.5)	61	(24.5)	0.011	1.47	1.09-1.98	0.011	0.83	0.59-1.16	0.266
Heart attack	179	(67.8)	85	(32.2)	0.880	0.98	0.75-1.28	0.880	0.59	0.44-0.80	0.001
Heart failure	42	(75.0)	14	(25.0)	0.272	1.40	0.63-2.58	0.274	0.96	0.50-1.83	0.892
Stroke	45	(66.2)	23	(33.8)	0.715	0.91	0.55-1.51	0.715	0.55	0.32-0.95	0.031
Diabetes	262	(73.6)	94	(26.4)	0.022	1.33	1.04-1.71	0.022	0.96	0.73-1.27	0.792
Hypertension	965	(72.6)	364	(27.4)	<0.001	1.39	1.19-1.61	<0.001	0.94	0.78-1.14	0.531
High Cholesterol	996	(75.5)	324	(24.6)	<0.001	1.72	1.48-2.00	<0.001	1.51	1.24-1.83	<0.001
Lung Disease	89	(65.0)	48	(35.0)	0.404	0.86	0.60-1.23	0.404	0.64	0.43-0.95	0.027
Asthma	192	(69.8)	83	(30.2)	0.554	1.08	0.83-1.42	0.554	0.83	0.62-1.11	0.204
Cataracts	233	(74.0)	82	(26.0)	0.022	1.36	1.04-1.76	0.023	0.83	0.62-1.13	0.242
Parkinson's Disease	19	(86.4)	3	(13.6)	0.067	2.97	0.88-10.04	0.081	2.41	0.67-8.73	0.179
Peptic Ulcer	212	(71.1)	86	(28.9)	0.258	1.16	0.90-1.51	0.259	1.03	0.78-1.38	0.813
Arthritis	588	(75.7)	189	(24.3)	<0.001	1.59	1.32-1.91	<0.001	1.23	0.99-1.52	0.058
Osteoporosis	56	(77.8)	16	(22.2)	0.079	1.65	0.94-2.88	0.081	1.17	0.65-2.10	0.598
Hip Fracture	106	(74.7)	36	(25.4)	0.094	1.39	0.94-2.04	0.096	1.07	0.71-1.60	0.760

Multivariate OR is adjusted for age (continuous), marital status (married/ single/separated or divorced/ widowed), education level attained (primary/ secondary/ third level), employment status (employed/retired/ other), smoking status(never/ past/ current), number of GP visits in the past year (continuous), receipt of influenza vaccine (ever/never), number of chronic illness reported (continuous).

APPENDIX 5: Sensitivity Analysis excluding men previously diagnosed with prostate cancer

TABLE A5: Assessment of the association between PSA testing (yes/no) and covariates associated with PSA testing having excluded men with prior prostate cancer.

Variables associated with PSA testing		Univariate Analysis			Multivariate Adjusted		
		OR	95% CI	p-value	OR	95% CI	p-value
Age at interview	years	1.03	1.02-1.03	<0.001	1.02	1.00- 1.03	0.013
Marital Status	Married	1.00	Ref		1.00	Ref	
	Single	0.58	0.47-0.71	<0.001	0.69	0.55-0.86	0.001
	Sep/Div	0.54	0.40-0.72	<0.001	0.68	0.50-0.93	0.014
	Widowed	0.88	0.68-1.15	0.342	0.70	0.52-0.93	0.014
Education	Primary	1.00	Ref		1.00	Ref	
	Secondary	1.13	0.96-1.33	0.141	1.30	1.08-1.56	0.005
	Third Level	1.57	1.31-1.89	<0.001	1.49	1.21-1.83	<0.001
Employment	Employed	1.00	Ref		1.00	Ref	
	Retired	1.47	1.26-1.72	<0.001	1.23	0.99-1.53	0.056
	Other	0.53	0.42-0.64	<0.001	0.67	0.53-0.86	0.002
Smoking Status	Never	1.00	Ref		1.00	Ref	
	Past	1.06	0.90-1.24	0.501	0.97	0.82-1.15	0.746
	Current	0.45	0.37-0.55	<0.001	0.56	0.46-0.69	<0.001
Number of GP visits	(Continuous)	1.03	1.02-1.05	<0.001	1.03	1.01-1.05	0.001
Influenza Vaccine	Ever	1.72	1.48-1.97	<0.001	1.35	1.14-1.60	0.001
Chronic illnesses	(Continuous)	1.22	1.16-1.30	<0.001	1.11	1.04-1.18	0.001
Prior Cancer diagnosis		1.78	1.13-2.82	0.013	1.48	0.91-2.40	0.112
Treated BPH		3.67	2.31-5.82	<0.001	2.64	1.64-4.25	<0.001
GMS Scheme Eligible		0.84	0.73-0.96	0.014	0.64	0.52-0.78	<0.001
No of medicines		1.11	1.08-1.14	<0.001			
Private Health Insurance		2.34	2.03-2.71	<0.001			
Cholesterol test		16.33	12.4-21.6	<0.001			

Multivariate OR is adjusted for age (continuous), marital status (married/ single/separated or divorced/ widowed), education level attained (primary/ secondary/ third level), employment status (employed/retired/ other), smoking status(never/ past/ current), number of GP visits in the past year (continuous), receipt of influenza vaccine (ever/never), number of chronic illness reported (continuous), prior cancer diagnosis (excluding other than prostate cancer)

APPENDIX 4: LOW DOSE ASPIRIN AND SURVIVAL IN MEN WITH PROSTATE CANCER: A STUDY USING THE UK CLINICAL PRACTICE RESEARCH DATALINK.

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Abstract

Background: Aspirin use is associated with reduced risk of, and death from, prostate cancer. Our aim was to determine whether low dose aspirin use after a prostate cancer diagnosis was associated with reduced prostate cancer-specific mortality.

Methods: A cohort of newly diagnosed prostate cancer patients (1998-2006) were identified in the UK CPRD (confirmed by cancer registry linkage). A nested case-control analysis was conducted using conditional logistic regression to compare aspirin usage in cases (prostate cancer deaths) with up to three controls (matched by age and year of diagnosis).

Results: Post-diagnostic low dose aspirin use was identified in 52% of 1,184 prostate cancer-specific deaths and 39% of 3,531 matched controls (unadjusted OR=1.51 95%CI 1.19, 1.90; P<0.001). After adjustment for confounders including treatment and comorbidities this association was attenuated (adjusted OR=1.02 95%CI 0.78, 1.34). Adjustment for oestrogen therapy accounted for the majority of this attenuation. There was also no evidence of dose response association after adjustments. Compared with no use, patients with 1-11 prescriptions, and 12 or more prescriptions had adjusted ORs of 1.07 (95%CI 0.78, 1.47) and 0.97 (95%CI 0.69, 1.33) respectively. There was no evidence of a protective association between low dose aspirin use in the year prior to diagnosis and prostate cancer-specific mortality (adjusted OR=1.04 95%CI 0.89, 1.22; P=0.60).

Conclusions: We found no evidence of an association between low dose aspirin use before or after cancer diagnosis and risk of prostate cancer specific mortality, after potential confounders were accounted for, in UK prostate cancer patients.

Introduction

Aspirin is one of the oldest commercially available drugs, and was the second most commonly prescribed agent in England in 2010.(1) It is indicated for its analgesic, anti-inflammatory, anti-pyretic and anti-thrombotic properties.(2) Inhibition of cyclooxygenase-2 (COX-2) by aspirin and other NSAIDS has been investigated extensively as an anticancer mechanism (3-5) but the antiplatelet effect of low dose aspirin, which is mediated through irreversible inhibition of COX-1, may also be important in the progression of cancer. Reduction in circulating platelets (6) or interference with platelet adhesion may impede the spread of tumour cells (7-9) and the antiplatelet activity of aspirin may also reduce neo-vascularisation and the formation of metastases.(10)

Recent meta-analyses of randomised trials of aspirin for cardiovascular indications have shown reduced incidence of, and mortality from, solid cancers in aspirin users and marked reductions for prostate cancer, although these results were based upon small numbers and were not significant.(11, 12) Similarly, meta-analyses of observational studies have reported that aspirin used at anti-platelet doses is associated with reduced incidence of prostate cancer.(13) A number of these studies have also suggested that men exposed to aspirin (though not specifically low dose) present with less advanced prostate tumours at diagnosis.(14-16)

Only three observational studies have examined aspirin use after prostate cancer diagnosis and prostate cancer survival and these have reported conflicting findings.(17-19) Dhillon et al. observed no association between aspirin use and prostate cancer specific mortality in a cohort of prostate cancer patients within the Health Professionals Follow-up study.(17) Grytli et al (19) observed a modest reduction in the risk of prostate cancer specific death with low dose aspirin use in a high risk subgroup of men with prostate cancer, whilst Choe et al observed marked reductions in cancer specific death with aspirin use in patients with localised prostate cancer and consequently recommended the conduct of clinical trials of aspirin in prostate cancer patients.(18)

As the preclinical and early epidemiological evidence indicates that aspirin may reduce prostate cancer progression, further investigations of the association between low dose aspirin use following diagnosis and prostate cancer specific mortality are required. We examined this association in a large population-based cohort of prostate cancer patients diagnosed in the UK between 1998 and 2006.

Materials and Methods

Study design

A cohort study was conducted utilising linkages between the English National Cancer Data Repository (NCDR), the UK Clinical Practice Research Datalink (CPRD), and the Office of National Statistics (ONS) death registrations. The NCDR data includes date and site of primary cancer diagnosis, and clinical data such as stage and treatment. The CPRD is the world's largest database of longitudinal patient records comprising around 8% of the UK population and includes demographic information, clinical diagnoses, and prescription data which is of documented high quality.(20) Ethical approval for all observational research using CPRD data has been obtained from a multicentre research ethics committee. Linkages between the datasets were conducted using a deterministic algorithm based upon NHS number, gender, date of birth, and postcode. Prostate cancer cases were included in the cohort if they had a CPRD prostate cancer diagnosis code which was confirmed by a NCDR diagnosis for prostate cancer (based upon a relevant ICD code) from 1998 to 2006. Cases with previous NCDR cancer diagnosis, apart from in situ neoplasms and non-melanoma skin cancers, were excluded. Date and cause of death up to 2011 were taken from ONS.

Exposure data

Aspirin use was determined from GP prescribing data. Aspirin preparations of 75 mg or less were classified as low dose (1% of all aspirin prescriptions were for 25mg, 96% for 75mg, 0.1% for 100mg, and 3% for 300mg or higher doses). The number of days use was determined from the quantity of tablets prescribed. A quantity of 28 tablets, based upon the average, was assumed for less than 1% of prescriptions where quantity was missing or assumed incorrect.

Confounders

Data available from the NCDR included histological grade, Gleason score, surgery, chemotherapy and radiotherapy in the six months after diagnosis. Gleason score was converted to grade to increase completeness.(21) GP prescribing data were used to determine androgen deprivation therapy (BNF chapter 8.3.4.2, including gonadorelin analogues and anti-androgens) and oestrogen therapy (BNF chapter 8.3.1, including diethylstilbestrol and ethinylestradiol) in the exposure period. Smoking, alcohol, and body mass index (BMI) were determined from the closest GP record prior to prostate cancer diagnosis (records older than ten years were ignored). Comorbidities prior to diagnosis were determined from GP diagnosis

codes on the basis of the eight most common diagnoses contributing to a recent adaptation of the Charlson comorbidity index for GPRD.(22)

Data analysis

The prostate cancer cohort was initially analysed using a nested case–control approach which accounts for immortal time bias.(23, 24) Cases were members who had died due to prostate cancer (with a prostate cancer ICD code as the underlying cause of death) and these were matched on age (in five year intervals) and year of cancer diagnosis to three controls who lived at least as long after their cancer diagnosis. The exposure period in cases was the period from prostate cancer diagnosis until six months prior to cancer-specific death. The exposure period in the controls was of the same duration as their matched cases starting from the date of prostate cancer diagnosis. Prescriptions in the six month period prior to death were removed as these may reflect end of life treatment or increased exposure to healthcare professionals. Analyses were restricted to individuals with at least one year of follow-up.

Conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (95%CIs). Adjusted analyses were conducted including potential confounders. Analyses were repeated classifying deaths as prostate cancer-specific if prostate cancer was recorded as any cause of death and not just the underlying cause. Similar analyses were also conducted for all-cause mortality. Additional analysis were conducted investigating aspirin usage in the year and 3 years prior to prostate cancer diagnosis, restricted to individuals with at least 1 year and 3 years, respectively, of medication records prior to diagnosis, not excluding deaths in the year after diagnosis. Various sensitivity analyses were conducted including varying the duration of the exposure exclusion period prior to death/index date and investigating exposure in various time intervals prior to death/index date. Analyses were also conducted investigating and stratifying by pre-diagnostic use of aspirin. Stratified analyses were also conducted by use of androgen deprivation therapy in the first six months after cancer diagnosis, by Gleason score and time to death. All stratified analyses were conducted after re-matching cases to controls within the strata of interest. An additional sensitivity analysis was conducted investigating only aspirin use prior to oestrogen therapy, by excluding aspirin prescriptions after first oestrogen therapy in each case-control matched set, to avoid the need to adjust for oestrogen therapy which has the potential for over adjustment. An additional analysis was also conducted analysing the prostate cancer cohort, without conversion to case-control data, and applying survival analysis to investigate aspirin exposure as a time varying covariate.(23) In this analysis individuals were considered non-users prior to

use and users after a lag of 6 months after their first aspirin prescription, to mimic the case-control analysis. A similar dose exposure analysis was conducted with individuals considered non-users prior to 6 months after first use, a short term user between 6 months after first use and 6 months after their 12th prescription and a longer term user after this time. A separate analysis was also conducted using the time varying covariate approach with prostate cancer-specific death as the outcome adjusting for the competing risk of deaths from other causes, using competing-risks regression based on Fine and Gray's proportional subhazards model (not shown as results were identical).(25) Finally, a stop\start time varying covariate analysis was conducted, with patient follow-up post diagnosis split into periods of aspirin use and non-use based upon the date and number of tablets, with a 6 month lag, adjusting for year of diagnosis, age, grade and oestrogen usage (user versus non-user with a 6 month lag).

The final analysis contained 1,184 prostate cancer-specific deaths and 3,531 matched controls, with aspirin usage of 25%. This allows over 80% power to detect as significant at the 5% level an odds ratio of 0.80 in patients receiving low dose aspirin. Statistical analyses were conducted in STATA 11 (StataCorp, College Station, Texas).

Results

Patient cohort

Overall, there were 8,128 primary prostate cancer cases occurring between 1998 and 2006 identified in NCDR and linked to CPRD. Of these, 633 cases were excluded because the diagnosis date preceded CPRD research quality records, 107 due to unavailability of death registration data, 875 because they had less than one year of follow-up post diagnosis and a further 174 because androgen deprivation therapy records preceded the prostate cancer diagnosis date by more than 60 days (suggesting an incorrect diagnosis date). The final cohort contained 6,339 prostate cancer cases, with an average follow-up of 6 years (range 1 to 13 years), in whom there were 1,194 cancer-specific deaths. This cohort was converted to case-control data with 1,184 cancer-specific deaths and 3,531 available controls.

Patient characteristics

Table 1 shows characteristics of prostate cancer-specific deaths (cases) and controls. The average duration of the exposure period was 3.8 years and varied from one to 11.9 years. Cases were more likely to have higher grade, higher Gleason scores and to have received chemotherapy, androgen deprivation therapy and oestrogen, compared with controls. In

contrast, cases were less likely to have had radical prostatectomy (2% versus 8%). There was little difference in receipt of radiotherapy between the groups. A slightly larger proportion of cases (19%) were current smokers compared with the controls (14%). Rates of comorbidities, alcohol consumption, and BMI levels prior to diagnosis were generally similar between cases and controls (Table 1).

Association between aspirin use and prostate cancer specific mortality

The association between aspirin usage and cancer-specific death is shown in Table 2. Overall, a greater proportion of patients dying from cancer were low dose aspirin users compared with controls (52.1% versus 38.7%, respectively) corresponding to an OR of 1.78 (95%CI 1.55, 2.04; P<0.001). After adjustment for confounders including treatment and comorbidities this association was attenuated and there was no association between low dose aspirin usage and prostate cancer specific mortality (adjusted OR=1.02 95%CI 0.78, 1.34). Further analysis revealed that adjustment for oestrogen therapy accounted for the majority of this attenuation (after adjustment for only oestrogen therapy OR=1.23 95%CI 1.05, 1.44). In unadjusted analyses there was evidence of substantial increases in the risk of cancer specific mortality in patients with 1-11 low dose aspirin prescriptions (OR=2.02 95%CI 1.71, 2.38) and of a lesser magnitude in individuals with 12 or more prescriptions (OR=1.53 95%CI 1.28, 1.83). However, after adjustments there was no evidence of a difference in risk of cancer specific mortality in individuals with 1-11 and 12 or more prescriptions (adjusted OR=1.07 95%CI 0.78, 1.47 and adjusted OR=0.97 95%CI 0.69, 1.37, respectively). Additional analysis again revealed this attenuation was largely due to adjustment for oestrogen therapy, (after adjustment for only oestrogen therapy OR for 1-11 prescriptions=1.19 95%CI 0.98, 1.45 and OR for 12 or more prescriptions =1.26 95%CI 1.03, 1.55). Similar findings were observed when the number of tablets and tablets per day were investigated.

Association between pre-diagnostic aspirin use and prostate cancer-specific mortality

Overall, there was some evidence of more frequent low dose aspirin use in the year prior to diagnosis (restricting analysis to individuals with 1 year of records) in patients dying from cancer compared with controls (27.1% versus 24.6%, respectively) but this difference was small corresponding to an unadjusted OR of 1.16 (95%CI 1.55, 2.04) and was no longer apparent after adjustment for confounders (adjusted OR=1.04 95%CI 0.89, 1.22, see Table 3). Although there was some evidence of a dose response association between low dose aspirin usage prior to diagnosis and risk of prostate cancer-specific mortality this association

disappeared after adjustment for confounders (see Table 3). When low dose aspirin usage in the 3 years prior to cancer diagnosis was investigated the unadjusted association was even weaker (OR=1.11 95% CI 0.95, 1.29) and, as before, this association was further attenuated after adjustment for confounders (adjusted OR 1.04 95% CI 0.87, 1.23).

Sensitivity/stratified analyses

Sensitivity analyses for the association between aspirin usage and cancer-specific death are shown in Table 3. The unadjusted association between aspirin usage and cancer specific mortality was attenuated after prescriptions in the year prior to death were excluded (unadjusted OR=1.55 95%CI 1.34, 1.80) and further attenuated after prescriptions in the two years prior to death were excluded (unadjusted OR=1.25 95%CI 1.05, 1.50) indicating that these associations reflected aspirin prescribing in the period immediately preceding death. After adjustments there was no evidence of an association when prescriptions in the year prior to death or 2 years prior to death were excluded (adjusted OR=0.96 95%CI 0.72, 1.28 and adjusted OR=0.81 95%CI 0.57, 1.15, respectively). There was little evidence of an association between post-diagnostic aspirin usage and cancer specific mortality in individuals who had used low dose aspirin prior to diagnosis (adjusted OR=1.01 95%CI 0.67, 1.53) or in those who had not (OR=0.77 95%CI 0.22, 2.62). There was no association between post diagnostic low dose aspirin usage and death in users of androgen deprivation therapy in the first 6 months after diagnosis and findings were similar across categories of Gleason score and across categories of time to death (as shown in Table 3). Analyses investigating pre-oestrogen aspirin usage (OR= 0.96 95%CI 0.75, 1.23) gave identical results to the main finding. Classifying deaths as prostate cancer specific if prostate cancer was recorded as any cause of death and not just the underlying cause had little impact on the main finding (adjusted OR=1.17 95%CI 0.94, 1.46). Table 3 also shows the main time varying covariate analysis conducted in the entire cohort which produced similar estimates to the main case-control analysis (adjusted HR=1.13 95%CI 0.95, 1.35). Finally, a stop\start time varying covariate also produced similar estimates for current use after adjustment for year of diagnosis, age, grade and oestrogen usage (adjusted HR=1.10 95%CI 0.94, 1.29).

Association between aspirin use and all-cause mortality

The association between low dose aspirin usage and all-cause mortality is shown in Table 4. After adjustments low dose aspirin users had a slight increase in the risk of all-cause mortality (adjusted OR=1.18 95% 1.00, 1.40), which to an extent followed a dose response in patients

using 1 to 11 and 12 or more aspirin prescriptions (adjusted OR=1.14 95%CI 0.93, 1.40 and adjusted OR=1.22 95%CI 1.00, 1.50, respectively). Similar results were observed for analyses of tablets and tablets per day.

Discussion

This study did not provide evidence of a reduction in the risk of prostate cancer specific mortality (or all-cause mortality) in UK prostate cancer patients receiving low dose aspirin after (or before) their prostate cancer diagnosis. Low dose aspirin use at any time after prostate cancer diagnosis was associated with a significantly increased risk of prostate cancer-specific mortality but the complete attenuation of this association after adjustment for potential confounders, especially use of oestrogen, suggests that this is not a causal relationship. In the UK, oestrogen therapy remains an important treatment option in castrate resistant prostate cancer (notably, one third of prostate cancer deaths in this study received oestrogen therapy) and low dose aspirin is frequently concomitantly prescribed to reduce the risk of thromboembolic side effects. (26)

Our findings support the study by Dhillon et al. which observed no association between any aspirin use, following cancer diagnosis and development of metastases or prostate cancer specific mortality within the Health Professionals Follow-up study after excluding aspirin use in the 2 years prior to death.(17) In contrast to our findings and those of Dhillon et al., Choe et al. observed a marked reduction in the risk of prostate cancer death in men exposed to aspirin at or following prostate cancer diagnosis (HR=0.28, 95% CI 0.19, 0.41).(18) Our study population was very different from these studies, both of which investigated patients diagnosed in the USA, where widespread PSA testing results in the diagnosis of very early stage prostate cancer. None of the patients included in the study by Choe et al had node positive or metastatic disease, 72% had T1 disease and virtually all patients had intracapsular disease. Similarly, none of the patients included in the Health Professionals Study had metastatic disease at presentation, 60% had stage T1 disease and more than 95% intracapsular disease. Stage at presentation was not available within our study but based on data from regional UK cancer registries it is likely that less than 1% of patients had T1 disease, between 50% and 70% had intracapsular disease and 25% to 50% had disease extending beyond the prostate, node positive or metastatic disease at presentation.(27, 28) Because of the lack of data on stage at presentation we were unable to restrict our analysis to a prostate cancer population with a stage distribution similar to that of Choe et al or Dhillon et al. We could not therefore rule out a protective effect in patients with early stage disease, as seen by

Choe et al. but not by Dhillon et al. It is possible that any benefit of inhibition of platelet aggregation by aspirin in prostate cancer patients may be restricted to patients with very early stage disease and without micro-metastases disseminated at the time of diagnosis.(29)

The findings of our study contrasts with that of Grytli et al (19) who observed a reduction in the risk of prostate cancer specific death in users of low dose aspirin (HR=0.81 95%CI 0.71, 0.93) in a subgroup of Norwegian prostate cancer patients at high risk of prostate cancer specific mortality. However, this study had significant methodological weaknesses.(30) Most importantly, an aspirin user was defined as someone using aspirin prior to diagnosis and "if they repeated prescription filling after diagnosis" and consequently their estimate will have incurred immortal time bias because prostate cancer patients who live longer will be more likely to get repeat prescriptions. Our analysis was conducted using techniques (such as the nested case-control analysis and the use of time varying covariates in survival models) recommended to avoid immortal time bias.(23)

The main strength of our study is that it contains the largest number of prostate cancer deaths in which low dose aspirin use post cancer diagnosis and survival have been investigated. In addition, prostate cancer diagnosis was verified from linkage to cancer registry data. Detailed aspirin prescription data were available, including the timing of prescriptions allowing us to investigate aspirin use after cancer diagnosis as this is the most relevant time point for clinical intervention (and clinical trials).

Some limitations must also be acknowledged. Adherence to aspirin cannot be determined as aspirin exposure is based on prescriptions issued. Furthermore aspirin is available over-the-counter, therefore some misclassification may have occurred. One previous CPRD study estimated that 70% to 80% (31) of aspirin use in the age-group we investigated was prescription based, whilst another showed little evidence of misclassification by aspirin usage when compared with patient recall. Also, methodological studies suggest that prescription data can give valid estimates of association even though drugs are available over the counter.(33) As with all observational studies is not possible to rule out the effect of confounding, or confounding by indication, which could have obscured a protective effect of aspirin however we were able to adjust for important confounders including Gleason score, treatment and comorbidities. Also reliable data was not gathered on disease progression or recurrence; therefore the findings of a recent study which demonstrated anticoagulant use including aspirin was associated with freedom from biochemical failure in men treated with

radiation could not be investigated.(34) However, it seems unlikely that aspirin could reduce the risk of recurrence or progression but not the risk of prostate cancer mortality.

Confounding by indication is a well-recognized limitation in pharmacoepidemiology. As discussed previously, it seems likely that the unadjusted increased risk of prostate cancer-specific mortality with post-diagnostic low dose aspirin use reflects confounding by indication as low dose aspirin will have been taken because oestrogen therapy has been used to treat advanced stage disease. Competing mortality could also influence our results as aspirin users may have increased risk of death from cardiovascular disease (due to confounding by indication) and hence higher competing mortality which could artificially reduce the risk of prostate cancer-specific mortality in aspirin users. Alternatively, aspirin users may have lower cardiovascular mortality (due to the medication), and reduced competing mortality, which could artificially increase the risk of prostate cancer-specific mortality in aspirin users. The former of these biases is of less concern as we did not observe protective effects of aspirin on prostate cancer-specific mortality. Moreover it is of some reassurance that when restricting the analysis to user of aspirin prior to cancer diagnosis (who are likely to have similar indications/experience similar reductions in cardiovascular mortality) no protective associations were observed. The principal limitation of our study was the lack of data on stage at presentation, which precluded an analysis in the subgroup of patients with early stage disease. Nevertheless, it is worth noting that previous studies have observed protective associations for aspirin in high risk prostate cancer patients [19] and in our study no protective associations were observed for low dose aspirin use prior to prostate cancer diagnosis (when disease is likely to be less advanced) or when the analysis was restricted to prostate cancer patients surviving for over 5 years (who are likely to have less advanced disease at onset).

In conclusion, there was no evidence that use of low dose aspirin after cancer diagnosis affected the risk of prostate cancer specific mortality in this study of UK prostate cancer patients. We could not, however, rule out a protective effect for post-diagnostic low dose aspirin use in prostate cancer patients diagnosed with early stage disease.

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Table 1. Characteristics of prostate cancer patients who die from prostate cancer (cases) compared with controls.

	Prostate cancer-specific deaths n (%)	Controls n (%)	*P*-value
	[n=1,184]	*[n=3,531]*	
Year of cancer diagnosis			
1998-2000	396 (33.5%)	1,178 (33.4%)	Matched
2001-2003	461 (38.9%)	1,374 (38.9%)	
2003-2006	327 (27.6%)	979 (27.7%)	
Age at cancer diagnosis			
< 50	6 (0.5%)	15 (0.4%)	Matched
50-59	71 (6.0%)	213 (6.0%)	
60-69	280 (23.7%)	840 (23.8%)	
70-79	532 (44.9%)	1,596 (45.2%)	
80-89	271 (22.9%)	808 (22.9%)	
≥ 90	24 (2.0%)	59 (1.7%)	
Post cancer diagnosis follow-up (years):			Matched
mean (sd)	3.8 (2.2)	3.8 (2.2)	
range	1-11.9	1-11.9	
Gleason score[a]			
2-6	112 (21.6)	943 (51.0)	<0.001
7	141 (27.2)	508 (27.5)	
8-10	265 (51.2)	398 (21.5)	
Missing	*313*	*715*	
Grade			
Well differentiated	34 (4.4%)	319 (12·3%)	<0·001
Moderately differentiated	217 (28.3%)	1,278 (49.3%)	
Poorly differentiated	516 (67.3%)	993 (38.3%)	
Missing	*417*	*994*	
Treatment within 6 months of cancer diagnosis			
Chemotherapy	49 (4.1%)	72 (2.0%)	<0.001
Radiotherapy	246 (20.8%)	743 (21.0%)	0.88
Androgen deprivation therapy	976 (82.4%)	2,092 (59.3%)	<0.001
Oestrogen therapy	352 (32.8%)	76 (2.4%)	<0.001
Radical prostatectomy[b]	20 (2.4%)	192 (7.6%)	<0.001
Smoking prior to cancer diagnosis			
Non-smoker	453 (46.8%)	1,530 (52.0%)	0.001
Ex-smoker	331 (34.2%)	1,000 (34.0%)	
Current smoker	185 (19.1%)	415 (14.1%)	

Missing	*215*	*586*	
Alcohol prior to cancer diagnosis			
Never consumed alcohol	95 (10.7%)	290 (10.7%)	0.80
Alcohol consumer	791 (89.3%)	2,415 (89.3%)	
Missing	*198*	*826*	
BMI (kg/m^2) prior to cancer diagnosis:	882	2736	
Mean (sd)	26.4 (4.0)	26.1 (3.8)	0.02
Comorbidity (prior to cancer diagnosis or during follow-up time)			
Cerebrovascular disease	124 (10.5%)	327 (9.3%)	0.24
Chronic pulmonary disease	229 (19.3%)	681 (19.3%)	0.95
Congestive heart disease	108 (9.1%)	231 (6.5%)	0.003
Diabetes	141 (11.9%)	393 (11.1%)	0.45
Myocardial infarction	130 (11.0%)	356 (10.1%)	0.39
Peptic ulcer disease	77 (6.5%)	240 (6.8%)	0.66
Peripheral vascular disease	113 (9.5%)	242 (6.9%)	0.003
Rheumatological disease	37 (3.1%)	166 (4.7%)	0.02

[a]Restricted to patients from the Thames Cancer Registry, South West Cancer Intelligence Service,
West Midlands Cancer Intelligence Unit and North West Cancer Intelligence Service.
[b]Excludes patients from the Thames Cancer Registry and Trent Cancer Registry as data not available

Table 2. Post-diagnostic exposure to aspirin and odds of prostate cancer specific death in prostate cancer patients.

Post-diagnostic aspirin usage	Prostate cancer specific deaths n (%)	Controls n (%)	Unadjusted OR (95%CI)	P	Adjusted [a] OR (95%CI)	P	Additionally adjusting for grade & radical prostatectomy [b] (95% CI)	p
No. prescriptions low dose								
0	567 (47.9)	2,166 (61.3)	1.00		1.00		1.00	
1 or more	617 (52.1)	1,365 (38.7)	1.78 (1.55, 2.04)	<0.001	1.19 (0.99, 1.43)	0.06	1.02 (0.78, 1.34)	0.86
No. prescriptions low dose								
0	567 (47.9)	2,166 (61.3)	1.00		1.00		1.00	
1-11	335 (28.3)	642 (18.2)	2.02 (1.71, 2.38)	<0.001	1.16 (0.94, 1.44)	0.17	1.07 (0.78, 1.47)	0.66
12 or more	282 (23.8)	723 (20.5)	1.53 (1.28, 1.83)	<0.001	1.23 (0.97, 1.56)	0.08	0.97 (0.69, 1.37)	0.88
No. tablets low dose[c]								
0	567 (47.9)	2,166 (61.3)	1.00		1.00		1.00	
1-365	252 (21.3)	444 (12.6)	2.19 (1.83, 2.64)	<0.001	1.16 (0.91, 1.47)	0.22	1.23 (0.87, 1.75)	0.25
366 or more	365 (30.8)	921 (26.1)	1.55 (1.32, 1.82)	<0.001	1.21 (0.98, 1.50)	0.08	0.90 (0.66, 1.23)	0.52
No. tablets per day[d]								
0	567 (47.9)	2,166 (61.3)	1.00		1.00		1.00	
0-0.5	269 (22.7)	443 (12.6)	2.42 (2.01, 2.90)	<0.001	1.08 (0.84, 1.40)	0.55	1.12 (0.79, 1.60)	0.53
0.5-1	228 (19.3)	586 (16.6)	1.55 (1.28, 1.86)	<0.001	1.25 (0.99, 1.58)	0.07	0.82 (0.58, 1.17)	0.28
>1	120 (10.1)	336 (9.5)	1.38 (1.10, 1.73)	0.006	1.26 (0.95, 1.66)	0.10	1.31 (0.85, 2.01)	0.22
Low dose aspirin category[e]								
Never	509 (46.9)	1,952 (60.3)	1.00		1.00		1.00	
Past (prescriptions pre-diagnosis)	12 (1.1)	54 (1.7)	0.88 (0.47, 1.67)	0.70	0.96 (0.49, 1.88)	0.90	1.43 (0.50, 4.12)	0.50
Current (prescriptions after diagnosis)	565 (52.0)	1,232 (38.1)	1.82 (1.58, 2.11)	<0.001	1.16 (0.96, 1.41)	0.12	1.18 (0.88, 1.57)	0.27

[a] Model includes chemotherapy within 6 months of diagnosis, radiotherapy within 6 months, androgen deprivation therapy during exposure period, oestrogen therapy during exposure period, comorbidities (pre-diagnosis or during exposure, including myocardial infarction, cerebrovascular disease

congestive heart disease, chronic pulmonary disease, peripheral vascular disease, peptic ulcer disease and diabetes), and smoking (pre-diagnosis, with missing included as a category). [b] Restricted to 577 prostate cancer specific deaths and 1,715 controls with available data. . [c] Total number of tablets taken in exposure period. [d] Total number of tablets taken in exposure period divided by duration of exposure period in days. [e] Restricted to individuals with 1 year of records prior to diagnosis, never includes individuals not using in the year prior to diagnosis or after diagnosis, past includes individuals using in the year prior to diagnosis but not after and current includes individuals using after diagnosis.

Table 3. Sensitivity analysis for association between low dose aspirin usage and prostate cancer-specific death in prostate cancer patients.

Comparison [a]	Prostate cancer-specific deaths	Controls	OR (95% CI) aspirin users vs. non-users	P-value	OR (95%CI) 1 to 11 aspirin prescriptions vs. none	P-value	OR (95%CI) 12 or more aspirin prescriptions vs. none	P-value
Main analysis								
Diagnosis to 6 months prior to death: Unadjusted	1184	3531	1.78 (1.55, 2.04)	<0.001	2.02 (1.71, 2.38)	<0.001	1.53 (1.28, 1.83)	<0.001
Diagnosis to 6 months prior to death	577	1715	1.02 (0.78, 1.34)	0.86	1.07 (0.78, 1.47)	0.66	0.97 (0.69, 1.37)	0.88
Diagnosis to 1 year prior to death [b]: Unadjusted	1021	3043	1.55 (1.34, 1.80)	<0.001	1.70 (1.42, 2.03)	<0.001	1.39 (1.15, 1.69)	0.001
Diagnosis to 1 year prior to death [b]	509	1,513	0.96 (0.72, 1.28)	0.77	0.96 (0.67, 1.36)	0.81	0.95 (0.66, 1.38)	0.81
Diagnosis to 2 years prior to death [c]: Unadjusted	738	2197	1.25 (1.05, 1.50)	0.01	1.33 (1.06, 1.65)	0.01	1.18 (0.93, 1.49)	0.17
Diagnosis to 2 years prior to death [c]	371	1,103	0.81 (0.57, 1.15)	0.25	0.76 (0.50, 1.15)	0.19	0.90 (0.56, 1.43)	0.65
No pre-diagnostic low dose aspirin use [d]	389	1161	1.01 (0.67, 1.53)	0.97	1.22 (0.76, 1.95)	0.41	0.71 (0.39, 1.30)	0.27
Pre-diagnostic low dose aspirin user [d]	140	400	0.77 (0.22, 2.62)	0.67	0.68 (0.20, 2.35)	0.54	1.04 (0.28, 3.85)	0.96
Pre-diagnostic low dose aspirin use [e]: Unadjusted	1371	4088	1.16 (1.00, 1.33)	0.05	1.14 (0.98, 1.32)	0.10	1.23 (0.93, 1.63)	0.14
Low dose aspirin use prior to oestrogen therapy [f]	577	1715	0.96 (0.75, 1.23)	0.76	0.88 (0.65, 1.19)	0.41	1.06 (0.78, 1.44)	0.72
Users of androgen deprivation therapy [g]	419	1241	0.97 (0.72, 1.32)	0.86	1.03 (0.71, 1.48)	0.87	0.90 (0.61, 1.34)	0.61
Gleason score 1 to 6 [h]	109	315	1.12 (0.60, 2.08)	0.72	1.22 (0.58, 2.56)	0.61	1.02 (0.46, 2.25)	0.97
Gleason score 7 [h]	135	389	0.97 (0.56, 1.68)	0.93	0.79 (0.39, 1.58)	0.50	1.17 (0.61, 2.25)	0.63
Gleason score 8 to 10 [h]	248	703	1.32 (0.89, 1.98)	0.17	1.30 (0.82, 2.04)	0.26	1.37 (0.81, 2.32)	0.24

In patients who died 1 to 2.49 years after diagnosis	206	612	1.42 (0.90, 2.25)	0.14	1.30 (0.80, 2.14)	0.29	1.93 (0.91, 4.12)	0.09
In patients who died 2.5 to 4.99 years after diagnosis	203	605	0.86 (0.54, 1.36)	0.51	0.59 (0.31, 1.11)	0.10	1.10 (0.65, 1.87)	0.72
In patients who died > 5 years after diagnosis	168	498	0.87 (0.51, 1.48)	0.60	1.60 (0.80, 3.22)	0.19	0.58 (0.31, 1.10)	0.09
Time varying covariate analysis [l]: Unadjusted	1194	5145	1.78 (1.58, 2.00)	<0.001	1.98 (1.73, 2.27)	<0.001	1.53 (1.32, 1.78)	<0.001
Time varying covariate analysis [l]	582	2852	1.13 (0.95, 1.35)	0.16	1.15 (0.93, 1.41)	0.19	1.12 (0.90, 1.38)	0.31

[a] All sensitivity analyses refer to low dose aspirin usage in the time period from prostate cancer diagnosis to 6 months before death, and are adjusted for matching criteria (age and year of cancer diagnosis), grade, radical prostatectomy, chemotherapy within 6 months of diagnosis, radiotherapy within 6 months, androgen deprivation therapy during exposure period, oestrogen therapy during exposure period, comorbidities (pre-diagnosis or during exposure, including myocardial infarction, cerebrovascular disease, congestive heart disease, chronic pulmonary disease, peripheral vascular disease, peptic ulcer disease and diabetes), and smoking (pre-diagnosis, with missing included as a category).

[b] Restricted to individuals with over 1.5 years of follow-up.

[c] Restricted to individuals with over 2.5 years of follow-up.

[d] Pre-diagnostic low dose aspirin use in 1 year prior to prostate cancer diagnosis, restricted to individuals with at least 1 year of medication records prior to diagnosis.

[e] Pre-diagnostic low dose aspirin use in 1 year prior to prostate cancer diagnosis, restricted to individuals with at least 1 year of medication records prior to diagnosis, not excluding deaths in the year after diagnosis.

[f] Adjusted for matching criteria (age and year of cancer diagnosis), grade, radical prostatectomy, chemotherapy within 6 months of diagnosis, radiotherapy within 6 months, androgen deprivation therapy during exposure period, comorbidities (pre-diagnosis or during exposure, including myocardial infarction, cerebrovascular disease, congestive heart disease, chronic pulmonary disease, peripheral vascular disease, peptic ulcer disease and diabetes), and smoking (pre-diagnosis, with missing included as a category) but not oestrogen therapy.

[g] Includes users of either gonadorelin analogue therapy or anti-androgen therapy first received between cancer diagnosis and 6 months after diagnosis.

[h] Adjusted for matching criteria (age and year of cancer diagnosis), chemotherapy within 6 months of diagnosis, radiotherapy within 6 months, androgen deprivation therapy during exposure period, oestrogen therapy during exposure period, comorbidities (pre-diagnosis or during exposure, including myocardial infarction, cerebrovascular disease, congestive heart disease, chronic pulmonary disease, peripheral vascular disease, peptic ulcer disease and diabetes), and smoking (pre-diagnosis, with missing included as a category).

[l] Reported estimates are hazard ratios and 95% CIs, adjusted for age and year of prostate cancer diagnosis, grade, radical prostatectomy and oestrogen therapy (as a time varying covariate).

Table 4. Post-diagnostic exposure to aspirin and odds of all-cause mortality in prostate cancer patients.

Post-diagnostic aspirin usage	All-cause deaths n (%)	Controls n (%)	Unadjusted OR (95%CI)	P	Adjusted [a] OR (95%CI)	P	Additionally adjusting for grade & radical prostatectomy [b] (95% CI)	p
No. prescriptions low dose								
0	1,033 (47.0)	3,904 (59.6)	1.00		1.00		1.00	
1 or more	1,164 (53.0)	2,646 (40.4)	1.69 (1.50, 1.92)	<0.001	1.20 (1.06, 1.35)	0.003	1.18 (1.00, 1.40)	0.05
No. prescriptions low dose								
0	1,033 (47.0)	3,904 (59.6)	1.00		1.00		1.00	
1-11	546 (24.9)	1,170 (17.9)	1.77 (1.56, 2.00)	<0.001	1.18 (1.02, 1.36)	0.02	1.14 (0.93, 1.40)	0.20
12 or more	618 (28.1)	1,576 (22.5)	1.63 (1.44, 1.84)	<0.001	1.21 (1.05, 1.40)	0.01	1.22 (1.00, 1.50)	0.05
No. tablets low dose[c]								
0	1,033 (47.0)	3,904 (59.6)	1.00		1.00		1.00	
1-365	402 (18.3)	799 (12.2)	1.92 (1.67, 2.21)	<0.001	1.23 (1.04, 1.44)	0.01	1.17 (0.93, 1.48)	0.17
366 or more	762 (34.7)	1,847 (28.2)	1.59 (1.42, 1.78)	<0.001	1.18 (1.03, 1.35)	0.01	1.18 (0.98, 1.43)	0.08
No. tablets per day[d]								
0	1,033 (47.0)	3,904 (59.6)	1.00		1.00		1.00	
0 to 0.5	479 (21.8)	871 (13.3)	2.15 (1.88, 2.47)	<0.001	1.31 (1.12, 1.53)	0.001	1.19 (0.96, 1.49)	0.11
>0.5	457 (20.8)	1,209 (18.5)	1.46 (1.28, 1.66)	<0.001	1.11 (1.00, 1.30)	0.18	1.09 (0.88, 1.41)	0.45
>1	228 (10.4)	566 (8.6)	1.54 (1.30, 1.82)	<0.001	1.21 (1.00, 1.46)	0.05	1.37 (1.05, 1.80)	0.02
Low dose aspirin category[e]								
Never	918 (45.5)	3,464 (57.6)	1.00		1.00		1.00	
Past (prescriptions pre-diagnosis)	33 (1.6)	63 (1.0)	1.98 (1.28, 3.05)	0.002	1.53 (0.97, 2.40)	0.07	1.47 (0.82, 2.61)	0.19
Current (prescriptions after diagnosis)	1,068 (52.9)	2,491 (41.4)	1.66 (1.50, 1.85)	<0.001	1.19 (1.05, 1.34)	0.01	1.22 (1.02, 1.45)	0.03

[a] Model includes chemotherapy within 6 months of diagnosis, radiotherapy within 6 months, androgen deprivation therapy during exposure period, oestrogen therapy during exposure period, NSAID use (post-diagnosis), comorbidities (pre-diagnosis or during exposure period, including myocardial

infarction, cerebrovascular disease, congestive heart disease, chronic pulmonary disease, peripheral vascular disease, peptic ulcer disease and diabetes), and smoking (pre-diagnosis, with missing included as a category). [b] Restricted to 1,153 cases and 3,420 controls with available data. [c] Total number of tablets taken in exposure period. [d] Total number of tablets taken in exposure period divided by duration of exposure period in days. [e] Restricted to individuals with 1 year of records prior to diagnosis, never includes individuals not using in the year prior to diagnosis or after diagnosis, past includes individuals using in the year prior to diagnosis but not after and current includes individuals using after diagnosis.

APPENDIX 5: ASPIRIN USE, LYMPH NODE METASTASIS AND MORTALITY IN WOMEN WITH STAGE I-III BREAST CANCER: A PROSPECTIVE COHORT STUDY

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Under Review

ABSTRACT

Background: In a recent meta-analysis of aspirin trials in cardiovascular disease, use of aspirin (COX-1/-2 inhibitor) was associated with a lower risk of distant metastasis in patients who developed cancer. In clinical studies, breast tumors that have spread to lymph-nodes are more likely to express COX-2 and in preclinical studies COX-2 inhibition prevents lymphatic metastasis. In this study, associations between aspirin use, the presence of lymphatic metastasis at breast cancer diagnosis, and mortality were examined. **Methods**: Women with stage I-III breast cancers diagnosed from 2001-2006 (N=2,796) were identified from Ireland's linked National Cancer Registry and prescription-refill database. Information on mammographic-screening was available from linked screening data. Relative risks (RR) were estimated for associations between pre-diagnostic aspirin use and lymph node-positive status. Hazard ratios (HR) were estimated for associations between aspirin use and mortality, stratified by lymph-node status. **Results**: Aspirin use was protective against node-positive disease. Women with pre-diagnostic aspirin use were significantly less likely to present with a lymph node-positive tumor than non-users (RR=0.89, 95%CI 0.81-0.97). The magnitude of association increased with aspirin dose (*P-trend*<0.01) and dosing-intensity (*P-trend*<0.001). Associations were consistent in women with and without screen-detected tumors (*P-interaction*=0.953). Aspirin use was associated with lower breast cancer-specific mortality only among women with lymph node-negative tumors (HR=0.53 95%CI 0.30-0.94; *P-interaction*=0.038). Overall associations between aspirin and mortality were non-significant. **Conclusion**: Consistent with preclinical studies, aspirin use prior to breast cancer diagnosis was protective against lymphatic metastasis. Furthermore, associations between pre-diagnosis aspirin use and breast cancer-specific mortality were significantly modified by lymph-node status.

Background

In a recent meta-analysis of randomized trials of aspirin in cardiovascular disease, the use of aspirin, a COX-1/-2 inhibitor, was associated with a 25% reduction in the risk of distant metastasis at initial presentation in patients with any cancer diagnosis.(1) In the same meta-analysis pre-diagnostic aspirin use was also associated with lower cancer-specific mortality, primarily among individuals with localized disease at diagnosis.(1) A similar but non-significant association was observed for specific cancer sites, including the breast, however the sample size was extremely limited. In other observational studies among postmenopausal women, aspirin use has been associated with significant reductions in breast cancer recurrence and mortality.(2,3)

In clinical studies, women with COX-2 expressing breast tumors were significantly more likely to present with lymph node metastases at diagnosis.(4,5) Preclinical data suggests that the cyclooxygenase/prostaglandin pathway is involved in the development of lymph node metastases through the regulation of vascular endothelial growth factor-C/-D (VEGF-C/-D) mediated lymphangiogenesis.(6,7) Inhibition of COX-2 has also been shown to suppress the development of lymphatic metastasis in breast cancer animal models.(6,7)

In this study we aimed to investigate, in women with breast cancer, associations between aspirin use prior to breast cancer diagnosis and the presence of lymph node metastasis at diagnosis; and also, whether lymph node status at diagnosis modifies associations between pre-diagnostic aspirin use and breast cancer mortality.

Methods

Setting & Data Sources

We conducted this study using linked patient records from the National Cancer Registry Ireland (NCRI) and prescription dispensing data from Ireland's General Medical Services (GMS) pharmacy claims database.(8) The NCRI records detailed information on all incident cancers diagnosed in the population usually resident in Ireland. Information is collected by trained, hospital-based, tumor registration officers from multiple sources; including pathology and radiology reports, medical records and death certificates. The use for research of anonymised data held by the NCRI is covered by the Health (Provision of Information) Act 1997.

Eligibility for the GMS prescription scheme is through means test or age (>70 years). The GMS database records details of all prescription drugs dispensed to GMS eligible patients since

2000. This includes all low-dose and most high-dose aspirin preparations, which are prescription-only in Ireland. Similar prescription-only regulations for aspirin exist in other European countries.(9) A small number of high-dose aspirin preparations are available over the counter, but only for specified short-term indications, in small pack sizes (≤24-50 doses) and at increased cost. Women with GMS eligibility can obtain high dose aspirin preparations on-prescription without charge or restriction.

We used two independent sources of information to identify women with breast tumors detected by organized or opportunistic(10) screening-mammography. Firstly, individual screening histories from Ireland's population-based organized screening-mammography program, BreastCheck,(11) were linked to NCRI patient records, allowing the accurate identification of all organized screen-detected breast cancers.(12) Secondly, the NCRI provided information, collected by tumor registration officers, identifying breast tumors detected by any screening mammography. There was close to 100% agreement for organized screen-detected tumors between linked BreastCheck records and data collected by the NCRI.(12) This enabled us to identify women with tumors detected by opportunistic screening-mammography (i.e. screening-mammography use outside of BreastCheck).

Cohort & Exposure Definitions

The study cohort included all women with a diagnosis of stage I-III invasive breast cancer (ICD-10 C50)(13) between 1st January 2001 and 31st December 2006, aged 50 to 80 years at diagnosis and with GMS eligibility from at least one year prior to diagnosis. Women were excluded if they had a prior invasive cancer other than non-melanoma skin cancer, or if their diagnosis was made at the time of death (Figure 1).

All prescriptions for aspirin, dispensed to women in the study cohort, were identified from the GMS database using WHO-ATC drug classifications(14) (Appendix-1). The dose and number of days' supply on each prescription were abstracted. We defined pre-diagnostic aspirin use as having at least one prescription for aspirin in the year prior to diagnosis. Aspirin dosing intensity, the proportion of days with a supply of aspirin available in the year prior to diagnosis, was also calculated.(15) Post-diagnostic aspirin use was defined as having at least one prescription for aspirin between diagnosis and the end of follow-up.

Outcomes & Covariates

We used information from the NCRI database to identify lymph node status at diagnosis (positive, negative). Women were identified as lymph node-positive if they had a pathologic

nodal status of pN1/2/3 or, if not available, a clinical nodal status of N1/2/3.(13) Death certificates were used to identify the date and cause of death (Appendix-1) for survival analyses. The NCRI database was also used to classify women by tumor size (T1, T2, T3, T4);(13) tumor stage (I, IIa, IIb, IIIa, IIIb-c);(13) tumor grade (low, intermediate, high, unspecified); tumor morphology (ductal, lobular, other; Appendix-1); tumor topography (outer, inner/central, unspecified; Appendix-1); ER, PR, HER2 status (positive, negative, unspecified; Appendix-1); age (years) and smoking status (never, past, current, unspecified). As described above, women were also classified by whether their tumor was screen-detected (organized screening, opportunistic screening, not screen-detected). We used prescription data to identify other medication use in the year prior to diagnosis (Appendix-1). Since diabetes has been associated with lymphatic metastasis,(16) the use of any anti-diabetic medication was taken to indicate a diagnosis of diabetes. The number of medication classes received in the year prior to diagnosis was used to generate a comorbidity score.(17)

Statistical Analyses

The distribution of clinical and socio-demographic covariates was compared between aspirin users and non-users. Univariate and multivariate log-binomial models(18,19) were used to estimate relative risks (RR) with 95% confidence intervals (CI) for associations between aspirin use and lymph node-positive breast cancer at diagnosis.(20,21) Covariates were identified for inclusion in the multivariate model based on prior knowledge of clinical, demographic and behavioral predictors of nodal status (tumor size; grade; morphology; topography; ER, PR, HER2 status; age; smoking status; screen- detection);(22–26) drugs associated with tumor invasiveness (beta-blockers, biguanides, bisphosphonates, statins, estrogen, estrogen/progesterone, NSAIDS);(27–32) comorbidities associated with lymphatic metastasis (diabetes);(16) and patient characteristics associated with extent of nodal evaluation (age, comorbidity score).(33) We selected the final multivariate model from these covariates using backwards elimination up to a 10% maximum cumulative change in the effect component of the fully adjusted RR.(34) Covariates consistently associated with nodal status in prior studies were fixed in the model (tumor size, grade, age, screen-detection).

Analyses were conducted by quartiles of aspirin dosing intensity; by low-dose (<150mg) and high-dose (at least one prescription ≥150mg) aspirin use; and by duration of pre-diagnostic aspirin use (0-1.5, 1.5-3, ≥3 years).(35) Effect modification of associations between aspirin use and nodal status was also assessed on an additive scale (risk difference, RD; interaction contrast, IC) with 95%CI (Wald test).(36) Breast tumor characteristics known to be associated

with COX-2 expression,(4,37,38) and therefore possibly more likely to respond to aspirin, were identified a priori and considered as potential effect modifiers. These were large tumor size, high grade, negative ER or PR status, positive HER2 status and morphology.

Multivariate Cox proportional hazards models were used to estimate hazard ratios (HR) with 95%CI for associations between aspirin use prior to diagnosis and (i) all-cause mortality, (ii) breast cancer-specific mortality. All women were followed from diagnosis to the first of either death or the 31st December 2007. Covariates were selected for inclusion in the multivariate model based on prior knowledge of clinical and demographic characteristics associated with breast cancer survival: age, comorbidity score, tumor stage (including nodal status), grade, ER, PR and HER2 status. Effect modification by nodal status at diagnosis was assessed on a multiplicative scale (ratio of hazard ratios, rHR) with 95%CI (Wald test). Analyses were repeated with adjustment for post-diagnostic aspirin use (unexposed, exposed; time varying; lagged 2 years). Cumulative mortality was also estimated from directly adjusted survival curves.(39) Analyses were conducted using SAS® v9.2 (SAS® Institute Inc, Cary, NC). Results were considered statistically significant at a two-sided α-level of 0.05.

Sensitivity Analyses

In addition to adjusting for screen-detection in analyses, the following sensitivity analyses were conducted to rule out early detection bias due to differential screening or intensity of medical surveillance among aspirin users as an explanation of our results: (i) associations between aspirin use and lymph node status were assessed in analyses stratified by screen-detection; (ii) a propensity-score matched analysis was conducted incorporating screening practices and comorbidities for aspirin users and non-users.

We also conducted sensitivity analyses to rule out bias due to the potential misclassification of nodal status based on clinical evaluation alone. In addition, to minimize the effect of any differential bias due to unrecorded nodal status (N=165) we took a conservative approach in the main analysis and classified all women with unrecorded lymph node status as lymph node positive (aspirin user 4.9%; aspirin non-user 8.6%). Sensitivity analyses using complete cases were also undertaken.

To assess the presence of bias due to possible misclassification of breast cancer-specific cause of death, we repeated survival analyses with the inclusion of: (i) deaths where breast cancer was listed as a secondary cause of death on the death certificate; (ii) deaths from ill-defined or secondary cancers, cancers of unknown behavior and unspecified causes. Post-diagnostic

aspirin use was lagged in survival analyses to allow an induction period for aspirin effect on mortality and to reduce the possibility that worsening prognosis influenced prescribing patterns. This lag time was varied from one to three years in sensitivity analyses.

Results

Cohort Characteristics

The characteristics of aspirin users (n=740) and non-users (n=2,056), stratified by dosing intensity, are presented in Table 1. Aspirin users were older and had a higher comorbidity score than non-users. However, the proportion of organized and opportunistic screen-detected tumors was similar between aspirin users and non-users (user/non-user; organized 11.0%/12.5%; opportunistic 3.9%/4.6%; P=0.38). There was also no difference in tumor size between aspirin users and non-users (P=0.781). The median proportion of days using aspirin in the year prior to diagnosis (dosing intensity) was 80.3%.

Aspirin & Nodal Status

RRs for associations between aspirin use and lymph node-positive breast cancer are presented in Table 2. The proportion of women with node-positive breast cancer in the aspirin non-user and user groups was 50.4% and 45.4%, respectively. In analyses adjusted for tumor size, tumor grade, screen detection, age and comorbidity score, women taking aspirin were significantly less likely to present with lymph node-positive breast cancer than women not taking aspirin (RR=0.89, 95%CI 0.81, 0.97). This translated to a 6% (95%CI 2%, 10%) lower adjusted absolute risk of lymph node metastasis between aspirin users and non-users.

The risk of presenting with lymph node metastasis decreased with increasing aspirin dosing intensity, dose and duration of use (Table 2). The adjusted RRs for node-positive disease for women in the lowest and highest quartiles of aspirin dosing intensity were 0.98 (95%CI 0.87, 1.10) and 0.81 (95%CI 0.68, 0.96) respectively (P-trend<0.001). The strength of association was also greater for women using higher aspirin doses (P-trend<0.001). We observed 20% and 33% reductions in the relative risk of node positive disease for regular use of low (<150mg) and high dose (≥150mg) aspirin respectively. Additionally, women initiating aspirin more than 1.5 years prior to their breast cancer diagnosis had a lower risk of lymph node metastasis (Table 2).

In sensitivity analyses, associations between aspirin use and lymph node metastasis were no different in women with and without screen-detected breast cancers (Appendix-2, P-

interaction=0.953). In addition our results were unchanged in analyses matched by propensity-score. The results were also unchanged in sensitivity analyses classifying women with only clinical assessment of nodal status as node positive. Full details of all sensitivity analyses are provided in Appendix-2.

Aspirin & Nodal Status – Effect Modification

Associations between aspirin use and a lower risk of lymph node metastasis were significantly stronger in women with larger tumors (Table 3; P-interaction=0.036); and PR-negative tumors (Table 3; P-interaction<0.001). Associations were also greater in women with ER-negative tumors (Table 3; P-interaction=0.056); HER2-positive tumors (Table 3; P-interaction=0.172); and high grade tumors (Appendix-3, Table A3-1; P-interaction=0.241), although these interactions did not reach statistical significance. There was no evidence of effect modification by tumor morphology (Table A3-1; P-interaction=0.619).

Aspirin & Mortality

Overall, pre-diagnostic aspirin use was associated with a non-significant reduction in the risk of breast cancer-specific and all-cause mortality (Table 4; Figure 2). In analyses of effect modification by nodal status, aspirin use pre-diagnosis was associated with a statistically significant 47% lower risk of breast cancer-specific mortality among women with node-negative tumors , and no reduction in women with node-positive tumors (P-interaction=0.038; Table 5; Figure 2). Post-diagnosis aspirin dosing intensity was similar for women with node-negative (84%) and node-positive tumors (78%). These results did not change after adjustment for post-diagnostic aspirin use (Appendix-2), or in sensitivity analyses for misclassification of cause of death (Appendix-2).

Discussion

In this study of 2,796 women with stage I-III breast cancer, we found that women taking aspirin prior to their breast cancer diagnosis were significantly less likely to present with a lymph node-positive tumor than non-users. Prior studies of daily aspirin use and nodal status are not available for comparison. In our analyses we also observed that the magnitude of association between pre-diagnostic aspirin use and risk of lymph node metastasis increased with increasing aspirin dosing intensity and was strongest in women with regular aspirin use. Our results also indicated that pre-diagnostic aspirin use was associated with a lower risk of

lymphatic metastasis in women taking <150mg/day, with the suggestion of a stronger association at higher doses.

Importantly, our results are unlikely to be explained by differences in screening-mammography use or breast cancer surveillance between aspirin users and non-users for the following reasons: (i) there was no difference in the proportion of screen detected tumors between aspirin users and non-users; (ii) there was no difference in the distribution of tumor size at presentation between aspirin users and non-users; (iii) associations between aspirin use and nodal-status were unchanged in propensity-score matched analyses; and (iv) we observed the same association between aspirin use and a reduced risk of lymphatic metastasis in women with and without screen-detected breast cancers.

In analyses of effect modification we observed significant interaction between aspirin use and a number of tumor characteristics previously associated with COX-2 expression in breast tumors. This suggests the possibility that inhibition of lymphatic metastasis by aspirin may be mediated at least partly through a COX-2 dependent pathway. Our findings are consistent with observations from in vivo breast cancer models which have shown that COX-2 inhibition suppresses the development of lymph node metastasis through the regulation of VEGF-C/-D mediated lymphatic dysregulation.(6,7) VEGF-C/-D overexpression has been shown to induce hyperplasia in peritumoral lymphatic vessels, increasing lymphatic flow and enhancing the rate of tumor cell delivery to lymph nodes, leading to increased lymph node metastasis.(7,40) Inhibition of lymphatic dysregulation represents one possible mechanism of action for aspirin in breast cancer, although a number other mechanisms have been proposed, including the inhibition of platelet function and reductions in serum estrogen concentrations.(41,42) Importantly, it is not clear whether regulation of lymphangiogenesis can restore dysregulated lymphatics in established tumors or inhibit the development of lymphatic metastases from tumor cells that have already seeded to the lymph nodes.(7,40) This may explain why associations with reduced lymph node metastasis were only observed in women with aspirin use for a sustained period prior to diagnosis.

In our survival analyses, pre-diagnostic aspirin use was associated with a significantly reduced risk of breast cancer-specific mortality among women with lymph node-negative disease at diagnosis, but not those with lymph node-positive disease. Adjustment for post-diagnostic aspirin use did not alter these findings. Although this study is the first to directly assess the modification of associations between aspirin and breast cancer mortality by nodal status, results from one previous study do support this observation.(3) Blair et al reported significant

associations between NSAID exposure and mortality in women with "local" breast cancer (100% node-negative; HR=0.37, 95%CI 0.16, 0.86) but not women with "non-local" breast cancer (1.6% node-negative; HR=0.67, 95%CI 0.31, 1.43; rHR 0.55). Together, these findings suggest the possibility that pre-diagnostic aspirin exposure inhibits the development of lymph node metastases; and that, in women using aspirin prior to a breast cancer diagnosis, negative nodal status is predictive of a subsequent survival benefit from aspirin use. This additional survival benefit may be mediated through possible effects of pre-diagnostic aspirin use on the presence of micro-metastatic disease,(6,7) and/or responsiveness to post-diagnostic aspirin use. Our results are also consistent with the findings from a recent meta-analyses of cardiovascular trials;(1) in which, associations between pre-diagnostic aspirin use and lower cancer-specific mortality, in a range of cancers, were primarily observed in patients without metastatic disease at diagnosis.

The strengths of this study include its prospective design, large sample size, high quality outcome data and the availability of high quality information on mammographic screening. In addition, the prescription-only status of low-dose aspirin in Ireland allowed the objective assessment of detailed aspirin exposure histories for all women. The study also has some limitations. Since aspirin use was based upon prescriptions dispensed, non-compliance with treatment or use of any non-prescription high dose aspirin preparations will have resulted in exposure misclassification. This will usually bias results towards the null. Information about obesity, which has been associated with lymph node metastasis in some prior studies,(43,44) was not available. Aspirin users are, however, likely to have higher body mass index than non-users;(45) and this would be expected to attenuate the observed associations. Finally, the results from effect modification analyses by PR and HER2 status should be interpreted with caution due to the number of women with unspecified receptor status.

In conclusion, aspirin use prior to breast cancer diagnosis was associated with a lower risk of lymph node metastasis at diagnosis. Furthermore, associations between pre-diagnostic aspirin use and breast cancer-specific mortality were only observed in women with lymph node-negative breast cancer at diagnosis. Our results provide insight into the potential mechanisms of action for aspirin in breast cancer progression. They also strongly suggest that pre-diagnostic use of aspirin may reduce mortality from breast cancer. These findings provide valuable information to inform the design of future clinical studies.

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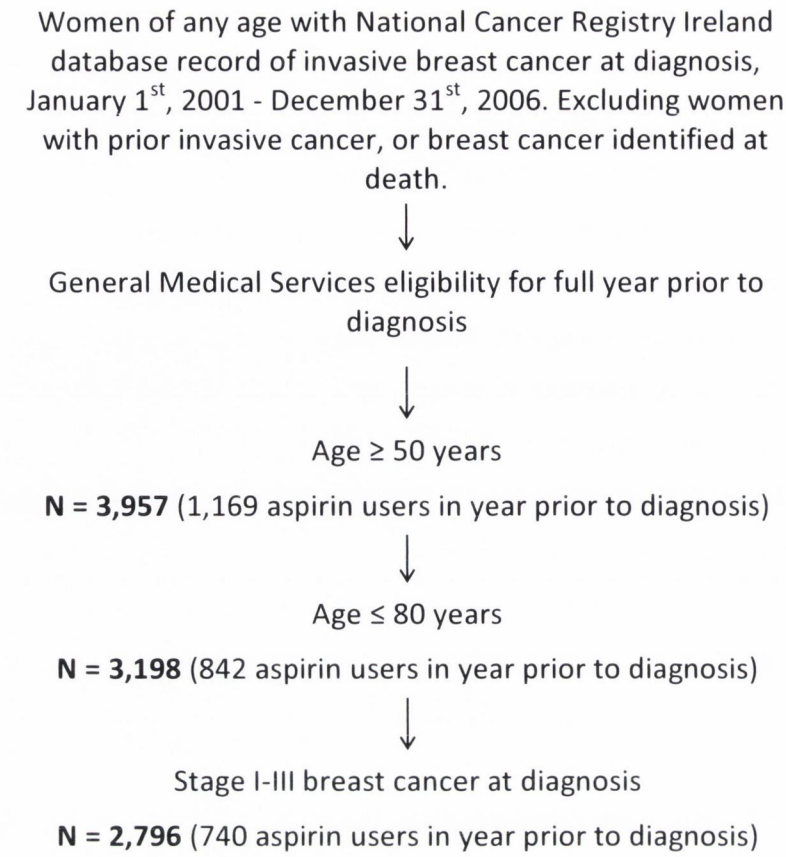

Figure 1: Flow chart for study cohort inclusion and exclusion critera

Table 1: Characteristics of women selected for inclusion in the study cohort

Characteristic at diagnosis		Aspirin use in the year prior diagnosis (dosing intensity by quartiles) [A]				
		Non-user N = 2,056	Dosing intensity (1%-37%) N = 186	Dosing intensity (38%-79%) N = 184	Dosing intensity (80%-97%) N = 185	Dosing intensity (98%-100%) N = 185
Patient details						
Age – Median (IQR)	Years	67 (58, 73)	72 (65, 77)	71 (64, 77)	73 (65, 76)	72 (66, 77)
Comorbidity – Median (IQR)	Drug classes	6 (3, 10)	10 (7, 14)	11 (8, 15)	11 (7, 15)	12 (9, 17)
Smoking status – (%)	Never	1,022 (49.7)	92 (49.5)	95 (51.6)	100 (54.1)	88 (47.6)
	Past	245 (11.9)	23 (12.4)	20 (10.9)	22 (11.9)	23 (12.4)
	Current	456 (22.2)	33 (17.7)	31 (16.8)	30 (16.2)	34 (18.4)
	Unspecified	333 (16.2)	38 (20.4)	38 (20.7)	33 (17.8)	40 (21.6)
Screen detected – (%)	Organized [B]	257 (12.5)	15 (8.1)	24 (13.0)	21 (11.4)	21 (11.4)
	Opportunistic	94 (4.6)	7 (3.8)	5 (2.7)	8 (4.3)	9 (4.9)
Concomitant drugs – (%) [C]	Estrogen	107 (5.2)	6 (3.2)	8 (4.3)	9 (4.9)	8 (4.3)
	Estrogen/Progesterone	164 (8.0)	9 (4.8)	7 (3.8)	6 (3.2)	10 (5.4)
	Statins	307 (14.9)	68 (36.6)	93 (50.5)	92 (50.5)	112 (60.5)
	NSAID	876 (42.6)	101 (54.3)	89 (48.4)	94 (50.8)	101 (54.6)
	Beta blocker	315 (15.3)	62 (33.3)	68 (37.0)	69 (37.3)	85 (45.9)
	Anti-diabetic	79 (3.8)	17 (9.1)	28 (15.2)	23 (12.4)	32 (17.3)
	- Biguanide	46 (2.2)	14 (7.5)	18 (9.8)	16 (8.6)	23 (12.4)
	Bisphosphonate	109 (5.3)	10 (5.4)	16 (8.7)	23 (12.4)	18 (9.7)
Tumor details						
Nodal status – (%) [D, E]	Negative	1,020 (49.6)	86 (46.2)	99 (53.8)	112 (60.5)	107 (57.8)
	Positive	1,036 (50.4)	100 (53.8)	85 (46.2)	73 (39.5)	78 (42.2)
Tumor size – (%) [D]	T1	848 (41.2)	71 (38.2)	84 (45.7)	78 (42.2)	62 (33.5)
	T2	916 (44.6)	87 (46.8)	81 (44.0)	84 (45.4)	98 (53.0)
	T3	130 (6.3)	9 (4.8)	12 (6.5)	12 (6.5)	13 (7.0)
	T4	162 (7.9)	19 (10.2)	7 (3.8)	11 (5.9)	12 (6.5)
Tumor stage – (%) [D]	I	624 (30.4)	51 (27.4)	66 (35.9)	60 (32.4)	48 (25.9)
	IIa / IIb	626/490 (30.4/23.8)	60/46 (32.3/24.7)	61/36 (33.2/19.6)	68/35 (36.8/18.9)	80/31 (43.2/16.8)
	IIIa / IIIb-c	130/186 (9.0/6.3)	9/20 (4.8/10.8)	13/8 (7.1/4.3)	8/14 (4.3/7.6)	10/16 (5.4/8.6)
Tumor grade – (%)	Low	207 (10.1)	18 (9.7)	18 (9.8)	27 (14.6)	17 (9.2)
	Intermediate	921 (44.8)	83 (44.6)	105 (57.1)	84 (45.4)	81 (43.8)
	High	692 (33.7)	59 (31.7)	50 (27.2)	51 (27.6)	64 (34.6)
	Unspecified	236 (11.5)	26 (14.0)	11 (6.0)	23 (12.4)	23 (12.4)
Tumor morphology – (%)	Ductal	1,462 (71.1)	135 (72.6)	122 (66.3)	128 (69.2)	131 (70.8)
	Lobular	270 (13.1)	29 (15.6)	26 (14.1)	24 (13.0)	21 (11.4)
	Other	324 (16.3)	22 (11.8)	36 (19.6)	33 (17.8)	33 (17.8)
Tumor topography – (%)	Outer	898 (43.7)	79 (42.5)	91 (49.5)	89 (48.1)	75 (40.5)
	Inner/Central	845 (41.1)	76 (40.9)	63 (34.2)	77 (41.6)	80 (43.2)
	Unspecified	313 (15.8)	31 (16.7)	30 (16.3)	19 (10.3)	30 (16.2)

ER – (%)	+ve/-ve/Unspecified	1,384/378/294 (67.3/18.4/14.3)	125/45/16 (67.2/24.2/8.6)	130/24/30 (70.7/13.0/16.3)	127/28/30 (68.6/15.1/16.2)	131/29/25 (70.8/15.7/13.5)
PR – (%)	+ve/-ve/Unspecified	918/497/641 (44.6/24.2/31.2)	85/57/44 (45.7/30.6/23.7)	79/38/67 (42.9/20.7/36.4)	82/41/62 (44.3/22.2/33.5)	75/51/59 (40.5/27.6/31.9)
HER2 – (%)	+ve/-ve/Unspecified	227/919/910 (11.0/44.7/44.3)	27/86/73 (14.5/46.2/39.2)	21/73/80 (12.1/42.0/46.0)	15/91/79 (8.1/49.2/42.7)	27/89/69 (14.6/48.1/37.3)
Aspirin exposure (year prior to diagnosis)						
Number of Rx dispensed		- -	522 -	1,587 -	2,166 -	2,353 -
Rx doses – (%)	75mg/300mg/Other	- -	379/107/36 (72.6/20.5/6.9)	1,247/264/76 (78.6/16.6/4.8)	1,914/186/66 (88.4/8.6/3.0)	2,137/160/56 (90.8/6.8/2.4)
Dosing intensity– Median(IQR)[A]	%	- -	16.4 (7.7, 26.6)	57.0 (47.0, 71.6)	90.4 (86.8, 94.2)	100.0 (99.2, 100.0)
Aspirin exposure (diagnosis to end of follow up)						
Dosing intensity– Median(IQR)[G]	%	0.0 (0.0, 0.0) [H]	23.6 (0.0, 75.3)	74.2 (44.5, 90.9)	89.4 (65.0, 96.2)	97.2 (88.5, 100.0)

IQR: Inter-Quartile Range. **ER**: Estrogen Receptor. **PR**: Progesterone Receptor. **HER2**: Human Epidermal Growth Factor Receptor 2. **Rx**: Prescription. **NSAID**: Non-Steroidal Anti-Inflammatory Drug.

A) Dosing intensity calculated as the number of days with a supply of aspirin available in year prior to diagnosis, divided by 365.

B) B) Identified from linked BreastCheck national screening program records.

C) In the year prior to breast cancer diagnosis.

D) AJCC Cancer Staging Manual 6th Edition. Springer, 2002.

E) Nodal status assessed pathologically, or if not available, assessed clinically.

F) Cumulative dose in year prior to diagnosis, divided by 365.

G) Post-diagnostic dosing intensity calculated as number of days with supply of aspirin available from diagnosis to end of follow-up, divided by the number of days from diagnosis to end of follow-up.

H) Mean post-diagnostic dosing intensity 6.2% (SD 18.1). Mean post diagnostic daily dose 5.1mg (SD 15.6).

Table 2: Univariate and multivariate relative risks for aspirin use and lymph node-positive breast cancer at diagnosis

	Risk-ratios for node-positive (N+ve) versus node-negative (N-ve)			
Aspirin Use	**N+ve (%)**	**N-ve (%)**	**Univariate RR (95%CI)**	**Multivariate RR (95%CI) [A]**
Non-user in year prior to diagnosis	1,036 (50.4)	1,020 (49.6)	Ref -	Ref -
Aspirin user in year prior to diagnosis	336 (45.4)	404 (54.6)	0.90 (0.82, 0.99)	0.89 (0.81, 0.97)
Aspirin dosing intensity				
Aspirin user in year prior to diagnosis				
Dosing intensity 1% - 37% [B, C]	100 (53.8)	86 (46.2)	1.07 (0.93, 1.23)	0.98 (0.87, 1.10)
Dosing intensity 38% - 79%	85 (46.2)	99 (53.8)	0.92 (0.92, 0.78)	0.96 (0.83, 1.11)
Dosing intensity 80% - 97%	73 (39.5)	112 (60.5)	0.78 (0.78, 0.65)	0.77 (0.65, 0.91)
Dosing intensity 98% - 100%	78 (42.2)	107 (57.8)	0.84 (0.84, 0.70)	0.81 (0.68, 0.96) **
Aspirin dose				
Aspirin user in year prior to diagnosis				
Low Dose ≤ 150mg [E]	288 (45.6)	344 (54.4)	0.90 (0.82, 0.99)	0.90 (0.82, 0.98)
High Dose > 150mg [F]	48 (44.4)	60 (55.6)	0.88 (0.71, 1.09)	0.82 (0.67, 1.00)*
Aspirin dosing intensity & dose				
Aspirin user in year prior to diagnosis				
Low dosing intensity 1% - 79% [B, D]				
Low dose < 150mg [E]	152 (49.8)	153 (50.2)	0.99 (0.88, 1.12)	0.99 (0.90, 1.10)
High dose ≥ 150mg [F]	33 (50.8)	32 (49.2)	1.01 (0.79, 1.28)	0.90 (0.72, 1.12)
High dosing intensity 80% - 100%				
Low dose < 150mg	136 (41.6)	191 (58.4)	0.83 (0.72, 0.95)	0.80 (0.71, 0.92)
High dose ≥ 150mg	15 (34.9)	28 (65.1)	0.69 (0.46, 1.04)	0.67 (0.45, 0.99)**
Aspirin duration [G]				
Non-user in 3 years prior to diagnosis	543 (49.5)	554 (50.5)	Ref -	Ref -
Aspirin user in 3 years prior to diagnosis				
Start aspirin <1.5 years prior to diagnosis	61 (50.8)	59 (49.2)	1.03 (0.85, 1.24)	1.01 (0.86, 1.18)
Start aspirin 1.5-3.0 years prior to diagnosis	89 (47.1)	100 (52.9)	0.95 (0.81, 1.12)	0.96 (0.83, 1.11)
Start aspirin ≥3.0 years prior to diagnosis	100 (46.1)	117 (53.9)	0.93 (0.80, 1.09)	0.89 (0.77, 1.03)
Aspirin dosing intensity & duration [G]				
Aspirin user in 3 years prior to diagnosis				
Low dosing intensity 1%-82% [D, H]				
Start aspirin <1.5 years prior to diagnosis	28 (47.6)	31 (52.5)	0.96 (0.73, 1.26)	1.01 (0.80, 1.28)
Start aspirin 1.5-3.0 years prior to diagnosis	60 (50.4)	59 (48.2)	1.02 (0.84, 1.23)	1.08 (0.91, 1.29)
Start aspirin ≥3.0 years prior to diagnosis	44 (51.8)	41 (48.2)	1.05 (0.84, 1.30)	0.97 (0.80, 1.16)
High dosing intensity 83%-100%				
Start aspirin <1.5 years prior to diagnosis	33 (54.1)	28 (45.9)	1.09 (0.86, 1.39)	1.01 (0.83, 1.22)
Start aspirin 1.5-3.0 years prior to diagnosis	29 (41.4)	41 (58.6)	0.84 (0.63, 1.11)	0.82 (0.64, 1.06)
Start aspirin ≥3.0 years prior to diagnosis	56 (42.4)	76 (57.6)	0.86 (0.70, 1.05)	0.83 (0.68, 1.01)

* *P-trend* <0.01; ** *P-trend* <0.001; **Ref**: Referent Group. **RR**: Relative Risk. **CI**: Confidence Interval. **N+ve**: Node-Positive. **N-ve**: Node-Negative.

A) Adjusted for age (years, continuous), tumor size (T1, T2, T3, T4), tumor grade (low, intermediate, high, unspecified), comorbidity score (number of medication classes, continuous) and screen-detected tumor (organized screening, opportunistic screening, not screen detected).

B) Dosing intensity calculated as the number of days with supply of aspirin available in the year prior to diagnosis, divided by 365.

C) Dosing intensity by quartiles.

D) Dosing intensity by median.

E) All prescriptions in the year prior to diagnosis were for doses of < 150mg. The 150mg cutpoint represents twice the standard low-dose aspirin strength (75mg) used in Ireland.

F) At least one prescription in the year prior to diagnosis was for a dose of ≥ 150mg.

G) Women with at least three years of continuous GMS eligibility prior to diagnosis were included in this exposure response analysis.

H) Dosing intensity calculated as number of days with supply of aspirin available from the first aspirin exposure in the three years prior to diagnosis up to diagnosis, divided by the number of days from the first aspirin exposure in the three years prior to diagnosis up to diagnosis.

Table 3: Aspirin Use & lymph node-positive breast cancer – Effect modification by tumor characteristics at diagnosis

Tumor size		Aspirin use in the year prior to diagnosis: Non-user	Low dosing intensity (1% - 79%) [A]	High dosing intensity (80% - 100%) [A]	Low dosing intensity v non-user within strata	High dosing intensity v non-user within strata
T1	N+ve/N-ve	266/582	55/100	40/100		
	RD (95%CI)	Ref -	0.02 (-0.06, 0.11)	-0.04 (-0.13, 0.04)	0.02 (-0.06, 0.11) p = 0.565	-0.04 (-0.13, 0.04) p = 0.288
T2-4	N+ve/N-ve	770/438	130/85	111/119		
	RD (95%CI)	0.24 (0.20 , 0.29)	0.21 (0.13, 0.28)	0.09 (0.01, 0.16)	-0.04 (-0.11, 0.04) p = 0.289	-0.16 (-0.23, -0.09) **p < 0.001**
	Aspirin*Tumor size	Additive scale: IC (95%CI)		T2-4 v T1	-0.06 (-0.17, 0.04) p = 0.255	-0.11 (-0.22, -0.01) **p = 0.036**
		Adjusted for age, tumor size, tumor grade, comorbidity, screen detection				

ER status		Aspirin use in the year prior to diagnosis: Non-user	Low dosing intensity (1% - 79%) [A]	High dosing intensity (80% - 100%) [A]	Low dosing intensity v non-user within strata	High dosing intensity v non-user within strata
ER Positive	N+ve/N-ve	681/703	121/134	110/148		
	RD (95%CI)	Ref -	-0.03 (-0.09, 0.03)	-0.08 (-0.14, -0.02)	-0.03 (-0.09, 0.03) p = 0.375	-0.08 (-0.14, -0.02) **p = 0.013**
ER Negative	N+ve/N-ve	196/182	39/30	21/36		
	RD (95%CI)	-0.01 (-0.06, 0.05)	0.03 (-0.09, 0.15)	-0.22 (-0.33, -0.10)	0.04 (-0.09, 0.16) p = 0.562	-0.21 (-0.34, -0.09) **p < 0.001**
	Aspirin*ER status	Additive scale: IC (95%CI)		ER negative v positive	0.06 (-0.07, 0.20) p = 0.358	-0.13 (-0.27, 0.00) p = 0.056
		Adjusted for age, tumor size, tumor grade, comorbidity, screen detection				

Table 3 Continued: Aspirin Use & lymph node-positive breast cancer – Effect modification by tumor characteristics at diagnosis

		Aspirin use in the year prior to diagnosis			Low dosing intensity v non-user within strata		High dosing intensity v non-user within strata	
PR status		**Non-user**	**Low dosing intensity (1% - 79%) [A]**	**High dosing intensity (80% - 100%) [A]**				
PR Positive	**N+ve/N-ve**	456/462	80/84	73/84				
	RD (95%CI)	Ref -	0.00 (-0.08, 0.08)	-0.05 (-0.12, 0.03)	0.00 (-0.08, 0.08)	p = 0.934	-0.05 (-0.12, 0.03)	p = 0.239
PR Negative	**N+ve/N-ve**	279/218	55/40	32/60				
	RD (95%CI)	0.06 (0.01, 0.11)	0.04 (-0.05, 0.14)	-0.19 (-0.28, -0.10)	-0.01 (-0.12, 0.09)	p = 0.775	-0.25 (-0.35, -0.15)	**p < 0.001**
		Aspirin*PR status	Additive scale: IC (95%CI)	PR negative v positive	-0.01 (-0.14, 0.12)	p = 0.863	-0.21 (-0.33, -0.08)	**p < 0.001**
			Adjusted for age, tumor size, tumor grade, comorbidity, screen detection					

		Aspirin use in the year prior to diagnosis			Low dosing intensity v non-user within strata		High dosing intensity v non-user within strata	
HER2 status		**Non-user**	**Low dosing intensity (1% - 79%) [A]**	**High dosing intensity (80% - 100%) [A]**				
Negative	**N+ve/N-ve**	483/486	87/82	80/100				
	RD (95%CI)	Ref -	-0.03 (-0.10, 0.04)	-0.10 (-0.17, 0.02)	-0.03 (-0.10, 0.04)	p = 0.432	-0.10 (-0.17, 0.02)	**p = 0.012**
Positive	**N+ve/N-ve**	132/95	27/21	17/25				
	RD (95%CI)	0.02 (-0.05, 0.09)	0.04 (-0.10, 0.18)	-0.18 (-0.31, -0.05)	0.01 (-0.14, 0.17)	p = 0.856	-0.21 (-0.35, -0.06)	**p = 0.005**
		Aspirin*HER2 status	Additive scale: IC (95%CI)	positive v negative	-0.04 (-0.21, 0.12)	p = 0.609	-0.11 (-0.27, 0.05)	p = 0.172
			Adjusted for age, tumor size, tumor grade, comorbidity, screen detection					

N+ve: Node-Positive. **N-ve**: Node-Negative. **RD**: Risk Difference. **IC**: Interaction Contrast. **CI**: Confidence Interval

A) Dosing intensity by median. Dosing intensity calculated as number of days with supply of aspirin available in year prior to diagnosis, divided by 365.

Table 4: Multivariate hazard ratios for pre-diagnostic aspirin use & all-cause and breast-cancer specific mortality.

Aspirin use			All-cause mortality		Breast cancer-specific mortality	
	N	Person years	Deaths	Multivariate HR (95%CI) [A]	Deaths	Multivariate HR (95%CI) [A]
Non-user in year prior to diagnosis	2056	7,287	380	Ref -	249	Ref -
Aspirin user in year prior to diagnosis	740	2,423	138	0.82 (0.07, 1.02)	83	0.84 (0.64, 1.10)

Ref: Referent Group. **HR**: Hazard Ratio. **CI**: Confidence Interval.
A) All multivariate hazard ratios are adjusted for age (years, continuous), tumor stage (I, IIa, IIb, IIIa, IIIb-c) tumor grade (low, intermediate, high, unspecified), estrogen receptor status (positive, negative unspecified), progesterone receptor status (positive, negative, unspecified), HER2 status (positive, negative, unspecified) and comorbidity score (number of medication classes, continuous)

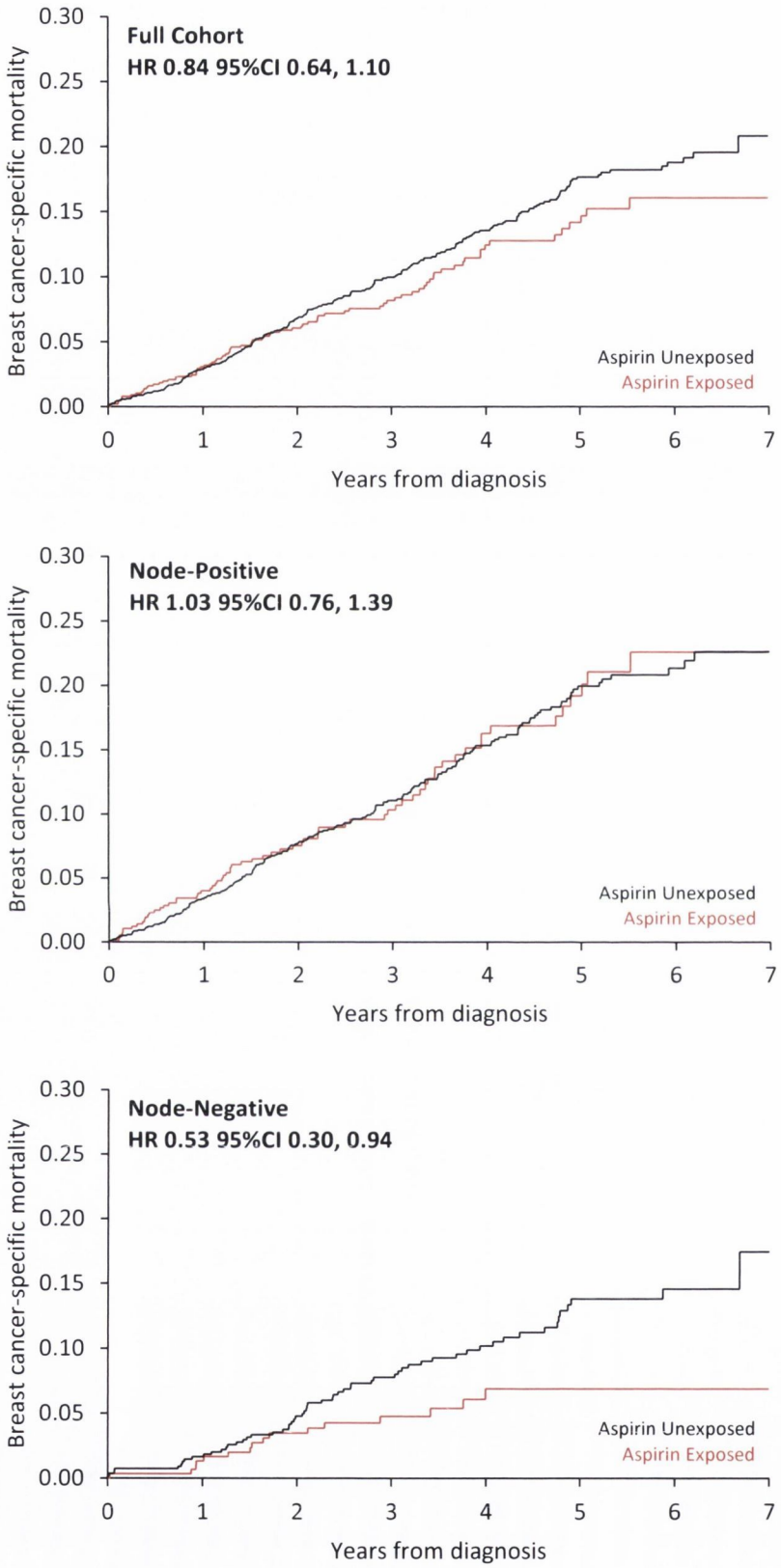

Figure 2: Adjusted Cumulative probability of Breast Cancer-Specific mortality for aspirin users and non-users in the Full cohort and by lymph node status at diagnosis (Positive, Negative). Adjusted for age, tumor stage, tumor grade, ER, PR, HER2 and Comorbidity.

Table 5: Pre-diagnostic Aspirin Use & Breast Cancer-Specific mortality – Effect modification by lymph node status at diagnosis

Nodal status		Aspirin use in the year prior to diagnosis: Non-user	Aspirin use in the year prior to diagnosis: User	User v non-user within strata	
Positive	**Person years**	3,486	1,008		
	Censored/Death	846/190	269/67		
	HR (95%CI)	Ref -	1.03 (0.76, 1.39)	1.03 (0.76, 1.39)	p = 0.840
Negative	**Person years**	3,801	1,415		
	Censored/Death	961/59	388/16		
	HR (95%CI)	0.62 (0.43, 0.91)	0.33 (0.19, 0.58)	0.53 (0.30, 0.94)	**p = 0.028**
	Aspirin*Nodal status	Multiplicative scale: rHR (95%CI)	Negative v Positive	0.52 (0.28, 0.96)	**p = 0.038**
		Adjusted for age, comorbidity, tumor stage, tumor grade, ER, PR, HER2			

HR: Hazard Ratio. **rHR**: Ratio of Hazard Ratios. **CI**: Confidence Interval.

APPENDIX 1

WHO-ATC DRUG CODES

Aspirin:	B01AC06, C10BX01, C10BX02, M01BA03, N02BA01, N02BA51, N02BA71
Bisphosphonates:	M05BA, M05BB
Statins:	C10AA
Beta-blockers:	C07
Anti-diabetic:	A10
Biguanides:	A10BA
Estrogen:	G03C
Estrogen/Progesterone:	G03FA, G03FB
Other NSAID:	M01A

ICD-O-2 TUMOR MORPHOLOGY CODES

Ductal:	8022/3, 8141/3, 8201/3, 8500/3, 8501/3, 8521/3
Lobular:	8520/3.

ICD-O-2 TUMOR TOPOGRAPHY CODES

Outer:	C50.4, C50.5, C50.6
Inner/Central:	C50.0, C50.1, C50.2, C50.3, C50.8
Unspecified:	C50.9.

ICD-10 CAUSE OF DEATH CODES

Breast cancer specific mortality:	C50

ER, PR, HER2 RECEPTOR STATUS

Estrogen and progesterone receptor activity was defined as positive if recorded by the NCRI database as unclear/possibly, some receptor activity or positive/strong. HER2 receptor activity was defined as positive by immunohistochemistry if recorded by the NCRI database as score 2+, weak/strong positive or weak/strong complete membrane staining in >10% of tumor cells. HER2 receptor activity was defined as positive by fluorescence in-situ hybridization if recorded by the NCRI database as weak/strong positive or some/strong amplification. Where IHC & FISH results were recorded, FISH results were used.

APPENDIX 2

SENSITIVITY ANALYSES

1. NODAL STATUS – MISCLASSIFICATION OF NODAL STATUS

Sensitivity analysis 1.1 – nodal status classified by clinical examination only

Nodal status was classified by clinical examination only, in 9.1% (N+ve 4.7%; N-ve 4.4%) of aspirin non-users and 9.5% (N+ve 3.1%; N-ve 6.4%) of aspirin users. Analyses were repeated classifying all of these women as node positive. The results from this analysis did not substantively change from the primary study analysis. Aspirin dosing intensity quartile: 1%-37% RR=1.01 (95%CI 0.92, 1.11); 38%-79% RR=0.95 (95%CI 0.85, 1.06); 80%-97% RR=0.93 (95%CI 0.82, 1.04); 98%-100% RR=0.85 (95%CI 0.75, 0.96).

Sensitivity analysis 1.2 – complete case analysis

Nodal status was unrecorded in 4.9% of aspirin non-users and 8.6% of aspirin users. A conservative approach to missing nodal status was taken in the main study analyses and these women were all classified as node positive. In addition to this, a complete case analysis was conducted excluding these women and adjusting for predictors of missing nodal status (age, comorbidity)(1) under the assumption that missing nodal status was missing at random.(2) The results from these analyses did not substantively change from the primary study analysis. Aspirin dosing intensity quartile: 1%-37% RR=0.95 (95%CI 0.83, 1.10); 38%-79% RR=0.93 (95%CI 0.78, 1.10); 80%-97% RR=0.76 (95%CI 0.62, 0.92); 98%-100% RR=0.77 (95%CI 0.63, 0.94).

2. NODAL STATUS – EARLY DETECTION BIAS

Sensitivity analysis 2.1 – Organized screening (BreastCheck)

Analyses were stratified by organized screen-detected tumors (BreastCheck) versus not screen-detected. The results from this analysis (Table A2-1) indicate that aspirin exposure is associated with the same reduced risk of node-positive disease for organized screen-detected tumors (RR=0.80, 95%CI 0.47, 1.35) and for tumors that are not screen-detected (RR=0.79, 95%CI 0.69, 0.90; P-interaction=0.967); although, the former did not reach statistical significance.

Sensitivity analysis 2.2 – organized and opportunistic screening

Analyses were also stratified by organized/opportunistic screen-detected tumors versus not screen-detected. The results from this analysis (Table A2-2) indicate that aspirin exposure is associated with the same reduced risk of node-positive disease for organized/opportunistic screen-detected tumors (RR=0.78, 95%CI 0.52, 1.16) and for tumors that are not screen-detected (RR=0.79, 95%CI 0.69, 0.90; P-interaction=0.953); although, the former did not reach statistical significance.

Table A2-1: Aspirin Use & lymph node-positive breast cancer – Effect modification by organized screen detection (BreastCheck)

Organized Screen-Detection		Aspirin use in the year prior to diagnosis			Low dosing intensity v non-user within strata		High dosing intensity v non-user within strata	
		Non-user	Low dosing intensity (1% - 79%) [A]	High dosing intensity (80% - 100%) [A]				
Not Screened	**N+ve/N-ve**	916/789	169/150	133/178				
	RR (95%CI)	Ref -	0.97 (0.88, 1.07)	0.79 (0.69, 0.90)	0.97 (0.88, 1.07)	p =0.587	0.79 (0.69, 0.90)	**p < 0.001**
BreastCheck	**N+ve/N-ve**	78/179	13/26	11/31				
	RR (95%CI)	0.79 (0.65, 0.96)	0.82 (0.53, 1.27)	0.63 (0.38, 1.04)	1.04 (0.65, 1.66)	p = 0.872	0.80 (0.47, 1.35)	p =0.404
	Aspirin* Organized Screen-Detection		Multiplicative scale: rRR (95%CI) Adjusted for age, tumor size, grade, comorbidity	No Screen v BreastCheck	1.07 (0.66, 1.72)	p = 0.788	1.01 (0.59, 1.74)	p = 0.967

N+ve: Node-Positive. **N-ve**: Node-Negative. **RR**: Relative Risk. **rRR**: Ratio of Relative Risks. **CI**: Confidence Interval.
A) Dosing intensity by median. Dosing intensity calculated as number of days with supply of aspirin available in year prior to diagnosis, divided by 365.

Table A2-2: Aspirin Use & lymph node-positive breast cancer – Effect modification by organized/opportunistic screen detection

Any Screen-Detection		Aspirin use in the year prior to diagnosis: Non-user	Low dosing intensity (1% - 79%) [A]	High dosing intensity (80% - 100%) [A]	Low dosing intensity v non-user within strata		High dosing intensity v non-user within strata	
Not Screened	**N+ve/N-ve**	916/789	169/150	133/178				
	RR (95%CI)	Ref -	0.97 (0.88, 1.07)	0.79 (0.69, 0.90)	0.97 (0.88, 1.07)	p = 0.573	0.79 (0.69, 0.90)	**p < 0.001**
Any Screen	**N+ve/N-ve**	120/231	16/35	18/41				
	RR (95%CI)	0.85 (0.73, 0.99)	0.78 (0.53, 1.17)	0.66 (0.46, 0.97)	0.92 (0.60, 1.40)	p = 0.691	0.78 (0.52, 1.16)	p = 0.216
	Aspirin* Any Screen-Detection	Multiplicative scale: rRR (95%CI)		No Screen v Any Screen	0.94 (0.62, 1.45)	p = 0.793	0.99 (0.65, 1.50)	p = 0.953
		Adjusted for age, tumor size, grade, comorbidity						

N+ve: Node-Positive. **N-ve**: Node-Negative. **RR**: Relative Risk. **rRR**: Ratio of Relative Risks. **CI**: Confidence Interval.
A) Dosing intensity by median. Dosing intensity calculated as number of days with supply of aspirin available in year prior to diagnosis, divided by 365.

Sensitivity analysis 2.3 – propensity score matched analysis

A propensity score model was developed to predict aspirin use in the 365 days prior to breast cancer diagnosis using an iterative approach as follows: (i) covariates were assessed for inclusion in the propensity score model based on prior knowledge of demographic and clinical covariates associated with higher intensity medical management (age, comorbidity score); increased breast cancer surveillance (screen-detection); medications commonly co-prescribed with aspirin (beta blocker, statin, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker, diuretic) and exposure to other medications that may be associated with potential confounding (anti-diabetic, biguanide, bisphosphonate, estrogen, estrogen/progesterone, NSAID).(3) (ii) Logistic regression models were used to estimate propensity scores for aspirin exposure using these covariates. Main effects, interaction terms and quadratic or cubic terms were assessed for inclusion as appropriate. (iii) Covariate balance within propensity score quintiles was assessed by standardized differences (d), with a $d<0.1$ being the desired limit.(4) The multivariate propensity score model which achieved the optimal balance of covariates between aspirin users and non-users was selected. (iv) Aspirin users and non-users were then matched (1:1) within a caliper of 0.2 standard deviations of the propensity score logit(5) using greedy matching without replacement.(6,7) Covariate balance between matched cohorts was assessed by standardized differences ($d<0.1$).(4)

The characteristics of matched aspirin users (n=613) and non-users (n=613) are presented in Table A2-3. Balance ($d<0.1$) was achieved for all matched covariates between aspirin users and non-users. Importantly, there was no difference in the distribution of tumor size ($d<0.01$), or screen-detected tumors ($d<0.1$) between aspirin users and non-users. Analyses of associations between aspirin use and nodal status were repeated using this propensity score matched cohort. Associations between aspirin use and node-positive status remained significant (Table A2-4) and were not substantively different to the primary (un-matched) analysis.

Table A2-3: Characteristics of propensity score matched aspirin users and non-users

Characteristic at diagnosis		Aspirin use in the year prior diagnosis [A]	
		PS-matched non-user (N=613)	PS-matched user (N=613)
Patient details			
Age – Median (IQR)	Years	72 (65, 76)	71 (64, 76)
Comorbidity – Median (IQR)	Drug classes	10 (7, 15)	10 (7, 14)
Smoking status – (%)	Never	318 (51.9)	312 (51.0)
	Past	105 (17.1)	110 (17.9)
	Current	89 (14.5)	69 (11.3)
	Unspecified	101 (16.5)	122 (19.9)
Screen detected – (%)	Organized [B]	62 (10.1)	72 (11.8)
	Opportunistic	22 (3.6)	24 (3.9)
Concomitant drugs – (%) [C]	Estrogen	30 (4.9)	28 (4.6)
	Estrogen/Progesterone	34 (5.6)	28 (4.6)
	Statins	238 (38.8)	255 (41.6)
	NSAID	311 (50.7)	328 (53.5)
	Beta blocker	183 (29.9)	185 (30.2)
	Anti-diabetic	58 (9.5)	71 (11.6)
	- Biguanide	35 (5.7)	46 (7.5)
	Bisphosphonate	50 (8.2)	51 (8.3)
Tumor details			
Nodal status – (%) [D, E]	Negative	301 (49.1)	340 (55.5)
	Positive	312 (50.9)	273 (44.5)
Tumor size – (%) [D]	T1	242 (39.5)	243 (39.6)
	T2	289 (47.2)	288 (47.0)
	T3	38 (6.2)	38 (6.2)
	T4	44 (7.2)	44 (7.2)
Tumor stage – (%) [D]	I	175 (28.6)	187 (30.5)
	IIa / IIb	203 / 146 (33.1 / 23.8)	220 / 122 (35.9 / 19.9)
	IIIa / IIIb-c	37 / 52 (6.0 / 8.5)	33 / 51 (5.4 / 8.3)
Tumor grade – (%)	Low	62 (10.1)	68 (11.1)
	Intermediate	286 (46.7)	291 (47.5)
	High	196 (32.0)	185 (30.2)
	Unspecified	69 (11.3)	69 (11.3)
Tumor morphology – (%)	Ductal	434 (70.8)	432 (70.5)
	Lobular	82 (13.4)	81 (13.2)
	Other	97 (15.8)	100 (16.3)
Tumor topography – (%)	Outer	247 (40.3)	278 (45.4)
	Inner/Central	159 (25.9)	164 (26.8)
	Unspecified	207 (33.8)	171 (27.9)
ER – (%)	+ve/-ve/Unspecified	413/116/84 (67.4/18.9/13.7)	426/105/82 (69.5/17.1/13.4)
PR – (%)	+ve/-ve/Unspecified	285/139/189 (46.5/22.7/30.8)	267/158/188 (43.6/25.8/30.7)
HER2 – (%)	+ve/-ve/Unspecified	56/293/264 (9.1/47.8/43.1)	78/287/248 (12.7/46.8/40.5)
Aspirin exposure details (year prior to diagnosis)			
Number of Rx dispensed		- -	5,363
Rx doses – (%)	75mg/300mg/Other	- -	4,637/561/165 (86.5/10.5/3.1)
Dosing intensity– Median(IQR) [A]	%	- -	78.4 (33.4, 97.0)

IQR: Inter-Quartile Range. **ER**: Estrogen Receptor. **PR**: Progesterone Receptor. **HER2**: Human Epidermal Growth Factor Receptor 2. **Rx**: Prescription. **NSAID**: Non-Steroidal Anti-Inflammatory Drug. **PS**: Propensity Score.

A) Dosing intensity calculated as the number of days with a supply of aspirin available in year prior to diagnosis, divided by 365.

B) B) Identified from linked BreastCheck national screening program records (www.breastcheck.ie).

C) In the year prior to breast cancer diagnosis.

D) AJCC Cancer Staging Manual 6th Edition. Springer, 2002.

E) Nodal status assessed pathologically, or if not available, assessed clinically.

Table A2-4: Univariate and multivariate relative risks for aspirin use and lymph node-positive breast cancer at diagnosis

	Risk-ratios for node-positive (N+ve) versus node-negative (N-ve)					
Aspirin use	**N+ve**	**(%)**	**N-ve**	**(%)**	**Univariate RR (95%CI)**	**Multivariate RR (95%CI) [A]**
Sensitivity analysis 2.3 (propensity score matched)						
Non-user in year prior to diagnosis	312	(50.9)	301	(49.1)	Ref -	Ref -
Aspirin user in year prior to diagnosis						
Dosing intensity 1% - 37%	89	(53.9)	76	(46.1)	1.06 (0.90, 1.25)	1.01 (0.89, 1.14)
Dosing intensity 38% - 79%	66	(44.3)	83	(55.7)	0.87 (0.72, 1.06)	0.92 (0.77, 1.10)
Dosing intensity 80% - 97%	61	(39.6)	93	(60.4)	0.78 (0.63, 0.96)	0.79 (0.64, 0.97)
Dosing intensity 98% - 100%	57	(39.3)	88	(60.7)	0.77 (0.62, 0.96)	0.79 (0.65, 0.95)

Ref: Referent Group. **RR**: Relative Risk. **CI**: Confidence Interval. **N+ve**: Node Positive. **N-ve**: Node Negative.
A) All multivariate relative risks and risk differences are adjusted for age (years, continuous), tumor size (T1, T2, T3, T4) tumor grade (low, intermediate, high, unspecified) and comorbidity score (number of medication classes, continuous).

3. SURVIVAL - MISCLASSIFICATION OF CAUSE OF DEATH

Sensitivity analysis 3.1 – misclassification of breast cancer-specific mortality

Analyses of breast cancer-specific mortality were repeated with the inclusion of all deaths where breast cancer was identified as a secondary/contributory cause of death (Definition 1). Analyses were also repeated with breast cancer-specific mortality additionally defined using ICD mortality site codes for ill-defined cancer sites (ICD-10 C76.1, C80) secondary cancer sites (ICD-10 C77-79), cancers of uncertain or unknown behavior (D48.6, D48.9) and unspecified causes of death (Definition 2). The results from these analyses are presented in Table A2-5. Hazard ratios for breast cancer-specific mortality are unchanged from those in the primary analysis.

Table A2-5: Multivariate hazard ratios for pre-diagnostic aspirin use & alternative definitons of breast cancer specific mortality

			Breast cancer-specific mortality	
Aspirin use	N	Person years	Deaths	Multivariate HR (95%CI) [A]
Breast cancer-specific mortality (Definition 1)[B]				
Non-user in year prior to diagnosis	2056	7,287	261	Ref -
Aspirin user in year prior to diagnosis	740	2,423	89	0.84 (0.65, 1.09)
Breast cancer-specific mortality (Definition 2)[C]				
Non-user in year prior to diagnosis	2056	7,287	268	Ref -
Aspirin user in year prior to diagnosis	740	2,423	85	0.79 (0.61, 1.03)

Ref: Referent Group. **HR**: Hazard Ratio. **CI**: Confidence Interval.
A) All multivariate hazard ratios are adjusted for age (years, continuous), tumor stage (I, IIa, IIb, IIIa, IIIb-c) tumor grade (low, intermediate, high, unspecified), estrogen receptor status (positive, negative unspecified), progesterone receptor status (positive, negative, unspecified), HER2 status (positive, negative, unspecified) and comorbidity score (number of medication classes, continuous).
B) Including all deaths where breast cancer was identified as a secondary/contributory cause of death.
C) Including all deaths from ill-defined cancer sites (ICD-10 C76.1, C80) secondary cancer sites (ICD-10 C77-79), cancers of uncertain or unknown behavior (D48.6, D48.9) and unspecified causes of death.

4. SURVIVAL– POST DIAGNOSTIC ASPIRIN USE

Sensitivity analysis 4.1 – adjustment for post diagnostic aspirin use

All survival analyses were repeated with adjustment for post-diagnostic aspirin use (unexposed, exposed; time varying; lagged 2 years). The results from the main analysis (Table A2-6) and the analysis of effect modification by nodal status at diagnosis (Table A2-7) were unchanged from analyses without adjustment for post diagnostic aspirin use.

Table A2-6: multivariate hazard ratios for pre-diagnostic aspirin use & all cause or breast cancer specific mortality, with adjustment for post-diagnostic aspirin use

Aspirin use			All-cause mortality		Breast cancer-specific mortality	
	N	Person years	Deaths	Multivariate HR (95%CI) [A]	Deaths	Multivariate HR (95%CI) [A]
Non-user in year prior to diagnosis	2056	7,287	380	Ref -	249	Ref -
Aspirin user in year prior to diagnosis	740	2,423	138	0.75 (0.59, 0.95)	83	0.83 (0.61, 1.12)

Ref: Referent Group. **HR**: Hazard Ratio. **CI**: Confidence Interval.
A) All multivariate hazard ratios are adjusted for age (years, continuous), tumor stage (I, IIa, IIb, IIIa, IIIb-c) tumor grade (low, intermediate, high, unspecified), estrogen receptor status (positive, negative unspecified), progesterone receptor status (positive, negative, unspecified), HER2 status (positive, negative, unspecified), comorbidity score (number of medication classes, continuous) and post diagnostic aspirin use (exposed, unexposed, time varying, lagged by 2 years).

Table A2-7: Pre-diagnostic Aspirin Use & Breast Cancer-Specific mortality – Effect modification by lymph node status at diagnosis, with adjustment for post diagnostic aspirin use

Nodal status		Aspirin use in the year prior to diagnosis		User v non-user within strata	
		Non-user	User		
Positive	**Person years**	3,486	1,008		
	Censored/Death	846/190	269/67		
	HR (95%CI)	Ref -	1.00 (0.73, 1.39)	1.00 (0.73, 1.39)	p = 0.990
Negative	**Person years**	3,801	1,415		
	Censored/Death	961/59	388/16		
	HR (95%CI)	0.62 (0.42, 0.91)	0.32 (0.18, 0.57)	0.51 (0.29, 0.92)	**p = 0.026**
	Aspirin*Nodal status	Multiplicative scale: rHR (95%CI)	Negative v Positive	0.51 (0.28, 0.96)	**p = 0.036**
		Adjusted for age, comorbidity, tumor stage, tumor grade, ER, PR, HER2 , post-diagnostic aspirin use (exposed, unexposed, time varying, lagged by 2 years)			

HR: Hazard Ratio. **rHR**: Ratio of Hazard Ratios. **CI**: Confidence Interval.

Sensitivity analysis 4.2 – post-diagnostic aspirin use lag time

Survival analyses were repeated, varying the length by which post-diagnostic aspirin use was lagged (1, 2 & 3 years). The results from these analyses did not substantively change from the primary study analysis (Table A2-8).

Table A2-8: multivariate hazard ratios for pre-diagnostic aspirin use & breast cancer specific or all causer mortality adjusted for time-varying post-diagnostic aspirin exposure (yes/no) lagged by 1, 2, or 3 years

			All-cause mortality		Breast cancer-specific mortality	
Aspirin use	**N**	**Person years**	**Deaths**	**Multivariate HR (95%CI) [A]**	**Deaths**	**Multivariate HR (95%CI) [A]**
Non-user in year prior to diagnosis	2056	7,287	380	Ref -	249	Ref -
Adjusted for post-diagnostic aspirin use: 1 year lag						
Aspirin user in year prior to diagnosis	740	2,423	138	0.69 (0.54, 0.89)	83	0.76 (0.55, 1.05)
Adjusted for post-diagnostic aspirin use: 2 year lag						
Aspirin user in year prior to diagnosis	740	2,423	138	0.75 (0.59, 0.95)	83	0.83 (0.61, 1.12)
Adjusted for post-diagnostic aspirin use: 3 year lag						
Aspirin user in year prior to diagnosis	740	2,423	138	0.77 (0.61, 0.96)	83	0.84 (0.63, 1.12)

Ref: Referent Group. **HR**: Hazard Ratio. **CI**: Confidence Interval.
A) In addition to adjustment for time varying post-diagnostic aspirin exposure (yes/no), all multivariate hazard ratios are adjusted for age (years, continuous), tumor stage (I, IIa, IIb, IIIa, IIIb-c) tumor grade (low, intermediate, high, unspecified), estrogen receptor status (positive, negative unspecified), progesterone receptor status (positive, negative, unspecified), HER2 status (positive, negative, unspecified) and comorbidity score (number of medication classes, continuous).

Appendix 2 References

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APPENDIX 3

Table A3-1: Aspirin Use & lymph NODE-positive breast cancer – Effect modification by tumor characteristics at diagnosis

ER/PR Negative		Aspirin use in the year prior to diagnosis: Non-user	Low dosing intensity (1% - 79%) [A]	High dosing intensity (80% - 100%) [A]	Low dosing intensity v non-user within strata		High dosing intensity v non-user within strata	
No	N+ve/N-ve	711/734	123/139	111/151				
	RD (95%CI)	Ref -	-0.03 (-0.09, 0.03)	-0.08 (-0.14, -0.02)	-0.03 (-0.09, 0.03)	p = 0.343	-0.08 (-0.14, -0.02)	**p = 0.012**
Yes	N+ve/N-ve	157/130	37/25	16/34				
	RD (95%CI)	0.03 (-0.04, 0.09)	0.06 (-0.06, 0.18)	-0.24 (-0.35, 0.13)	0.03 (-0.10, 0.16)	p = 0.621	-0.27 (0.39, -0.14)	**p < 0.001**
	Aspirin*ER/PR Negative	Additive scale: IC (95%CI)		yes v no	0.06 (-0.08, 0.20)	p = 0.392	-0.19 (-0.32, -0.05)	**p = 0.006**
		Adjusted for age, tumor size, tumor grade, comorbidity, screen detection						

ER/PR/HER2 Negative		Aspirin use in the year prior to diagnosis: Non-user	Low dosing intensity (1% - 79%) [A]	High dosing intensity (80% - 100%) [A]	Low dosing intensity v non-user within strata		High dosing intensity v non-user within strata	
No	N+ve/N-ve	770/767	133/147	117/162				
	RD (95%CI)	Ref -	-0.03 (-0.09, 0.03)	-0.09 (-0.15, -0.03)	-0.03 (-0.09, 0.03)	p =295	-0.09 (-0.15, -0.03)	**p = 0.002**
Yes	N+ve/N-ve	59/63	18/12	9/19				
	RD (95%CI)	-0.04 (-0.13, 0.05)	0.05 (-0.01, 0.22)	-0.23 (-0.37, -0.09)	0.09 (-0.10, 0.28)	p = 0.357	-0.19 (-0.35, -0.03)	**p =0.021**
	Aspirin*ER/PR/HER2 Negative	Additive scale: IC (95%CI)		yes v no	0.12 (-0.08, 0.32)	p = 0.234	-0.10 (-0.26, 0.07)	p = 0.267
		Adjusted for age, tumor size, tumor grade, comorbidity, screen detection						

Table A3-1 Continued: Aspirin Use & lymph node-positive breast cancer – Effect modification by tumor characteristics at diagnosis

Tumor Morphology		Aspirin use in the year prior to diagnosis			Low dosing intensity v non-user within strata		High dosing intensity v non-user within strata	
		Non-user	Low dosing intensity (1% - 79%) [A]	High dosing intensity (80% - 100%) [A]				
Ductal	**N+ve/N-ve**	730/720	128/129	106/153				
	RD (95%CI)	Ref -	-0.01 (-0.07, 0.05)	-0.10 (-0.16, -0.04)	-0.01 (-0.07, 0.05)	p = 0.692	-0.10 (-0.16, -0.04)	**p < 0.001**
Lobular	**N+ve/N-ve**	148/122	30/25	20/25				
	RD (95%CI)	-0.01 (-0.07, 0.06)	-0.01 (-0.13, 0.11)	-0.15 (-0.29, -0.01)	-0.01 (-0.14, 0.13)	p = 0.928	-0.14 (-0.29, 0.00)	**p = 0.058**
		Aspirin*Tumor Morphology	Additive scale: IC (95%CI)	lobular v ductal	0.01 (-0.14, 0.15)	p = 0.932	-0.04 (-0.20, 0.12)	p = 0.619
			Adjusted for age, tumor size, tumor grade, comorbidity, screen detection					

Table A3-1 Continued: Aspirin Use & lymph node-positive breast cancer – Effect modification by tumor characteristics at diagnosis

Tumor Grade		Aspirin use in the year prior to diagnosis			Low dosing intensity v non-user within strata		High dosing intensity v non-user within strata	
		Non-user	Low dosing intensity (1% - 79%) [A]	High dosing intensity (80% - 100%) [A]				
Low	**N+ve/N-ve**	66/141	16/20	14/30				
	RD (95%CI)	Ref -	0.05 (-0.11, 0.22)	-0.06 (-0.20, 0.08)	0.05 (-0.11, 0.22)	p = 0.529	-0.06 (-0.20, 0.08)	p = 0.428
Intermediate	**N+ve/N-ve**	458/463	85/103	70/95				
	RD (95%CI)	0.08 (0.01, 0.14)	0.04 (-0.05, 0.14)	-0.02 (-0.11, 0.08)	-0.03 (-0.11, 0.04)	p = 0.409	-0.09 (-0.17, -0.01)	**p = 0.023**
High	**N+ve/N-ve**	391/303	61/48	48/67				
	RD (95%CI)	0.11 (0.03, 0.18)	0.08 (-0.02, 0.19)	-0.05 (-0.15, 0.05)	-0.02 (-0.12, 0.07)	p = 0.599	-0.16 (-0.25, -0.07)	**p < 0.001**
		Aspirin*Tumor Grade	Additive scale: IC (95%CI)	Intermediate v Low	-0.09 (-0.27, 0.10)	p = 0.359	-0.03 (-0.19, 0.13)	p = 0.685
			Adjusted for age, tumor size, tumor grade, comorbidity, screen detection					
		Aspirin*Tumor Grade	Additive scale: IC (95%CI)	High v Low	-0.08 (-0.27, 0.11)	p = 0.418	-0.10 (-0.27, 0.07)	p = 0.241
			Adjusted for age, tumor size, tumor grade, comorbidity, screen detection					

N+ve: Node-Positive. **N-ve**: Node-Negative. **RD**: Risk Difference. **IC**: Interaction Contrast. **CI**: Confidence Interval

A) Dosing intensity by median. Dosing intensity calculated as number of days with supply of aspirin available in year prior to diagnosis, divided by 365.

APPENDIX 6: STUDY PROPOSAL REGISTERED WITH ENCEPP E-REGISTER OF STUDIES:

Aspirin use and prostate cancer mortality in men with high grade prostate cancer

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Background & Research Question

Aspirin exposure has been associated with reduced incidence of prostate cancer[1,2] and less advanced prostate tumours at diagnosis.[3] More recently, large meta-analysis and observational studies have reported associations between aspirin use and reduced mortality from cancer.[4,5] In particular, aspirin use in men with localised prostate cancer, has been reported to be most significantly associated with reduced mortality from prostate cancer in both meta-analysis[6] and observational studies.[7] In the latter study the most significant findings were in men with high-risk disease i.e. larger tumours, high PSA and high Gleason Score.

The hypothesis for the present study has evolved from this evidence and the findings of another study by the authors assessing associations between aspirin exposure and prostate cancer mortality in men with localised prostate cancer. The results suggested aspirin exposure to be associated with a modest non-significant reduction in prostate cancer-specific mortality (HR=0.90, 95% CI 0.68, 1.20) with high dose (>75mg) of aspirin having a more pronounced association with reduced mortality (HR=0.59, 95% CI 0.35, 1.00).

The mechanism attributed to aspirin's anticancer activity which has been investigated most extensively is the inhibition of cyclooxygenase enzyme 2 (COX-2). COX-2 expression in cancerous prostate cells is associated with higher Gleason Score,[8,9] distant metastasis,[10] biochemical failure and treatment failure.[11] It is biologically plausible that there is a stronger association between aspirin use and reduced prostate cancer mortality in men with high-grade prostate cancer. The vasculature close to the tumour and the newly generated tumour vasculature have been shown to express COX-2.[12] Another mechanism of proposed anti-cancer activity is the anti-platelet property of aspirin, which may inhibit the spread of tumour cells through the vasculature.[13] Considering these mechanisms through which aspirin may mediate an effect on prostate tumours, it is of interest to examine whether associations between aspirin use and prostate cancer mortality differ depending on if the cancer is localised or has progressed beyond the prostate to lymph nodes or other sites.

This cohort study will be carried out in men aged 50-80 years diagnosed with high-grade prostate cancer in Ireland using the linked database of the National Cancer Registry Ireland (NCRI) and the Primary Care Reimbursement Services (PCRS) pharmacy claims database. The study aims to assess whether there is an association between aspirin use and mortality, in men with high-grade prostate cancer and whether there is a difference in the association between aspirin use and mortality in men with localised compared to advanced disease.

Methods

Setting and Data Sources:

The National Cancer Registry Ireland (NCRI) database, which has been linked to Ireland's Health Services Executive (HSE) – Primary Care Reimbursement Services (PCRS) pharmacy claims database, will be used to conduct this study. The NCRI database is nationally representative. Detailed data on all incident cancers in the population of the Republic of Ireland is complied, with five-year tumour registration of prostate cancer estimated to be in excess of 96% complete.[14] Hospital-based tumour registration officers collect information on patient characteristics, tumour details and treatment received from hospital medical records. Tumours are recorded using the ICD-O system (Prostate neoplasm, C61).[15] The national death certificate register, which includes patient cause of death, coded as ICD-9 or ICD-10, is linked to the data at the NCRI.

The general medical services (GMS) scheme, provided by the HSE-PCRS, delivers state-funded universal healthcare, including prescription medicines, to approximately one third (1.4 million) of the Irish population. GMS scheme eligibility is assessed through means test and age; all persons over the age of 70 years were entitled to the GMS scheme prior to January 2009. The GMS database contains claims for all prescription drugs dispensed from community pharmacies to GMS patients. Drugs are coded according to the WHO Anatomical Therapeutic Chemical Classification (ATC) system.[16]

Linkage of various files is by an identifier generated by the NCRI. Cancer cases diagnosed from January 1st 2001 to December 31st 2006 have had prescription claims have been linked using probabilistic matching techniques. Follow-up of vital status is until December 31st 2010. This linked database has been used for similar studies before.[17] The use of data held by the NCRI for research purposes is covered by the Health (Provision of Information) Act 1997. Data utilisation agreements have been established with the NCRI. All potential patient identifiers are removed from the datasets prior to use. The data is to be stored on an encrypted drive on a desktop computer available only to the researcher.

Study cohort

Men aged 50-80 years at the time of prostate cancer diagnosis (ICD-O, C61),[15] diagnosed as having a tumour with Gleason Score histology > 7,[18] between 1st January 2001 and 31st December 2006 will be included in the study. Continuous eligibility for the GMS scheme for a full year prior to diagnosis is also required for inclusion. Men who received a prostate cancer

diagnosis at death or autopsy only and men with a prior invasive tumour other than non-melanoma skin cancer will be excluded.

Sample size will depend on the number of cases in the dataset which meet the inclusion criteria i.e. all men in the population who meet the inclusion criteria will be included. Formal power calculations have not carried out *a priori*.

Exposure definition

Prescriptions for aspirin and aspirin combinations dispensed to eligible men will be identified from the GMS database using WHO-ATC codes (see Appendix 1).[16] Aspirin users will be defined as men who have a supply of aspirin available in the year prior to prostate cancer diagnosis. The date, dose and number of days' supply on each prescription are recorded and will be used to stratify pre-diagnostic aspirin use by: (i) dosing intensity (high/low) split on the median proportion of days covered (PDC) with a supply of aspirin available in the year prior to diagnosis;[19] (ii) dose prescribed (low: only received dose ≤75mg / high: any received dose > 75 mg).

As low-strength aspirin indicated for anti-platelet activity is licensed as a prescription only medicine in Ireland, very low levels of misclassification of aspirin use due to over the counter purchases are anticipated. New aspirin use in the six months prior to diagnosis will be censored as a sensitivity analysis to guard against bias introduced by new aspirin users receiving aspirin for pain which may be due to cancer progression.

Outcome Definitions

Information from death certificates, provided by the General Register Office to the NCRI, will be used to identify the date and primary cause of death. Primary outcome: prostate cancer death (ICD 9 185; ICD 10 C61); Secondary outcome: any cause death. All men will be followed from the date of diagnosis to death or the end of follow-up (31st December 2010).

Study Covariates

The following patient demographics and tumour characteristics at diagnosis will be identified from the NCRI database: patient age (years); smoking status (current/ former/ non-smoker/ unspecified); and AJCC tumour stage (tumour size, nodal status, metastases).[18] Treatment type and date received in the year post-diagnosis is also captured in the NCRI data: prostate surgery (yes/no), radiation (yes/no) androgen deprivation therapy (ADT) (yes/no) or

chemotherapy (yes/no). Where data is missing for a covariate it will be retained in the analysis and classified as unspecified.

The prescription claims data will be used to determine a medication-based comorbidity score, based on the sum of distinct medication classes (as defined by the 5 character ATC code) received by each man in the year prior to diagnosis.[20] Prescription dispensing data will be used to identify exposure (yes/no) to other, potentially confounding, medication in the year prior to prostate cancer diagnosis: anti-diabetic agents, statins, non-aspirin anti-coagulants, non-aspirin NSAIDs, medication for the treatment of Benign Prostatic Hypertrophy (BPH). See Appendix 1 for WHO-ATC codes.

Statistical Analyses

Cohort characteristics will be tabulated to assess univariate differences between aspirin users and non-users. Cox proportional hazards models will be used to estimate hazard ratios (HR) with 95% confidence intervals (CI) for prostate cancer-specific mortality associated with aspirin use. Covariates are to be considered for inclusion in multivariate models based on prior knowledge of clinical and demographic predictors of prostate cancer mortality: age;[21] comorbidity score;[20] smoking status;[22,23] tumour size;[21] diabetes;[24] and exposure to beta-blockers,[17] statins,[25] non-aspirin anti-coagulants,[7,26] non-aspirin NSAIDS[25] and drugs used in BPH.[27] Also considered for inclusion in the model will be the year of prostate cancer diagnosis (continuous) and treatment received in the year following diagnosis: prostate surgery / radiation / androgen deprivation therapy (time-varying). A backward deletion method, with a 10% maximum change in the effect component of the fully adjusted HR will be used to select the final multivariate model.[28] The proportionality of hazard functions will be assessed by testing for the interaction between aspirin use and the logarithm of person-time (Wald test for product term).

Effect Modification

Analyses will be stratified by tumour stage to assess the potential for modification of the association between aspirin use and prostate cancer mortality according to whether the tumour has progressed to involve lymph nodes or metastases. Multiplicative interactions across strata of tumour stage will be determined (ratio of hazard ratios, rHR) with 95%CI.

Sensitivity Analyses

Due to the potential for misclassification of prostate cancer death on death certificates, sensitivity analyses are to be carried out. Firstly other cancer causes of death by which

prostate cancer may reasonably be misclassified will considered as prostate cancer deaths (See appendix 2);[29] and secondly death certificates where prostate cancer is recorded as a secondary or contributory cause of death will be considered as prostate cancer deaths.

Sensitivity analyses around aspirin exposure will also be examined to guard against the potential for protopathic bias which may occur as a result of men being prescribed aspirin as an analgesic for pain prior to the diagnosis of cancer.

Limitations

Although the cancer registry captures population-based cancer cases, the subset of men for whom data on medication exposure exists are those men eligible for the GMS scheme. As eligibility for the scheme is based on means test and age, older men and men of lower socioeconomic status are likely to be over-represented. However this is unlikely to confound the potential association between aspirin and prostate cancer mortality. It should be noted that only medicines dispensed on the GMS scheme have been linked, and medicines dispensed under other community drugs schemes are not captured. This is not considered to differ greatly between aspirin users and non-users. As the data is based on medicines dispensed, it does not necessarily mean men were adherent, however determining (high/low) dosing intensity does stratify men on their level of exposure.

Although measures have been taken to account, as far as possible, for confounding by comorbidities using a medication-based comorbidity score, there may be some unmeasured confounding associated with comorbidity. The comorbidity score to be used has been validated as a medication-based means of prediction of mortality, hospitalisation and long-term care admissions.[20]

There may be selection bias based on the selection of only men who had a histologically graded prostate biopsy, thus men who were not deemed fit for a biopsy may have been excluded, which may affect external validity

Time-frame, planning and dissemination

The analysis is to commence in February 2013 with write-up anticipated to be complete by April 2013. Further amendments to the data in this time frame are not anticipated.

This work is to be disseminated as an original research article in a peer-reviewed journal and conference presentations.

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Appendix 1

WHO ATC[16] Drug codes for medication exposures

Drug Exposure	WHO ATC Code
Aspirin & Combinations	B01AC06, M01BA03, N02BA01, N02BA51, N02BA71
Anti-diabetic medication	A10
Statins	C10AA
Non-aspirin anti-coagulants	B01A, excluding B01AC06
Non-aspirin NSAIDs	M01A
Benign Prostatic Hypertrophy	G04C

Appendix 2

Table S2.1: Potential other cancer sites which prostate cancer death may be misclassified:[29]

Cancer Site	ICD 9 Code	ICD 10 Code
Malignant neoplasm of prostate	185	C61
Malignant neoplasm of other male genital organs, site unspecified	187.9	C63.9
Malignant neoplasm of pelvis	195.3	C41.4
Secondary malignant neoplasm	196-198	C76-C80
Malignant neoplasm without specification of site	199	C80.9
Benign neoplasm of prostate	222.2	D29.1
Benign neoplasm of male genital organs, site unspecified	222.9	D29.9
Neoplasm of uncertain behaviour of prostate	236.5	D40.0
Neoplasm of uncertain behaviour of other and unspecified male genital organs	236.6	D40.9
Neoplasm of uncertain behaviour, site unspecified	238.9	D48.9
Neoplasm of unspecified nature of other genitourinary organs	239.5	D40.7, D41
Neoplasm of unspecified nature, site unspecified	239.9	D48.9

APPENDIX 7: LIST OF MEDICATION EXPOSURES REFERRED TO IN THIS THESIS

World Health Organisation Collaborating Centre for Drugs Statistics and Methodology, Anatomical Therapeutic Classification (WHO-ATC)[143] Drug codes used to define medication exposures referred to in this thesis

Drug Exposure	WHO ATC Code
Anti-diabetic medication	A10
Biguanides	A10BA; A10BD01; A10BD02; A10BD03; A10BD05; A10BD07
Aspirin and combinations	B01AC06; M01BA03; N02BA01; N02BA51; N02BA71
Non-aspirin anti-coagulants	B01A, (excluding B01AC06)
Warfarin	B10AA03
Digoxin	C01AA05
Antiarrhythmic agents	C01B
Antiarrhythmic agents Class I a	C01BA
Antiarrhythmic agents Class I c	C01BC
Class III antiarrhythmic agents	C01BD
Cardiac stimulants	C01C
Nitrates	C01DA
Other Cardiac agents	C01E
Low-ceiling diuretic	C03B
High-ceiling diuretic	C03C
Aldosterone antagonists	C03DA
Peripheral Vasodilators	C04
Beta-blocker	C07
Calcium Channel Blocker Vascular	C08C
Calcium Channel Blocker Cardiac	C08D
Verapamil	C08DA01; C08DA51
Angiotensin converting enzyme inhibitors	C09A; C09B
Angiotensin II receptor blockers	C09C; C09D
Statin	C10AA; C10B
Benign prostatic hypertrophy Medication	G04C
Non-aspirin NSAIDs	M01A

APPENDIX 8: PRESENTATIONS PERTAINING TO THIS DOCTORAL RESEARCH

ORAL PRESENTATIONS

Flahavan EM. *Cancer Pharmacoepidemiology* Trinity College Dublin June 2013. Trinity 3-Minute Article Competition, Trinity Graduate Students Union.

Flahavan EM, Bennett K, Sharp L, Barron TI. *A Matched Cohort Study Examining Digoxin Exposure and Prostate Cancer Mortality.* Barcelona, August. 2012. International Society of Pharmacoepidemiology Annual Meeting

Flahavan EM, Barron TI, Sharp L, Bennett K. *Socio-economic and marital status and their association with prostate cancer survival: A population-based study*. Dublin, November 2011, National Conference on Population-Based Cancer Research in Ireland

POSTER PRESENTATIONS

Flahavan EM, Bennett K, Sharp L, Barron TI. *Aspirin use and mortality in men with localised prostate cancer: A cohort study.* Chicago, May 2013. American Society for Clinical Oncology Annual Meeting

Flahavan EM, Bennett K, Sharp L, Barron TI. *Aspirin exposure and prostate cancer outcomes: a population based study in Irish men*. Cork, September 2012. International Association of Cancer Registries Annual Conference

Flahavan EM, Barron TI, Sharp L, Bennett K. *Socio-demographic factors associated with prostate cancer survival: a population based study*. London, October. 2011. The Prostate Cancer Charity, National Research Conference

Flahavan EM,* Spillane SC,* Bennett K, Sharp L, Barron TI. *Digoxin and Breast Cancer Mortality: a population based study*. Dublin, September 2011. Trinity College Dublin Cancer Conference 2011,

PROSTATE CANCER PHARMACOEPIDEMIOLOGY: DIGOXIN, ASPIRIN AND PATIENT OUTCOMES

Eva Flahavan December 2013

ABSTRACT

Pharmacoepidemiology is the study of the effects of medicines in a real-world population; combining pharmacology, the study of medicines, with epidemiology the study of diseases. Prostate cancer is the most commonly diagnosed non-cutaneous malignancy in Irish men and the second most common cause of cancer death. This thesis contains the first pharmacoepidemiology studies to be carried out in a cohort of Irish prostate cancer patients. These studies were carried out using linked patient records from the National Cancer Registry of Ireland (NCRI) and prescription claims data from the Primary Care Reimbursement Services (PCRS) General Medical Services (GMS) scheme. Exposure to two medicines, digoxin and aspirin, commonly used for the treatment and prevention of cardiovascular disease were examined in relation to prostate cancer patient outcomes.

Digoxin is a member of the cardiac glycoside family, and is prescribed as second line therapy in the treatment of atrial fibrillation and heart-failure. Digoxin and other cardiac glycosides have been shown to impede cancer cell growth and tumour progression in a variety of cancer types and in mouse tumour models. These anti-cancer activities have been attributed to the pharmacological activity of digoxin on the sodium/potassium ATPase pump, and the more recently documented effects of digoxin on gene transcription; demonstrated through inhibition of Hypoxia Inducible Factor-1α (HIF-1α) expression. Digoxin exposure has also been associated with reduced risk of prostate cancer.

In this thesis, two studies were carried out investigating digoxin exposure in men with prostate cancer. The first study examined the association between digoxin exposure prior to cancer diagnosis and tumour characteristics (stage or grade) at diagnosis; digoxin exposure was not found to be associated with tumour stage or grade at diagnosis. The second study investigated the association between digoxin exposure at diagnosis and prostate cancer-specific mortality. In this study no association was observed between digoxin exposure and prostate cancer-specific mortality in the main analysis or in a propensity score matched cohort. There are a number of possible reasons why improved outcomes were not observed in men with prostate cancer exposed to digoxin; the most critical of these is that the therapeutic plasma concentrations of digoxin in humans are much lower than those used in pre-clinical studies. However clinical research is on-going, investigating digoxin in patients with breast cancer and in the treatment of recurrent prostate cancer.

Aspirin is the most commonly prescribed drug on community drugs schemes in Ireland. It was originally used for its anti-inflammatory and anti-pyretic properties, mediated through the inhibition of cyclooxygenase enzyme-2 (COX-2). Currently aspirin is most commonly prescribed at low doses for its anti-thrombotic effects, as it reduces the risk of stroke and myocardial infarction. This effect is mediated through the inhibition of COX-1 in platelets. Inhibition of COX-1 and/or COX-2 by aspirin has been proposed to impede the development, growth and dissemination of a number of cancers, including prostate cancer.

The findings of observational studies investigating aspirin exposure and prostate cancer incidence have been equivocal; meta-analyses of these studies have reported aspirin to be associated with an approximately 10% reduction in risk of prostate cancer. Recent studies have also reported aspirin exposure to be associated with reduced prostate cancer mortality. The studies carried out in this thesis examined the association between aspirin exposure prior to diagnosis and prostate cancer-specific mortality in two cohorts; firstly in men diagnosed with stage I-III prostate cancer and secondly in men with prostate cancer of Gleason score >7.