ANTI-CANCER ACTIONS IN COMMONLY USED DRUGS: EPIDEMIOLOGY LED BY LABORATORY SCIENCE

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Abstract

Despite considerable research on cancer treatments and preventatives, poor outcomes in cancer patients are common. The vital search for effective cancer drugs often begins in the laboratory, where unfortunately the effects of a drug in humans cannot be perfectly modelled. Epidemiology can play a vital role in determining the real world efficacy of a drug currently used for other purposes before clinical trials begin. This thesis therefore used primarily laboratory evidence to identify potential anti-cancer uses for existing common drugs. The drugs and cancers studied were; tricyclic antidepressants and both incidence and survival in a number of cancer types, particularly glioma; aspirin and colorectal cancer survival; and angiotensin converting enzyme (ACE) inhibitors and hepatocellular carcinoma (HCC) incidence.

A series of studies using The General Practice Research Database as a data source assessed any potential associations: A case-control study for tricyclic antidepressant use and cancer incidence; cohort studies to examine mortality in colorectal cancer and glioma in relation to tricyclic use, and for colorectal cancer mortality in aspirin users; and a case-control study in relation to ACE inhibitor use and HCC

A strong, cancer type specific, dose and time dependant protective effect was found for the incidence of glioma and colorectal cancer. This led to a further study examining mortality for these cancer types in tricyclic users. While no significant protective effects in all-cause mortality of tricyclic users were found, a larger study could still find such an effect in glioma. For aspirin and colorectal cancer mortality, a small but significant reduction in mortality was observed, though these effects were not entirely consistent throughout the study. There were no significant associations found between ACE inhibitors and HCC. These findings contribute to the knowledge of the anti-cancer effectiveness of these drugs, and may assist in designing future clinical studies.

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Contributions

This work was carried out by myself, aside from the contributions detailed above. The normal supervisory process was carried out by my various supervisors and included assistance such as advice on statistical analyses and diversion toward appropriate literature and other resources. The work carried out by me included: writing study protocols, acquisition of data for all studies except the hepatocellular carcinoma study, data processing, data analysis,

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Abbreviations

←	Indicates a statistically significant result
ACE	Angiotensin Converting Enzyme
BMI	Body Mass Index
BNF	British National Formulary
CI	Confidence Interval
COX	Cyclooxygenase
GI	Gastrointestinal
GP	General Practice/General Practitioner
GPRD	General Practice Research Database
HCC	Hepatocellular Carcinoma
HR	Hazard Ratio
KM	Kaplan-Meier
NHS	National Health Service
NSAID	Non-Steroidal Anti-Inflammatory Drug
OR	Odds Ratio
OXMIS	Oxford Medical Information Systems

1 Introduction

Cancer, conventional therapy and new approaches

1.1 Introduction

Effective treatment and prevention of cancer is amongst the most sought after aims in medicine. It is certainly an area of research in which a vast amount of money is being spent. Cancer Research UK alone spent £334 million on cancer research activities in 2009/10. Advances in knowledge of the molecular biology of cancer have led to identification of drug targets. I*n vitro* and *in vivo* laboratory drug testing can give important indications of their effects on cancer models. But while these approaches are vital, they cannot perfectly model of how a drug will behave in humans.

However, testing the effects of a treatment in humans using clinical trials is extremely expensive. Despite good pre-clinical work and great expense, many drugs entering into clinical trials are found to be ineffective, for example, marimastat in treating breast cancer (Sparano et al. 2004). To reduce the likelihood of this occurring gaining as much data as possible about the effect of a drug in man before beginning full scale clinical trials is of great value. It is here that high-quality epidemiology can provide an important link between the lab and clinic, in providing real world data on patients, without the expense and long timescales inherent in clinical studies. In addition to this, epidemiological studies are inherently good for looking at long term effects of drugs, as data sources often have long patient follow up times, compared to clinical trials.

This chapter provides an overview of cancer, its treatment, and advances in the molecular biology of cancer that are leading to increasingly targeted treatment. This ever improving understanding leads to in depth knowledge of the molecules involved in cancer development and how they interact with drugs. It is this mechanism led approach that has helped to identify the drug and cancer combinations found in this study.

The first such mechanism to be identified in this study is mitochondrial modulation. This led to the identification of tricyclic antidepressants as a potential anticancer drug (Daley et al. 2005), and subsequently to two of the studies in this thesis involving incidence and survival in various cancer types. Recent interest in the effect of non-steroidal anti-inflammatory drugs (NSAIDs) and cancer, as well as their putative cyclooxygenase-2 inhibition mechanism led to another of the studies in this thesis, investigating NSAID use and colorectal cancer survival. Finally, angiogenesis is a vital step in carcinogenesis and while drugs targeting this process are already in use, other drugs that are thought to do so are being identified, such as ACE inhibitors.

With this in mind, the aims of this thesis are:

1. To determine the effects of tricyclic antidepressants on cancer incidence
2. ...as well as determining their effect on post diagnosis cancer survival.
3. To investigate aspirin and colorectal cancer survival.
4. To determine if ACE inhibitors have an effect on hepatocellular carcinoma incidence.

This chapter also contains an outline of each of the subsequent chapters, which include each of the studies carried out for this thesis. It also contains a description of the source of all data for this study, the General Practice Research Database (GPRD), as well as an outline of the commonly used methods within the thesis.

1.2 Cancer

1.2.1 What is it?

Cancer is a vast and diverse spectrum of diseases, all of which are characterised by the uncontrolled growth of cells, often involving invasion into surrounding tissues and sometimes metastasis into distant organs. One particular review in the journal Cell has become almost ubiquitously cited in any description of cancer (Hanahan et al. 2000). This describes the 6 major changes that occur in virtually all cancer types during cancer development:

- Evading apoptosis
- Self-sufficiency in growth signals
- Insensitivity in antigrowth signals
- Sustained angiogenesis
- Limitless replicative potential
- Tissue invasion and metastasis.

Typically, development of cancer occurs in stages, from precancerous lesions through to metastatic malignancies. These stages often correspond to the changes described above and occur at a molecular level within the cells. This is driven by mutations in DNA, accumulated usually over a long time. These occur in two classes of genes known as oncogenes and tumour suppressor genes. Sometimes one of the above changes can be attributable to a mutation in a single gene, as in the case when activation of the telomerase gene causes cells to have limitless replicative potential. In many cases however, multiple mutations are required in order to circumvent the redundancy commonly found in biological pathways.

1.2.2 Causes

The causes of cancer mostly involve environmental exposures. This can be either through exposure to DNA damaging agents, such as ionising radiation or chemical carcinogens, like the polycyclic aromatic hydrocarbons found in tobacco smoke. For certain cancers, dietary exposure to carcinogens is thought to be an important factor, for example, heterocyclic amines found in red meat may be responsible for an increase in risk of colorectal and other cancers (Sinha 2002).

Genetic background is also an important factor in the development of cancer, though environmental factors always play a key role in development, regardless of genetic background. There are a number of examples of commonly occurring mutations in genes, which can make people particularly susceptible to particular cancer types. Perhaps the best characterised of these are BRCA I and BRCA II genes, which greatly increase the chance of an individual getting breast or ovarian cancer when mutated (King et al. 2003; Kadouri et al. 2007).

1.2.3 Cancer staging/grading

At diagnosis, cancer is typically given a number of classifications in order to aid in determining prognosis and the most effective treatment option. The stage of cancer describes how much a cancer has spread, usually taking into account factors such as tumour size, penetration into surrounding tissues, lymph node involvement and presence of distant metastases. The staging system differs depending on cancer type, but the most commonly used is the TNM system:

T relates to the tumour size (numbered 1-4)

N describes lymph node involvement (numbered 0-3)

M describes metastasis (numbered 0-1)

Other letters can be used to describe various parameters of the cancer, such as grade. Grading depicts the level of anaplasia in a cancer, which is an important prognostic indicator. A patient with a well differentiated (low grade) tumour will generally have a better prognosis than on one with a poorly differentiated (high grade) one.

1.3 Conventional cancer treatment

The mainstay of cancer treatment for the majority of solid tumours is surgery. This usually occurs early during treatment, with the aim of removing the bulk of cancerous cells. This can occur alongside other therapies, such as radiotherapy and chemotherapy, although for some cancer types one of these treatment options may be used in isolation.

Outside of surgery, the basic premise of all cancer therapy lies in the presence of a therapeutic index. This means that a treatment is more toxic to cancer cells than normal cells. Generally the wider this 'gap' in toxicity, the more effective the treatment. For conventional therapy this therapeutic index exists due to cancer cells being particularly sensitive to things relating to their high rate of cell division, such as DNA damage.

Radiotherapy aims to damage DNA by bombarding the cancer tissue with ionising radiation. This happens either directly (i.e. the radiation directly breaks bonds in DNA) or through creation of free radicals which then go onto damage DNA (Dunne-Daly 1999). It is usually administered in a number of fractions, separated by a time period which allows the normal tissues to recover.

"Conventional" chemotherapy drugs act through a variety of mechanisms, which can be classified into a number of different categories. Cytotoxic drugs aim to cause catastrophic damage to cancer cells, either through damage to DNA (e.g. alkylating agents, topoisomerase inhibitors), or by preventing DNA replication/division (antimetabolites, antimitotic agents). Hormonal therapies are used where the cancer type is reliant on

hormonal signalling for continued growth/existence (e.g. some prostate and breast cancers). Removal of these hormone signals by for example hormone receptor antagonists can lead to growth inhibition or death of the cancer cells.

While often effective in treating cancer, all three of these treatment options are well known for their adverse side effects. These are due to damage to normal cells which occurs alongside damage to the cancer cells. For surgery, often a wide margin of surrounding normal tissue is removed with the tumour, in order to try to remove any cancer cells which have invaded the surrounding tissue. With radiotherapy, there is inevitably some surrounding tissue which gets exposed to the radiation. This can cause acute problems such as nausea or blood cell depletion, which are specific to the site being irradiated. Problems can also emerge later, such as increased tissue fibrosis, tissue damage and a small increase in risk of another cancer developing. As chemotherapy is almost always administered systemically, it is renowned for its detrimental effects to other rapidly dividing tissues, such as hair follicles, immune suppression. Though these can be managed to some extent during treatment, these side effects are often a limiting factor in chemotherapy and can lead to an enforced reduction or premature halting of therapy, while factors such as white blood cell count recover. This can of course lead to reduced efficacy.

1.4 Targeted cancer treatment

The relentless drive of cancer research has gradually begun to change cancer treatment to be increasingly targeted. This targeting is made possible by the vastly increased knowledge of the molecular biology of cancer cells. The development of new targeted agents typically follows a path involving identification of a target gene or protein involved in cancer, and then determining an agent which will act on it to repress or induce its function. Though many agents with potential for treating cancer have been identified, as yet relatively few have actually made it into clinical use.

Perhaps the most successful class of early targeted agents are monoclonal antibodies. These drugs are designed to bind to cell surface proteins and thus block their action An example of this is bevacizumab (trade name Avastin). This drug binds to a protein called vascular endothelial growth factor (VEGF), which is involved in induction of angiogenesis. Bevacizumab is often used in combination with cytotoxic drugs, and was first shown to be effective in metastatic colorectal cancer (Kabbinavar et al. 2003; Kabbinavar et al. 2005; Tol et al. 2010), but has also found use in other cancer types (Van Meter et al. 2010). Such versatility is due to angiogenesis being a vital step in cancer development for all solid tumours.

Trastuzumab (trade name Herceptin) is another targeted cancer drug that has made it into clinical use. Another monoclonal antibody, it is primarily used in late stage breast cancers that over-express the HER2 protein. HER2 is a cell surface protein involved in cancer related signalling pathways such as the PI3K/Akt pathway (Bange et al. 2001; Menard et al. 2003).

1.5 Chemoprevention

Given the sometimes ineffective and side effect riddled nature of current cancer treatments, prevention is clearly a desirable aim. Cancer chemoprevention can encompass a multitude of different methods, from lifestyle choices to drug treatment.

There have so far been few successful examples of drugs that can be used to protect against cancer. Aspirin is one of the only truly successful examples of a working chemopreventative drug, though vast amount of research, public interest and media attention is directed at this area. While some of the media interest may be somewhat sensationalist, the list of potential cancer chemoprevention compounds like resveratrol (Jang et al. 1997), antioxidants, curcumin (Patel et al. 2010), dietary fibre (Asano et al. 2002) and capsaicin (Athanasiou et al. 2007b) is ever growing. There is of course some basis

for these claims, but it remains difficult for any nutritional compound to be proven as efficacious, as they are by nature not patentable and therefore of little interest to drug companies.

The most obvious alternative to this is a novel drug, which may be patented and developed by drug companies. However, a third option exists in that there is a vast array of drugs already in common use, many of which may have as yet undiscovered functions. These functions can be assessed using epidemiology.

1.6 Pleiotropy

Due to the complexity inherent in all biological systems, it is a near certainty that any drug will have multiple pharmacological effects. This can be due to many factors such as drug binding to multiple target molecules, multiple downstream effects after binding with one target or differential effects in different cell types. In most cases, this pharmacological complexity leads to unwanted side effects. A good example of this is aspirin, which can bind to both the cyclooxygenase-1 and cyclooxygenase-2 (COX-1 and COX-2) proteins. This causes different effects in different cell types, due to variation in expression of these proteins among tissues, and leads to unwanted side effects, like gastrointestinal bleeding. It is therefore valuable in many cases to develop drugs that are as selective as possible to their intended target, in order to reduce such side effects.

However, in the case of some drugs these alternative effects may be positive, and may lead to alternative uses for these drugs. Somewhat amazingly, aspirin is once again an example of this. Its Inhibition of the COX enzymes regulates compounds such as thromboxanes, which are involved in platelet aggregation. Through this mechanism, aspirin is thought to be beneficial in reducing cardiovascular events.

Aspirin is not alone in this amazing ability to perform multiple functions. All sorts of functions could exist for a huge array of drugs. It is simply a matter of testing for these functions.

1.7 Chapter outline

Chapter 2 is a detailed review of mitochondrial modulation in cancer therapy, as it was pivotal in the initiation of this study and is relevant to chapters 3 and 4.

Chapter 3 describes a case control study investigating whether tricyclic antidepressant use modifies the risk of subsequently developing nervous system, breast, colorectal, lung and prostate cancers. The study takes a particular interest in glioma (a subcategory of brain tumour), which is thought to be particularly sensitive to them.

Chapter 4 describes a cohort study to determine whether tricyclic antidepressants affect survival in glioma and colorectal cancer patients. These cancer types were chosen after some associations were found between drug use and their incidence in the Chapter 3 study.

Chapter 5 describes a cohort study to determine whether aspirin and other non-steroidal anti-inflammatory drugs affect survival in colorectal cancer patients. Recent evidence has emerged to suggest that aspirin may improve post-diagnosis survival.

Chapter 6 describes a case control study to investigate the effect of ACE inhibitor use on hepatocellular carcinoma (HCC) incidence. HCC is a cancer with well-defined high risk groups, meaning patients could benefit if laboratory evidence suggesting an anticancer action for ACE inhibitors translates to an effect in humans.

Chapter 7 contains a summary of the findings in this thesis and attempts to put these findings into perspective in terms of implications for clinical use of the studied drugs. The chapter also includes recommendations for further studies relating to these drugs and cancers.

1.8 Cancer types in this study

The cancer types used in these studies are a mixture of the most commonly occurring cancers such as breast cancer and less common cancers like glioma. These types of cancer were all chosen on the basis of previous evidence that they may be affected by specific drugs, as described in the introduction to each chapter. Statistics for cancer incidence, prevalence and death rates amongst other things are compiled routinely by Cancer Research UK (CRUK). All incidence and mortality statistics in this section relate to the 2008 version of these CRUK data unless otherwise stated (http://info.cancerresearchuk.org/cancerstats/).

Breast cancer is the most common cancer in the UK, with a crude annual incidence of around 78 per 100,000, making up around 16% of all cancer cases. This is despite affecting almost exclusively women (there are ~350 cases in males each year). The importance of this cancer can therefore not be overstated. As a result, screening for breast cancer by mammography is common in many countries including the UK. The benefits of this screening are questionable however due to the small absolute risk reduction, combined with problems such as over diagnosis caused by false positive results (Olsen et al. 2001). Prognosis for people with this cancer has been improving steadily over the last 40 years, with 5-year survival increasing from around 50% during 1971-75 to around 80% during 2001-2006.

Risk factors for breast cancer include intrinsic factors such as increasing age, later menarche, later menopause and genetic predisposition (such as BRCA 1 and 2 mutations). Lifestyle factors such as alcohol use, smoking, obesity contraceptive pill and childbearing have also been implicated in breast cancer development to some extent (Key et al. 2001).

Lung cancer has a crude annual incidence of ~75 per 100,000, only fractionally less than that for breast cancer. However, prognosis for lung cancer patients is dramatically worse than for breast cancer patients, with a 5 year survival of around 8% between 2001 and 2006. This makes it the most common cause of cancer related death in the UK so it is clearly an important public health issue.

Smoking is overwhelmingly the greatest risk factor for lung cancer, and in fact has been implicated in development of at least 12 other cancer types (Doll et al. 2005). Risk of lung cancer is strongly associated with the intensity and duration of smoking (Lubin et al. 2006), though this risk reduces substantially or even entirely after cessation of smoking (Crispo et al. 2004). Other more minor risk factors for lung cancer include exposure to air pollution and radon gas, physical activity, poor diet and alcohol consumption.

Colorectal cancer is also common, with a crude annual incidence of ~65 per 100,000. It is the second most common cause of cancer related death after lung cancer, with a 5 year survival rate of around 50%.

Diet is thought to be an important risk factor in colorectal cancer development, though gaining reliable data on dietary factors in patients is inherently difficult, due to factors such as recall bias. There are however large, multinational studies such as the European Prospective Investigation into Cancer and Nutrition (EPIC), which have investigated dietary components such as red and processed meat (Norat et al. 2005), and fibre (Bingham et al. 2005).As alluded to above, tobacco use is thought to increase risk of colorectal cancer (Botteri et al. 2008), as is alcohol use (Fedirko et al. 2011). Obesity is also thought to be significant in colorectal cancer development, with increasing level of obesity corresponding to increased risk (Moghaddam et al. 2007). In contrast with these risk factors, regular use of non-steroidal anti-inflammatory drugs is thought to decrease risk of colorectal cancer (Cuzick et al. 2009; Half et al. 2009).

Prostate cancer is the most common cancer in the male population in the UK with an incidence of around 120 per 100,000. Although this statistic seems higher than for the above cancers, it does exclude women as for obvious reasons the prostate cancer occurs exclusively in men. 5 year survival in prostate cancer patients is around 77%, though due to the often advanced age of many prostate cancer patients it is common for patients to die of factors not related to the cancer itself. Hence these patients may be described as dying *with* cancer rather than *from* cancer.

Age is the most influential factor in determining risk of prostate cancer, with the incidence rising sharply from the age of 50 to peak at 751 per 100,000 between 75 and 79. Research on alcohol use and prostate cancer has been conflicting (Dennis 2000; Bagnardi et al. 2001), and the same is true for smoking (Doll et al. 2005; Gong et al. 2008).

Brain tumours are substantially less common than the above cancers, making up just 2% of diagnosed cancers. With an annual incidence of 8 per 100,000 this may seem like a less important public health issue. However, with 5 year survival rates as low as they are (11-16% between 2000 and 2001) and little improvement in this survival in the previous 30 years, brain tumours appear to have had relatively little research directed at them. Gliomas make up around half of brain tumour cases and late stage high grade versions of these such as glioblastoma multiforme have especially low survival rates.

Risk factors for brain tumours are not well defined. Age does play a role, with increasing age corresponding to greater incidence. However some types of brain tumour (such as medulloblastoma) are more common in younger patients. Previous radiotherapy treatment is identified as a possible risk factor (Little et al. 1998). Despite extensive research, evidence for other well publicised potential risk factors, such as mobile phone use is thus far unsubstantiated (Swerdlow et al. 2011).

Hepatocellular carcinoma is a subcategory of primary liver cancers and is the most common of these. Annual incidence of liver cancers in the UK is 5.9 per 100,000. 5 year survival for liver cancers is less than 6%, making the situation for liver cancer patients even worse than for those with brain tumours.

Hepatocellular carcinoma is commonly a condition caused by chronic conditions which damage the liver. This can include cirrhosis, hepatitis B and C infection, alcohol use, and haemochromatosis. While these chronic liver diseases contribute greatly to risk of hepatocellular carcinoma, other predictors of the cancer include male gender, diabetes (El-serag et al. 2004) and increasing age (Fattovich et al. 2004).

1.8.1 Covariates

The covariates used in this study were justified to a large extent on the basis of the risk factors detailed above. Covariates that are common to all the studies are age, smoking status, BMI and alcohol use. These factors were chosen as *a priori* confounders, due to their very frequent implication in cancer development. Age is known for 100% of the participants in these studies. Having a known age is a requirement of GPRD quality controls and is therefore effectively an inclusion criterion. It is also likely that the patient age is accurate, as this is recorded as year of birth and calculated using the date of (cancer) diagnosis.

Smoking status recording in the GPRD is variable depending on when the data were exacted from the database. Early versions have only around 50% recording, whereas in later versions, recording is almost complete. Accuracy of smoking recording is potentially questionable however, as it is dependent on several factors, such as accurate patient reporting and frequency of status updates. Additionally it is possible that those with for example lung disease will have different reporting patterns than those without, which

potentiates some bias in the reporting. However, regardless of whether the categories of smoking status are accurately coded, it is certain that there will be differences in smoking habits between the people in different categories. This means that some assessment of the impact of smoking on an outcome can be made, so long as it is remembered that the data are not perfect and therefore residual confounding may exist.

The issues with smoking status recording are essentially the same for alcohol use and BMI reporting. Alcohol use relies again on patient reporting, while BMI is measured by the GP taking weight and height measurements, which while common, are not carried out universally. Once again people with health issues relating to these factors may be have these factors reported more frequently. As with smoking though these data can still be used to inform about the effect of these factors, provided their limitations are remembered in interpretation.

Other covariates that were used include:

- Depression- this may be linked to cancer development (Reiche et al. 2004) and is clearly related to prescription of tricyclic antidepressants. This was defined by a medical code for depression.

- Non-steroidal anti-inflammatory drug use- which is thought to reduce incidence of colorectal cancer (Elwood et al. 2009; Iwama et al. 2009; Din et al. 2010) and may well be concurrent with other drug use, such as tricyclic antidepressants. This was defined by a record or two or more NSAID prescriptions.

- Comorbidity (Charlson Index)- is thought to be a predictor of prognosis to some extent (Charlson et al. 1987). It was derived using various code lists which code for multiple diseases, such as AIDs, heart disease, lung disease, diabetes and stroke. When the index is calculated, a score is created for each patient with each detected

disease in an individual adding 1, 2, 3 or 6 to their score. These scores depend on the perceived increase in risk of death caused by each disease (for example AIDs gives a score of 6, whereas dementia only increases the score by 1). The total score can then be used in a multivariate model to adjust for comorbidity, which should in part account for the differing prognosis of patients at the start of a survival study. The algorithm used to determine Charlson Index was adapted by me from an existing algorithm in my department (developed previously by Dr Tim Card).

- Chronic liver disease- is a well-known predictor of HCC (Tsukuma et al. 1993; Fattovich et al. 2004). This was defined by a diagnosis code for: alcoholic liver disease, cirrhosis, haemochromatosis, hepatitis, cholangitis or alcoholic/non-alcoholic fatty liver. Relatively few of the patients in the study had one of these codes, and the decision was taken to combine these into one category in order to maximise the limited power which exists for these variables and simplify interpretation.

- Diabetes is thought to increase risk of hepatocellular carcinoma (El-serag et al. 2004) and ACE inhibitors are sometimes prescribed as part of treatment for diabetes. They therefore could be a confounder between HCC and ACE inhibitor use.

1.9 The GPRD

The GPRD is a prospectively gathered, anonymised database encompassing around 500 GP practices throughout the UK and is the largest of its type, with around 43 million patient years of data spread across approximately 4.8 million patients. This amounts to about 8% of the UK estimated population and covers around 7% of UK GPs.

1.9.1 History

Originally known as the VAMP (Value Added Information Medical Products Ltd) Research Databank, it was set up to supply participating GPs with computers and software, in return for providing anonymised patient data of a specified standard. After a brief spell under ownership of Reuters Health Information, the database was donated to the Department of Health. It is currently operated by the Medicines and Healthcare Products Regulatory Agency (MHRA). The business model has also changed since its inception. Rather than directly providing computing equipment in return for their data, the GPRD now gives GPs a financial contribution for each patient, as well as feedback on the quality of their data.

1.9.2 Recording cancer in the NHS

Cancer is initially identified and diagnosed in a number of different ways. These vary by things such as the type of cancer, the stage of cancer at diagnosis and on the presence or absence of screening programs.

The most obvious first point of contact is a GP. If a patient is symptomatic, they will often see their GP, who would then determine whether it is appropriate to refer the case to a specialist. Where referral does take place, this usually causes a record to be created to document the occurrence. These records are however often non-specific and/or uncertain in nature due to the very early stage of the diagnostic process. For example, the read code 1J0E.00 (Suspected lower gastrointestinal cancer) is commonly used to record a referral.

However the recording of this code is relatively uninformative for research, as it neither confirms the presence of a cancer nor does it specify the precise type of cancer.

More useful records are usually generated when the specialist to whom the patient has been referred reports back to the GP. In this situation, the specialist may for example write to the GP, who would then interpret this to determine the appropriate code to record in the patient record. This process could return a code which confirms the presence of cancer, sometimes with detail of what type of cancer, or may return a code such as 1I2..00 (No evidence of cancer found). This is clearly more useful for research purposes.

As cancer is usually a condition that requires extensive treatment and often multiple consultancies to determine the exact type, there is often a great deal of dialogue between secondary care and GPs. This dialogue leads to further entries being recorded in the patient record, with more specific diagnoses, or treatments such as surgery or chemotherapy. These records are potentially useful to research, to give further detail or validation to the diagnosis of a patient.

An alternative to identification of cancer than directly through the GP is through screening programmes. These only exist for some common cancers, notably breast, colorectal and prostate cancer. There are often read codes generated by the GP to denote attendance at such screening programmes. These codes are not in themselves informative, as patients are routinely invited to screening, regardless of the presence of cancer symptoms. However, the results of the screening are sent to the GP, which would provide information on the presence or absence of cancer. It is worth mentioning however that if there were a positive finding in a screening there would invariably be further diagnostic tests, such as a fine needle aspiration, biopsy, or radiological test to confirm the diagnosis. These would of course also create electronic records.

It is of course possible that information received from secondary care might be misclassified by the GP. This could occur either through miscommunication, or by a lack of expertise in the interpreter (communication from secondary care can be recorded at the GP by administrative staff rather than by a doctor. However, given that there are often multiple codes recorded for each patient, this allows for some checking of diagnoses. Also the multiple validations of GPRD diagnoses give some reassurance that events are accurately coded in most cases (Jick et al. 1991; Jick et al. 2003; Fombonne et al. 2004; Herrett et al. 2010).

1.9.3 GPRD data collection and quality control

All currently contributing practices use the same software program to collect data. This allows for easier and more consistent data collection across GPs. The Vision clinical system (In Practice Systems Ltd) is used in around 22% of UK GPs as a tool for practice management.

Each patient has a unique record within each practice, which contains basic clinical details such as age, gender and height, all of which are entered by the GP. Each time a patient visits a GP, a consultation record is opened, which allows the clinician to enter any number of details about the patient, including symptoms, diagnoses and referrals. In addition to this, any prescription that is created for the patient is automatically added to the consultation record, which results in a very complete prescription record. For each type of record, standardised forms and pop up boxes are used to encourage data collection to be as complete as possible.

Data from each contributing practice is collected at monthly intervals by the GPRD. At the point of collection, all records are anonymised by stripping out the name, NHS number and any other data allowing patient identification. The data is assessed by the GPRD at this

point and must reach certain standards in terms of completeness and accuracy of data. If the practice does not meet these standards, it is marked as not up to standard and data is not used until the required standard is reached.

1.9.4 Advantages

Perhaps the greatest advantage of the GPRD, over other data sources is its size. With around 11 million patients (both current patients and those who have died or left the practice) and also 66 million person years in most recent version, many studies using the database can have good statistical power. The additional benefit of this is that it allows the study of rare diseases and/or exposures, for example glioma.

The database has been widely used in research, resulting in the publication of more than 750 original research articles and reviews and its validity has been well documented in a number of studies (Jick et al. 1991; Jick et al. 2003; Fombonne et al. 2004; Herrett et al. 2010). Due to the various requirements and controls specified by the GPRD, the quality of data is generally very high. For example, prescribing data is automatically recorded for every prescription, which allows all such data to be recorded with little potential for things such as recall bias. This makes the prescribing data much more accurate than patient recorded exposures.

The database contains records of all data necessary for daily clinical management of patients. This allows various factors to be taken into account during study design/analysis. For example during cohort selection, patients can be included or excluded according to factors such as previous cancer diagnoses. Additionally, these clinical details can be used to adjust for confounding during analysis.

Dose received can be derived from the data. This is in contrast to patient reported drug use data, which are often only qualitative or semi-quantitative. The studies in this thesis could

use the precise dose and number of prescriptions for a high proportion of cases. This allowed for more reliable assessment of dose response, which can give a better indication of whether an effect is causal.

The data in the GPRD are readily available, in that it is a by-product of health care delivery. This saves money due to no need for additional data collection or surveys. It also has the additional benefit that there is no waiting for data to accrue as with a prospective cohort study, thus saving a substantial amount of time. The data are inherently less prone to selection bias than questionnaire based studies, as no informed consent is required from the patients, so a whole cohort can be selected without worrying about response rates etc.

Practices from the GPRD are reasonably distributed to cover the whole geographical population, meaning that studies are therefore more easily generalised. Additionally any imbalance that does exist in the geographical distribution is unlikely to create false associations as patients can be matched by practice to virtually eliminate any biases. This matching of control patients within the same practice also helps to make them more comparable with cases. For example, it may limit differences in socioeconomic status to some extent.

1.9.5 Disadvantages

While data quality and completeness within the database is continually improving, there are some drawbacks to using the data.

Despite the database being the largest of its kind, there are still insufficient data in some cases. For example in this thesis, only a small number of exposed glioma cases existed in the database (see chapters 3 and 4), though still the largest glioma study in humans. Additionally, there were only a small number of certain HCC patients (chapter 6).

The database only contains data that is routinely recorded by GPs and this limits particularly aspects in a patient's background. For example lifestyle data such as exercise and nutrition are recorded only sporadically. In addition, socioeconomic data, while available, is currently only recorded at a practice level and therefore generalises the socioeconomic status of every patient at a particular practice. It is therefore not possible to rule out confounding due to these factors. However, in for example the tricyclics and incidence study (chapter 3), good lifestyle is likely to be linked to both lower cancer incidence and lower antidepressant use. It is therefore an unlikely explanation for my findings, though the effect estimate may still be biased despite this. The recording of some other confounders may also be somewhat unreliable. For example alcohol use has far too many non-users, suggesting that these may be misclassified as ex-heavy users. These data may still be used to partly adjust for confounding, as there will still be differences in patient alcohol use between categories. Recording of data such as this has been getting better over time. For example the oldest data used in this thesis (Chapter 6) has ~45% missing smoking data, whilst the two subsequent versions have ~10% missing (Chapter 3) and ~5% missing (Chapters 4 and 5).

While the database has been running for over 20 years, it is quite common for patients to move practices, and this can limit the duration of a patient's follow up. This limited long term follow up means that for survival studies, it is difficult to say what the effects of treatment are after a few years.

Data in the core database is also limited to things recorded at a primary care level and this does not reliably include important information such as reason for death or indications of disease severity, such as cancer grade or stage. This is important in that it would allow assessment of the effect of drugs on cancers prescribed at different stages. For example, early stage may respond to treatments better than late stage. Also cancers diagnosed in

late stage may be equivalent to one diagnosed in early stage before the study period (i.e. it is effectively a prevalent cancer, but was just not diagnosed early enough).Development of linkages to secondary care databases, such as hospital episode statistics and cancer registry data is on-going, and should help to address some of these issues.

Despite good validation of diagnoses within the GPRD, Read codes were not designed for use in research. This means that some of them are ambiguous and/or give little information on the diagnosis. As a consequence, some patients could be included as cases erroneously, which would likely reduce any size of effect found by adding random 'noise'. Additionally, some true cases may be missed which would reduce the power of the study.

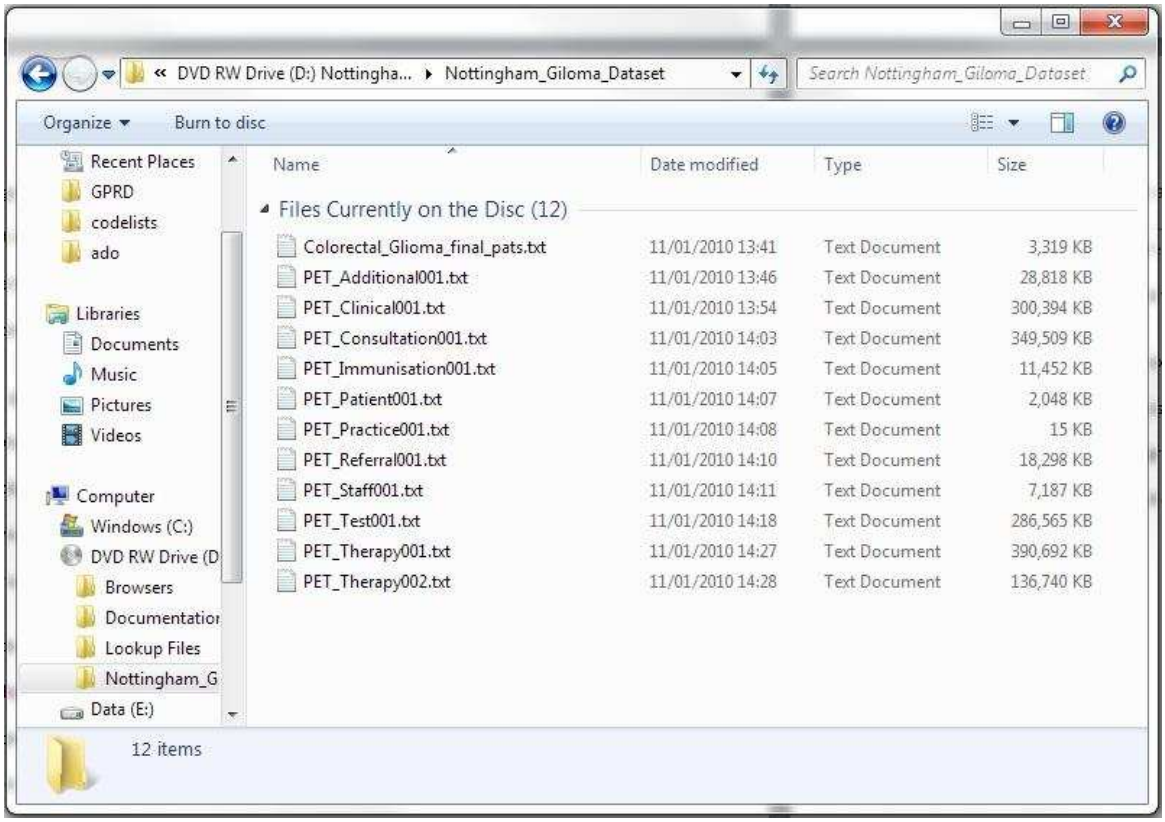
The data do not include over the counter (OTC) drug use. This is more significant for some drugs than others, for example aspirin OTC use may be important, although studies on chemoprevention of colorectal cancer have shown that regular use is necessary for effective prevention. OTC use would most likely be occasional, for a headache etc., whereas GP prescribing would mostly be for low dose. The data also do not include hospital prescribing, though this is less significant for these studies as the drugs studied are most frequently prescribed for chronic conditions, and would therefore be infrequently prescribed in hospital. There is also some missing data which relates to prescription dosing. This missing data is usually due to non-completion by the GP. This means some patients would have to be excluded from the dose-response analysis. It is likely that exclusion of these patients is unlikely to introduce bias, but would reduce power.

Though prescribing data are accurately recorded in the GPRD, there are no data on whether prescribed drugs are actually used. This may however reflect real world prescribing and use. Furthermore, my use of ≥2 prescriptions in determining exposure will limit the effect of patients who receive one prescription and do not use it.

1.9.6 Data format and access to the data

The database provides data on patients including clinical diagnoses, treatments and outcomes. This is received on a disc, which contains multiple files (Figure 1.1). These files contain separate types of data, such as clinical details, referral events, therapy events and a file giving general information on each patient in the dataset.

Figure 1.1 Files contained in a dataset distributed by the GPRD



Within each file there is a variable containing a unique anonymised code to identify the patient that each record relates to. Linked to these are various details relating to that patient. These details vary depending on the purpose of the file. For example the patient file (Figure 1.2) contains details such as dates of registration, death dates, year of birth, gender and various other data relating to the patient's registration.

Figure 1.2 Contents of the patient file from the GPRD

patid	vmid	gender	yob	mob	marital	famnum	chsreg	chsdate	prescr	capsup	ses	frd	crd	regstat	reggap	internal	tod	toreason	deathdate
392001	2661	1	136	0	6	1152	2		0	4	0	11/03/1988	11/03/1988	0	0	0		0	
1005001	9888	1	138	0	2	4088	2		0	4	0	23/02/1972	23/02/1972	0	0	0	19/01/2006	1	05/01/2006
1560001	2663	2	119	0	3	1154	2		3	4	0	27/04/1971	27/04/1971	0	0	0	23/06/2000	1	04/06/2000
2229001	514	1	124	0	6	235	2		4	4	0	11/03/1988	11/03/1988	0	0	0		0	
2371001	7167	1	140	0	6	2994	2		0	4	0	30/03/1991	30/03/1991	0	0	0	14/04/2005	1	23/03/2005
2536001	7797	1	125	0	2	3245	2		0	4	0	29/04/1964	29/04/1964	0	0	0	27/03/2003	1	08/03/2003
2815001	5557	1	116	0	3	2358	2		4	4	0	14/06/1972	14/06/1972	0	0	0	22/09/2004	1	09/09/2004
2831001	14625	1	123	0	2	7325	2		4	4	0	30/03/1991	30/03/1991	0	0	0		0	
2866001	4216	1	129	0	6	1812	2		0	4	0	04/08/1960	04/08/1960	0	0	0	27/11/2008	1	15/11/2008
2926001	4083	2	163	0	1	1757	2		0	4	0	08/06/1965	08/06/1965	0	0	0		0	
2927001	4081	1	135	0	6	1757	2		0	4	0	08/06/1965	08/06/1965	0	0	0		0	
3212001	9180	1	132	0	2	3795	2		0	4	0	30/03/1991	05/08/1999	1	93	0		0	
3313001	10939	1	130	0	6	4636	2		0	4	0	30/03/1991	30/03/1991	0	0	0	24/02/2004	1	03/02/2004
3638001	3955	1	141	0	6	1708	2		0	4	0	11/03/1988	11/03/1988	0	0	0	30/06/2005	1	11/06/2005
4249001	2189	1	142	0	6	954	2		0	4	0	30/03/1991	30/03/1991	0	0	0	16/07/2009	1	01/07/2009
4839001	5004	1	124	0	6	2140	2		4	4	0	30/03/1991	30/03/1991	0	0	0	13/01/2005	1	19/12/2004
5141001	10369	2	140	0	6	4302	2		0	4	0	30/03/1991	30/03/1991	0	0	0		0	
5172001	3338	1	126	0	6	1443	2		0	4	0	30/03/1991	30/03/1991	0	0	0		0	
5189001	761	1	134	0	6	346	2		0	4	0	28/09/1979	28/09/1979	0	0	0	01/01/2006	1	02/12/2005
5502001	14112	1	144	0	2	7039	2		0	4	0	30/03/1991	30/03/1991	0	0	0		0	
5660001	4851	1	127	0	6	2069	2		0	4	0	12/05/1965	12/05/1965	0	0	0	21/07/2005	1	29/06/2005
5870001	13978	2	129	0	2	6963	2		3	4	0	10/11/1989	10/11/1989	0	0	0	31/07/2008	1	15/07/2008
5871001	13979	1	125	0	2	6963	2		4	4	0	10/11/1989	10/11/1989	0	0	0	12/03/2009	27	
6169001	10666	1	122	0	6	4490	2		4	4	0	24/10/1966	24/10/1966	0	0	0	06/11/2006	27	
6467001	2683	2	138	0	2	1163	2		0	4	0	13/05/1975	13/05/1975	0	0	0	09/06/2009	1	01/06/2009
6503001	3152	2	136	0	6	1364	2		0	4	0	30/03/1991	30/03/1991	0	0	0	15/07/2004	1	25/06/2004
6537001	10311	1	130	0	6	3956	2		0	4	0	11/03/1988	11/03/1988	0	0	0		0	
6687001	14117	2	132	0	2	7042	2		0	4	0	30/03/1991	30/03/1991	0	0	0	30/07/2008	1	23/07/2008
7263001	7820	2	150	0	6	3255	2		0	4	0	30/03/1991	30/03/1991	0	0	0		0	
7288001	20606	1	137	0	2	10304	2		0	4	0	20/03/1995	20/03/1995	0	0	0		0	
7644001	143	2	148	0	6	64	2		0	4	0	30/03/1991	30/03/1991	0	0	0	30/09/2003	2	
8119001	5774	2	130	0	6	2444	2		0	4	0	10/02/1970	10/02/1970	0	0	0	19/02/2004	1	03/02/2004
8293001	4405	2	128	0	2	1878	2		3	4	0	20/01/1975	20/01/1975	0	0	0		0	
8841001	10177	2	126	0	6	4199	2		3	4	0	11/03/1988	11/03/1988	0	0	0		0	
9209001	20617	2	194	10	6	10305	1	30/03/1995	0	4	0	22/03/1995	22/03/1995	0	0	0		0	
9270001	5641	1	115	0	6	7760	2		4	4	0	30/03/1991	30/03/1991	0	0	0	20/02/2006	1	31/01/2006
9652001	8638	1	140	0	2	3573	2		0	4	0	25/05/1971	25/05/1971	0	0	0	20/09/2001	1	01/09/2001
9708001	9437	1	131	0	6	3900	2		0	4	0	11/03/1988	11/03/1988	0	0	0		0	
9877001	4607	1	125	0	6	1970	2		0	4	0	11/03/1988	11/03/1988	0	0	0	19/11/2007	1	01/11/2007
10319001	4707	1	146	0	6	2004	2		0	4	0	30/03/1991	30/03/1991	0	0	0		0	
10416001	3957	2	132	0	6	1709	2		0	4	0	30/03/1991	30/03/1991	0	0	0		0	
10466001	14158	2	140	0	2	7064	2		0	4	0	30/03/1991	30/03/1991	0	0	0		0	

At the time of data extraction for the datasets used in this study, free access to GPRD datasets for academics was granted by the licence agreement with the Medical Research Council (MRC). This agreement has now ended, although it is believed funding for datasets is still available from the MRC via individual grant applications.

1.10 Common methods

1.10.1 Medical code lists

Diagnostic electronic records in the NHS are recorded largely using a medical term classification system known as Read codes. This is a hierarchical system, with similar conditions grouped together and sharing the earlier characters of the code. The most recent version uses 5 primary characters, with 2 additional characters to denote subtypes of a code. Each code has an associated description to allow the GP or researcher to identify what condition the code represents.

While there is some organisation to the codes, they can be difficult to work with from a research point of view. This is because there can be many different codes for almost identical conditions, and some codes can be somewhat vague or ambiguous in their descriptions. For example Z4B3.00 denotes “Cancer counselling”, which neither confirms that the patient has cancer (they may be receiving counselling regarding a friend or relation’s cancer) nor is it informative about what type of cancer. Such codes are avoided wherever possible and instead more specific codes are used.

Another type of coding system, known as Oxford Medical Information Systems (OXMIS) codes exist in some versions of the GPRD. These codes are much less organised than Read codes, and were only in use early in the history of the GPRD. This means that there are relatively few diagnoses that are coded using this system. In later versions of the GPRD (as used in Chapters 4 and 5), OXMIS codes have been entirely converted into Read codes, making identification simpler.

In the GPRD each Read/OXMIS codes have been converted further into a unique GPRD code set, in which all medical codes are recorded. The reasoning for this is not entirely clear, but it is easy to convert precisely between the two sets of codes.

Code lists for these studies were derived using a software tool developed by the GPRD, known as the medical browser. This is essentially a searchable database

Initially, the codes for these studies were identified using common names for the disease or organ of interest and common cancer types associated with it. The medical browser accepts wild cards, so for example using *brain* would identify all codes containing the word brain. In addition to this, more obscure cancer codes can then be identified due to the hierarchical nature of READ codes. For example, the code for 'Malignant neoplasm of the tapetum' (B51y100) can be found as it is adjacent to the code for 'Malignant neoplasm of medulla oblongata' (B517100). It is then possible to then re-enter any the newly found terms in the medical browser to potentially find more. Using this iterative process it is highly likely that all relevant codes will be found.

1.10.2 Acquisition/extraction of data

All data from this study were sourced from the GPRD and were in the form of 3 datasets, each extracted separately. Data for chapter 2 were acquired after a successful application to the Independent Scientific Advisory Committee (ISAC), which regulates access to the GPRD. For chapters 3 and 4 a different dataset was acquired in the same way, though the same dataset could be shared for these two studies. For the chapter 5 study, data were obtained from an existing dataset within the Division of Epidemiology and Public Health.

Data used in chapters 2-4 were extracted by the GPRD in accordance with a data specification (see appendices I and II), which was set out by staff at the GPRD in accordance with protocols written by me. For chapter 5 data were kindly extracted from the existing dataset by Dr Joe West, also from the Division of Epidemiology and Public Health.

1.10.3 Data 'cleaning'

Though the data obtained from the GPRD were generally in good order, it is still good practice to check for anomalous data. This was done for each dataset by checking for the following situations:

- Patient registration date anomalies, e.g. 'start of registration' date being before 'end of registration' date (any such patients were removed entirely)
- Extreme ages (any patient with an age greater than 120 was removed)
- Data inconsistent with inclusion or exclusion criteria

Also, throughout the data analysis process, care was taken to spot other anomalies which might be encountered. Notable examples included; a female prostate cancer patient (this patient was removed from the dataset), and BMI values for patients that were beyond what could be might be considered physiologically possible (any values greater than 100 or less than 8 were discarded)

1.10.4 Software used

Virtually all data handling and analysis was carried out using the software program Stata (initially version 10.1, later version 11.1). This is a commonly used statistical software package, with a number of capabilities, including data management, statistical analysis and statistical graphics generation.

For code list generation, software provided by the GPRD was used. This was in the form of 'medical' and 'product' browsers. These browsers allowed various search terms to be used to find codes, including Read/OXMIS codes and terms for the medical browser and BNF codes and drug names for the 'product' browser.

1.10.5 Conditional logistic regression

For case-control studies, investigating cancer incidence (chapters 3 and 6), conditional logistic regression was used to determine differences between drug use in cases and controls. This was possible because the cases in both studies were individually matched to controls by various factors. This technique allows factors such as age and sex to be adjusted for, as it means that each case is compared to controls that have the same characteristics. Other variables were taken into account by adjusting for them by including them as covariates in the conditional logistic regression model.

1.10.6 Cox regression

The cohort studies used Cox regression to determine differences in survival between patients with different categories of drug use. Multivariate modelling adjusted for various factors, some of which were *a priori* confounders and others which were included in the model according to the level of confounding they appeared to exhibit.

1.10.7 Kaplan-Meier curves

In the studies investigating mortality (chapters 4 and 5), Kaplan-Meier (KM) curves were drawn to visualise differences in survival for different drug exposures. These were created using Stata 11.1. Initially survival curves were drawn in an unadjusted fashion, giving results which approximate the univariate results from the Cox regression. They were then drawn adjusting for the covariates used in the multivariate Cox regression, which more closely reflected these analyses. KM curves were also used to give an initial impression of the validity of proportional hazards assumptions, while log-log plots and observed/predicted KM curves were used to assess this more closely.

1.10.8 Number needed to treat

It can be difficult to determine the usefulness of a treatment or preventative drug using only odds or hazard ratios. It can therefore be useful to create additional statistics in order to assess the impact of a treatment. Number needed to treat (NNT) is an estimate of the number of patients in a population that would need to be treated in order to prevent one outcome (for example disease occurrence or death). NNT is calculated using the equation:

$$NNT = \frac{1}{incidence\ in\ control\ group - incidence\ in\ drug\ treated\ group}$$

In this study incidence values for the control group were taken from CRUK annual incidence data, as sated for each of the cancer types in Section 1.8. This means that values given are the number of people to be treated to prevent one outcome *per year*. Incidence in the drug treated group can be calculated using:

$$incidence\ in\ drug\ treated\ group = incidence\ in\ control\ group \times odds\ ratio$$

Where the odds ratio used is taken from the results of the study. Taking the example of glioma, the incidence in the control population is $4/100{,}000 = 0.00004$ (glioma makes up around half of brain tumour cases). The largest odds ratio found was 0.36 (>117 days in Table 3.9). This therefore gives the final equation:

$$\frac{1}{0.00004 - (0.00004 \times 0.36)} = \frac{1}{0.0000256} = 39{,}062$$

Incidentally, the reciprocal of NNT (in this example 0.0000256) is known as the absolute risk reduction.

2 Mitochondrial modulators

An emerging anti-cancer field

2.1 Introduction

Mitochondria are emerging as an important new target in the treatment and prevention of cancer. Compounds which act on the mitochondria have been known about for a long time, but many of these compounds have only recently been linked with an anti-cancer action. There are several types of compound with a proposed anti-mitochondrial action. These include existing anti-cancer drugs, other existing drugs such as antidepressants, novel drugs and naturally occurring compounds.

The potential of naturally occurring compounds for mitochondrial inhibition has only been partly explored. However, there are good indications that there is a great deal of potential in some of these compounds as mitochondrial inhibitors. This hypothesis may go some way towards explaining the anecdotal and epidemiological evidence that the dietary sources of these compounds such as fruits and vegetables can have potential health benefits. This review details a number of compounds which have been shown to have actions on mitochondria. The focus of this literature review is on compounds which occur naturally and may be dietary, or may be obtained from plant extracts or other sources. This of course does not preclude man-made compounds from being mitochondrial inhibitors. One such class of compounds, the tricyclic antidepressants, is explored in detail in subsequent chapters.

Despite the potential of naturally occurring compounds for therapeutic benefit in cancer patients, it is difficult to develop these compounds beyond the laboratory because of the difficulty of obtaining robust intellectual property in the form of patents which thus severely limits their sales and marketing potential. However, this does not rule them out from being useful. If enough evidence is accumulated that a particular molecule can be useful, then it may be that a drug company could develop a new, structurally related

analogue to the molecule, to increase its efficacy and allow a patent to be issued on such newly developed related compounds.

2.2 Mitochondria as targets for cancer therapy

Regardless of mechanism, the ultimate aim of any cancer drug is always induction of cell death by apoptosis or necrosis. Apoptosis is also known as “programmed cell death” and is a common physiological process which occurs in various pathological (such as after viral infection (Everett et al. 1999)) and non-pathological processes (such as during development (Meier et al. 2000)). It is a tightly regulated process which results in a series of morphological changes to the cell, including cell shrinkage, DNA fragmentation, apoptotic body formation and eventual phagocytosis of resultant components by immune cells such as macrophages. In contrast, necrosis is a rapid and uncontrolled form of cell death and results in cell membrane rupture, organelle destruction and leakage of intracellular contents into the extracellular space, although some evidence now exists to suggest that necrosis is more tightly regulated than first thought (Henriquez et al. 2008). Because of this membrane leakage of intracellular molecules, necrotic cell death can have undesirable clinical consequences, such as inflammation. Apoptosis induction is therefore a better target for novel cancer drugs, as apoptosis is associated with limited or no inflammatory changes (Kanduc et al. 2002). Induction of apoptosis is however a complex process which can involve both intrinsic and external signals and numerous internal interactions, eventually leading to an apoptotic effector pathway (Fulda et al. 2006; Moffitt et al. 2010).

The extrinsic apoptotic pathway involves cell surface receptors such as the TNF-related apoptosis-inducing ligand-receptor (TRAIL). These receptors then interact with downstream signalling molecules within the cell, which eventually leads to the activation of a family of proteins called caspases. These caspases (such as caspase 9) are the effectors of apoptosis. Amongst their functions is activation of enzymes which can cause DNA fragmentation,

which lead to the chromosomal breakdown characteristic of apoptotic cell death (Fulda et al. 2006).

The intrinsic apoptotic pathway is arguably more important in cancer therapy. It is also known as the mitochondrial apoptotic pathway and can interact with the extrinsic pathway, or act independently of it. Mitochondria are central to the intrinsic pathway in that they mediate both pro- and anti- apoptotic signalling and provide a means by which a discrete commitment to apoptosis in an individual cell is made. This done through interaction with external stimuli such as oxidants and DNA damage, and is effected by the release of proteins, such as cytochrome c and caspases (Moffitt et al. 2010).

An important point of intrinsic apoptosis is known as the mitochondrial permeability transition (MPT), which is a large increase in the permeability of the mitochondrial inner membrane to molecules of molecular weight less than 200 Daltons. A MPT is not absolutely necessary for apoptosis to occur but is sometimes described as a 'point of no return' in the process (Kroemer 2002). A MPT often occurs along with processes such as inner mitochondrial membrane depolarisation and release of cytochrome c, all of which are markers of apoptosis. The MPT is thought to occur through formation of a permeability transition pore (PTP), which is a large multi-protein complex. The formation of a PTP complex is thought to make the mitochondrial membrane permeable to small molecules such as water and can lead to morphological changes, such as mitochondrial swelling. Initiation of a mitochondrial permeability transition is a complex and as yet not fully understood process. However, it is known that induction occurs under conditions of cellular stress such as DNA damage.

With mitochondria being so central in the process of apoptosis, they are a promising target for novel cancer drugs. Not only are they central to apoptosis, but they also exhibit a number of advantages as therapeutic targets.

There is a vast array of potential target proteins and complex metabolic pathways such as oxidative phosphorylation, the Kreb's (Tricarboxylic cycle) cycle and fatty acid oxidation processes that can be targeted within the mitochondria. For instance, it is known that mitochondrial protein content is approximately 10% of total cellular protein on average for all cell types in the body and in some tissues, such as the heart accounts for 50% of the total cellular protein (Bates T.E., personal communication). Although drug inhibition/interaction will not necessarily lead to apoptosis with just any protein, it is reasonable to postulate that sufficient disruption of suitable mitochondrial target proteins/processes may lead to effective apoptosis induction.

It is well known that most cancer cells exhibit some sort of defect in the apoptotic process (Hanahan et al. 2000), which allows them to replicate without the usual control of non-neoplastic cells. Therefore another advantage of the mitochondrial targeting approach is that relatively direct and specific induction of apoptosis can be achieved. This may allow a 'side stepping' of many apoptotic defects in cancer cells, which may be associated with a lack of specific proteins associated with receptor mediated (extrinsic) apoptosis, thus overcoming a major cause of resistance to cancer drugs.

Perhaps the greatest advantage of mitochondria as a drug target is that there is great potential for development of drug specificity. This is an important aspect of any cancer drug as it allows high levels of drug to be used with minimal toxicity and therefore potentially a higher therapeutic index. Therapeutic index is an important term here, as it is a vital determinant of the effectiveness of any drug. Therapeutic index is essentially the concentration 'gap' between an agent having a therapeutic effect (in this case killing cancer cells) and the agent causing toxicity (i.e. killing or damaging normal cells).

There are two potential approaches to achieving a high therapeutic index in targeting mitochondria. The first is perhaps generic to many cancer drug types. This would involve

selective *delivery* of the drug to cancer cells, perhaps through a differentially expressed cell surface receptor. Drug delivery to the mitochondria of cancer cells may be more effective for some types of drug molecules, as it is known that lipophilic cations can accumulate in mitochondria (due to the mitochondrial membrane potential) (Modica-Napolitano et al. 2001). This could also provide increased selectivity due to the higher membrane potentials often exhibited by cancer cell mitochondria (although this is often heterogeneous within a population of cancer cells).

The second approach could exploit phenotypic differences between mitochondria of normal and neoplastic cells. These differences include lower levels of oxidative phosphorylation, greater reliance on glycolysis, often higher mitochondrial membrane potential and high production of reactive oxygen species (ROS) (reviewed in (Fantin et al. 2006)). The significance of these phenotypic differences is that the concentration at which a mitochondrial inhibitor is toxic to a cancer cell may be lower than that for a normal cell (Daley et al. 2005; Athanasiou et al. 2007a; Athanasiou et al. 2007b). Studies have shown that inhibition of particular enzymes within the mitochondria, such as cytochrome c oxidase will cause a relatively small drop in respiratory function at low inhibitor concentrations, but once a threshold concentration is reached, a dramatic reduction in respiratory function is observed (Mazat et al. 1997). It may be that this threshold concentration is lower in a cancer cell than a normal cell, due to the mitochondrial defects often exhibited in cancer cells. This 'concentration gap' is vital in determining the therapeutic potential of a drug. Both targeted delivery and exploiting phenotypic differences between cells are common approaches in pharmacology.

2.3 Naturally occurring mitochondrial modulators

2.3.1 Cyanide

Well known as a highly toxic poison, cyanide (CN^-) is a potent inhibitor of cytochrome c oxidase (mitochondrial complex IV). It occurs naturally (in small amounts) in apple seeds, mangoes and almonds. As cyanide is such a well described inhibitor of mitochondrial activity, it could potentially be a candidate as a mitochondrial inhibitor with anti-cancer activity, though there has been little research into such effects. However it is often used in research if artificially induced mitochondrial dysfunction is required.

2.3.2 Rotenoids

Rotenoids are a class of compounds of the flavonoid family. Rotenone is the best known of these compounds and has been used for many years as a pesticide and insecticide. Rotenone occurs naturally in the roots of several plants including *Lonchocarpus utilis* (commonly known as cubé). The anticancer properties of rotenoids have been demonstrated in a mouse model, where a dose dependant tumour size reduction in hepatocellular carcinoma was shown (Cunningham et al. 1995). Rotenoids found in cubé include: rotenone and deguelin, which are known to exhibit very strong mitochondrial complex I inhibition (Fang et al. 1998).

2.3.3 Vanilloids

Capsaicin is a vanilloid receptor agonist which occurs in chilli peppers and is the main component responsible for the burning sensation occurring when eating food containing chilli. The capsaicin molecule is highly lipid soluble and is part of a small family of related compounds also found in smaller amounts in chillies. A major molecular target of capsaicin is the cell membrane bound VR1 receptor (also known as the transient receptor potential V1 channel TRPV1). The VR1 receptor and its various ligands may in fact have anti-

proliferative and/or apoptotic functions themselves (Athanasiou et al. 2007b), but capsaicin is also thought to have other potential apoptotic effects independent of the VR1 receptor. Although vanilloid receptors are thought to be expressed in a number of cell types, there is significant evidence that suggests that mitochondrial mediated mechanisms may be responsible for their apoptotic action.

An example of this is the demonstration of a reduction in oxygen production and an increase in hydrogen peroxide levels when cells were treated with vanilloids (Hail et al. 2002). These occurrences are associated with apoptosis and are indicative of inhibition of mitochondrial respiration. The precise mechanism for the apoptotic effects of vanilloids is not yet certain. While membrane depolarisatrion and decreased oxygen consumption have been observed (Athanasiou et al. 2007b) along with caspase 3 activation and intracellular Ca^{2+} release (Wu et al. 2006), the actual function of the vanilloid is less clear. It has been postulated that activity inhibition of mitochondrial complex I of the respiratory chain may be a vanilloid target (Athanasiou et al. 2007b).

2.3.4 Cannabinoids

Cannabinoids are compounds found naturally in the *Cannabis sativa* plant and are ligands for the CB_1 and CB_2 receptors (Howlett et al. 2002). Their interactions are complex however as they have also have been shown to interact with vanilloid receptors such as TRPV1. Like vanilloids, they are also known to be able to induce cell death. Another similarity with vanilloids is that they are also thought to induce apoptotic cell death through a mitochondrial mechanism. This was shown in a study using isolated rat heart mitochondria (Athanasiou et al. 2007a). Here it was demonstrated that cannabinoids can cause a reduction in oxygen consumption and decreased mitochondrial membrane potential. Their mode of action was thought to be inhibition of complex I and complex II-III activity.

2.3.5 Resveratrol

Resveratrol was originally identified as a potential anticancer agent for its ability to inhibit cyclooxygenase (COX). Resveratrol is found in the skin of grapes and in high levels in red wine and has therefore received extensive attention for its potential chemopreventative role. Resveratrol has been found to exert a number of effects of cells, including cycloxygenase-1 (COX-1) inhibition and modulation of antioxidant activity (through a variety of mechanisms (Rubiolo et al. 2008)). It has also been found to inhibit cancer in both *in vitro* and *in vivo* models (Jang et al. 1997). More recently, evidence for a mitochondrial mechanism in inhibiting cancer has been revealed. A number of studies have shown the ability of resveratrol to specifically induce apoptosis in a number of cancer cell types such as acute lymphoblastic leukaemia cells (Dorrie et al. 2001), U251 glioma cells (Jiang et al. 2005) and neuroblastoma cells (van Ginkel et al. 2007). In these studies it was shown that resveratrol has the ability to induce mitochondrial membrane depolarisation along with activation of caspase 3 and caspase 9.

2.3.6 Jasmonates

Jasmonates are a group of plant stress hormones with putative anti-cancer activity. One group has demonstrated its anti-cancer activity and the mechanism of action of the compound. In their initial paper, Fingrut and Flescher describe high levels of cytotoxicity in 4 different cancer cell lines. This was particularly notable when treating with methyl jasmonate. The study also investigated mice bearing EL-4 mouse lymphoma, where a significantly higher survival rate was observed in the mice treated with methyl jasmonate (Fingrut et al. 2002).

Evidence for the mitochondrial action of jasmonates is demonstrated in a further paper from the group (Rotem et al. 2005). This mitochondrial action was demonstrated by measurement of mitochondrial swelling, membrane depolarisation and cytochrome c

release. The group also showed that the mitochondrial toxicity is dependent on the PTP, as inhibition of the PTP prevented membrane depolarisation. The study also demonstrates selective mitochondrial toxicity towards chronic lymphocytic leukaemia cells expressing both wild type and mutant forms of the tumour suppressor protein p53 (Rotem et al. 2005).

2.3.7 Arsenic trioxide

Arsenic trioxide is a major active component in Traditional Chinese Medicine and is found in Arsenicum *Sablimatum*. It can also be made synthetically. The compound has long been known to have anti-cancer properties and has been used for some time in the treatment of acute promyelocytic leukaemia (Antman 2001), with high levels of clinical complete remission observed (Niu et al. 1999) (known commercially as Trisenox). As with so many cancer drugs, this compound has been used extensively before its mechanism of action is known. There is still debate over this matter.

Various different proposals have been put forward to explain the anti-cancer action of arsenic trioxide. Inhibition of thioredoxin reductase and therefore downstream induction of oxidative stress and inhibition of DNA synthesis is one possibility (Lu et al. 2007). Other groups have suggested that autophagic cell death may be responsible rather than or alongside apoptotic cell death in malignant glioma cells (Kanzawa et al. 2003) and in leukaemia HL-60 cells (Yang et al. 2008a). There is also data to suggest the involvement of mitochondrial toxicity and several groups suggest that mitochondria involved in the apoptosis induction (Zhu et al. 1999; Shen et al. 2002; Yu et al. 2007). Moreover, other groups have investigated the direct involvement of arsenic trioxide in mitochondrial interactions (Korper et al. 2004). One such group demonstrates more direct effects on the PTP, as in a cell free system, apoptosis only occurs in the presence of the mitochondria and inhibition of the PTP abolishes induction of apoptosis by arsenic trioxide (Larochette et al.

1999). A good short review of these mechanisms can be found in (Kroemer 1999). All these mechanisms are not necessarily mutually exclusive, as it is possible that the compound acts on multiple parts of the cell to elicit apoptosis (either extrinsic and/or intrinsic) and/or necrosis, dependant on both cell type and concentration of the agent concerned.

2.4 Summary conclusion

Within all of these compounds exists the potential to be anti-cancer drugs. Some compounds are further along this path than others, but in all cases strong anti-cancer activity at least *in vitro* has been demonstrated. What connects all of these compounds is that they all have putative mitochondriotoxic activity. However, some of the compounds have been more strongly linked with this activity than others. Some caution must be exercised in determining if a compound *directly* interacts with the mitochondria. There can be a clear distinction between a compound that directly interacts with the mitochondria and one which affects the mitochondria via a different, possibly parallel cellular process which leads to mitochondrial apoptotic cell death. The implication of this is that it is not sufficient to measure changes in the mitochondria brought about by a particular agent within a cell. For example, an observation showing membrane depolarisation in the mitochondria of a target cell does not demonstrate direct mitochondrial inhibition. Methods to determine direct interactions therefore have to involve using isolated mitochondria and measurement of effects such as depolarisation and cytochrome c release in absence of any upstream pathways which may indirectly act on the mitochondria. Methods involving detection of direct mitochondrial protein interactions and measurement of mitochondrial enzyme kinetics may also be valuable in this approach. Another important consideration when studying naturally derived products is their source. Studies using isolated purified compounds are far more valuable than those using extracts that may contain a large number of different compounds, often at varying concentrations depending on the source and methods of extraction.

2.5 Importance to this thesis

The natural compounds reviewed above are not generally used extensively in medicine. This means that their putative anti-cancer actions are not testable in a primary care

database such as the GPRD. However there are compounds that are known to modulate mitochondrial activity, that are also commonly prescribed in the general population. The best known example of this is tricyclic antidepressants (Daley et al. 2005). The next two chapters explore the potential anticancer action of these drugs, in terms of both their effect on cancer incidence and mortality

3 Tricyclic antidepressants and cancer incidence

A case control study using the GPRD

3.1 Introduction

3.1.1 Study background

This study was conceived after laboratory findings demonstrated an anti-cancer action for tricyclic antidepressants, coupled with a mitochondrial inhibition mechanism (Daley et al. 2005). This led to further investigation into such anti-cancer actions, which gave further indication of their potential (detailed below). Although there is extensive evidence for the anti-cancer action of tricyclics, it was felt that there were few satisfactory studies in humans and the obvious choice to remedy this situation was a large-scale epidemiological study. The background contained in this introduction is also largely applicable to chapter 4, which is also based around tricyclics.

Tricyclic antidepressants are widely prescribed for a variety of conditions, including depression, anxiety, insomnia and certain types of chronic pain management. They have been in clinical use for over 40 years, and were originally developed as derivatives of the antipsychotic chlorpromazine, with imipramine being the first tricyclic to be used as an antidepressant. Their psychoactive mechanism of action is thought to involve inhibition of serotonin and norepinephrine reuptake. Side effects under normal dosing are generally mild, but include dry mouth, constipation, urinary retention and restlessness. However, in overdose they can cause severe cardiovascular and neurological toxicity.

3.1.2 *In vitro* evidence

Tricyclics have long been known to interact with the mitochondria (Eto et al. 1985), but their anti-cancer action is somewhat more recently discovered. Chlorimipramine is probably the most studied of the tricyclics with regard to its anti-cancer action. One such study shows that chlorimipramine has the ability to kill cultured brain tumour cells and also demonstrates the mechanism by which the drug acts (Daley et al. 2005). It demonstrates

inhibition of the mitochondrial complex III, which is accompanied by a decrease in mitochondrial membrane potential and morphological changes to the mitochondria. Intriguingly, it is noted that primary human glia (non-cancerous cells) are not affected by the drug, although the reason for this selectivity is not entirely clear. This has been further investigated using various tricyclic drugs in combination with dexamethazone (an anti-inflammatory drug often used in the treatment of glioma), where it was found that amitryptyline, chlorimipramine and dexamethazone were all able to inhibit cellular respiration, leading to cell death and a possible synergistic effect existed between chlorimipramine and dexamethazone (Higgins et al. 2010).

Another example of combination therapy in glioma used chlorimipramine along with imatinib, though in this case a murine model of glioma was used (Bilir et al. 2008). This glioma killing action is also aptly demonstrated in a study which found that chlorimipramine, amongst other drugs caused apoptosis induction in glioma and neuroblastoma cell lines (Levkovitz et al. 2005). The group also suggest in this paper that proteins involved in mitochondrial pathway of apoptosis, such as cytochrome *c* and caspase-3 are involved in the cell death triggered by the drugs.

Other studies using other tumour types support the findings of Daley et al for both chlorimipramine and other antidepressants. Human peripheral lymphoblasts and acute myeloid leukaemia cells have been used to demonstrate the ability of the drugs imipramine, chlorimipramine and citalopram to induce apoptosis (Xia et al. 1998b; Xia et al. 1999). The later study here shows that the apoptosis occurs via caspase-3 activation, although it does not demonstrate the mechanism by which this occurs. Caspase-3 is known to be intimately involved in apoptosis. Other studies by this group give further confirmation that a mitochondrial pathway is involved in apoptosis induction (Xia et al. 1998a).

Other tricyclic drugs such as amitriptyline and desipramine, have been shown to induce apoptosis in human colon carcinoma cells (Arimochi et al. 2006). In this case, desipramine has been shown to act through both mitochondrial and non mitochondrial mechanisms, depending on the colon cancer cell line used (Arimochi et al. 2008). This gives an insight into the reasons for observing differential sensitivity, in that cancer cells may or may not be sensitive to a drug according to their mitochondrial phenotypes.

3.1.3 *In vivo* studies

In vivo studies provide further evidence for the efficacy of these drugs. Interestingly, many of the *in vivo* studies precede many of the *in vitro* studies. Also, rather than look at the efficacy of the antidepressant drugs alone, they are used as an adjuvant treatment, in order to overcome drug resistance. One such study demonstrates the ability of chlorimipramine to partly overcome vincristine resistance in P388/VCR-bearing mice (Tsuruo et al. 1983). This study showed an increased survival time of around 30%, and therefore only a partial response. Another study used clorimipramine in combination with another drug, verapamil, to overcome actinomycin resistance (Merry et al. 1991). Further evidence of *in vivo* efficacy is provided by a group looking at doxorubicin resistance. They describe a "significant reduction in growth of doxorubicin-resistant tumours" (Pommerenke et al. 1995).

3.1.4 Epidemiological evidence

A number of epidemiological studies have been carried out to investigate tricyclic use and cancer incidence. Oddly however, despite the greatest amount of laboratory research being directed at glioma, there are no epidemiological studies relating to this cancer type. Breast cancer is the most widely studied in this area, mostly due to an apparent concern that antidepressants may increase cancer risk (Cotterchio et al. 2000; Bahl et al. 2003; Lawlor et al. 2003; Gonzalez-Perez et al. 2005; Fulton-Kehoe et al. 2006; Wernli et al. 2009). Consensus here seems to be that there is little meaningful increase in risk.

Colorectal cancer risk has also been investigated in relation to tricyclic use and small but non-significant decreases in risk among tricyclic users have been observed in three separate studies (Xu et al. 2006; Coogan et al. 2009; Chubak et al. 2011). Both lung (Toh et al. 2007b) and prostate (Tamim et al. 2008) cancer have also been involved in similar studies, but neither of these demonstrated a significant protective effect.

3.1.5 Clinical use

Despite there being no formal clinical trials using tricyclics as an anti-cancer agent, there are anecdotal reports of clinical use of chlorimipramine in the treatment of primary brain tumour. Around 350 patients are thought to be using chlorimipramine in this way (detailed in (Higgins et al. 2010)). Though relatively few of these cases are well documented, a study on a small group of glioma patients (n=27), has shown a good partial response from chlorimipramine treatment in around 80% of patients (Beaney et al. 2005).

3.1.6 Conclusion

There is strong evidence for activity of some antidepressants in reducing incidence of or even in treating cancers such as glioma. This evidence would be further supported by an epidemiological study, which would allow widespread analysis of antidepressant usage and brain tumour incidence. If positive results were to emerge from such a study it would provide a very clear indication of the efficacy of antidepressants and would most likely lead to further studies into their potential therapeutic usage. There would also be increased incentive for the investigation of other mitochondrial inhibitors many of which show promise.

3.1.7 Cancer types in the study

Due to the large volume of research involving glioma and tricyclics, this was the first cancer type to be selected, along with all other primary brain tumours. There are also *in vitro* and epidemiological suggestions that some types of colorectal cancer may be sensitive to tricyclics, so this was also chosen to be included in this study.

The other cancer types, breast, lung and prostate cancer were included mainly due to their high incidence, giving a great deal of power to the study. Because there is relatively little evidence that they are sensitive to tricyclic antidepressants, these may appear to be of secondary interest, but in fact provide an important role in determining whether any observed effects are selective to cancer type.

3.2 Methods

3.2.1 Study design

A matched case-control study was be used to determine the relationship between tricyclic usage and cancer incidence.

3.2.2 Patients

Cases were defined as any person with a recorded diagnosis of brain tumour, breast cancer, colorectal cancer, lung cancer or prostate cancer within the GPRD (for diagnosis codes see Appendix I). Gliomas were also considered separately. I excluded cases with less than 5 years of data contributions prior to the first recorded diagnosis of the relevant cancer. This was to assure sufficient duration of data on aspects such as therapy for each patient, and also to increase the likelihood that cancer diagnoses relate to incident, rather than prevalent cases. I also excluded patients aged less than 18 years, or with a diagnosis of any other cancer prior to the index cancer.

Controls were contributing data at the time of the case index date, had at least 5 years of follow-up prior to that date and had no recorded medical diagnosis of cancer. Controls were matched to cases by year of birth, gender and GP practice in a ratio of 2:1 where possible. In addition, a pseudo-diagnosis date was assigned to each control, which corresponded to the diagnosis date of the matched case.

3.2.3 Variables

The primary exposure was the prescription of any tricyclic antidepressant (section 4.3.3 of the British National Formulary). I abstracted data on all such prescriptions at least one year

prior to the date of diagnosis of the index cancer (or the equivalent pseudo-diagnosis date of the cancer for controls).

A number of approaches were taken to classify these exposures for analysis. Firstly, I created a binary variable defining a tricyclic user as anyone with ≥6 prescriptions for any tricyclic antidepressant, and comparing these to non-users (those with <6 prescriptions). This was later refined to ≥2 prescriptions (and <2 prescriptions for non-users), in order to be more consistent with other similar studies. For example, the same exposure definition of ≥2 prescriptions was used by Fulton-Kehoe et al (2006) and Chubak et al (2011).

Assessment of dose response was initially handled by grouping the number of prescriptions that each patient received into 4 categories (unexposed, 1–10 prescriptions, 11–50 prescriptions and >50 prescriptions). Though this provided some indication of the total dose received by each patient, it was felt that it did not truly reflect whether patients were receiving high or low dose prescriptions. This was amended by determining the dose received for each prescription. This was standardised across drug types by dividing by the maximum recommended doses for each drug (determined from the BNF). These standardized doses were then used to calculate the mean dose across all prescriptions for each patient individually. Patients with prior tricyclic use were divided into 'high' or 'low' dose groups based on the median corrected dose, which was 0.35 times the maximum recommended dose.

The time of exposure was also categorised, initially with exposures divided into 1–5 year, 5–10 year and >10 year categories according to the time of the first prescription for each drug type. This somewhat crude method of categorisation did give some indication of the length of time patients had been exposed for, but did not give an indication of the consistency or overall length of exposure. This was assessed by determining the number of days of exposure over a 10 year period before the index date. Any patient contributing data

for less time was excluded for this part of the analysis. Exposed patients were then divided into 2 cohorts around the median level of exposure time.

3.2.4 Drug specificity/confounding by indication

Confounding by indication is a situation where another condition (such as depression) is linked to both the outcome (cancer) and the exposure (in this case tricyclic drug use). Clearly depressed patients are often prescribed antidepressants, and depression may be linked to cancer development (Reiche et al. 2004). It is therefore important that this potential confounding is assessed, as it is a potential alternative explanation for any effects found.

In order to assess any potential confounding by indication, use of another class of antidepressants, the Selective Serotonin Reuptake Inhibitors (SSRIs) was investigated in parallel with tricyclic use. This is because their use is also associated with depression, and any similar patterns in the results for this drug may indicate confounding by indication.

As well as doing some of the analysis directly using SSRI as the primary exposure in place of tricyclics, further investigation was carried out by re-categorising exposures into patients exposed to only SSRIs, only tricyclics, and both SSRIs and tricyclics. This helps to further scrutinise the effect of SSRIs and tricyclics, and may help to clarify if there is confounding by indication.

Stratification of the tricyclic analysis according to whether a patient had diagnosed depression was also carried out. This was in order to determine the indication for tricyclic prescription. Any differences in the results between the strata may indicate whether depression is a confounder.

Finally the relationship between depression and tricyclic dose was investigated, in order to determine if depression was a marker for the dose prescribed.

Other indications for tricyclic prescriptions (i.e. chronic pain management) were not investigated. This is because chronic pain is virtually impossible to classify in the GPRD as most patients will have a code relating to pain at some point in their record (there are over 27 million events relating to it in the GPRD), and there are very few codes specifying *chronic* pain.

3.2.5 Other covariates

I extracted data on smoking status, body mass index (BMI), alcohol use, diagnosed depression, and prescriptions for non-steroidal anti-inflammatory drugs and statins which I considered as possible confounders.

3.2.6 Statistical methods

Data were analysed with conditional logistic regression, initially using univariate analysis, then using a multivariate model. Results were presented as odds ratios (ORs), with accompanying 95% confidence intervals (CIs). Potential confounders were retained in the model if their inclusion altered the effect size by more than 10% in either direction. Analyses were performed on all cancer types together, followed by individual cancer types to look for specific effects. All data handling and analysis was done using Stata v10.1 SE (Statacorp, 4905 Lakeway Drive, College Station, Texas 77845 USA).

3.3 Results

3.3.1 Study population

31,953 cases were matched to 61,591 controls by age, gender and general practice. The cases were broken down by cancer site, into 1,372 cancers of the nervous system (of which 773 were glioma), 10,293 of the breast, 6,232 colorectal, 6,537 of the lung and 6,537 of the prostate. Median age of patients across all cancer types was 68.2. Female patients made up 50.7% (16,212) of the study, and had a median age of 65.6, male patients had a median age of 70.9. 18.9% of cases and 17.6% of controls were exposed to one or more prescriptions for a tricyclic prior to a year before the index date. These data are described, stratified by cancer type in Table 3.1.

Table 3.1 Population characteristics

Cancer		Cases		Controls	
		Number	%	Number	%
All	Total	31,953		61,591	
	Male	15,740	49.3	29,998	48.7
	Female	16,212	50.7	31,593	51.3
	Mean age		68.3		
Glioma	Total	773		1,502	
	Male	468	60.3	906	60.5
	Female	305	39.7	596	39.5
	Mean age		60.1		
Colorectal	Total	6,232		12,010	
	Male	3,496	56.1	6,704	55.8
	Female	2,736	43.9	5,306	44.2
	Mean age		70.9		
Brain	Total	599		1,164	
(excluding	Male	214	35.7	413	35.5
glioma)	Female	385	64.3	751	64.5
	Mean age		65.8		
Breast	Total	10,293		20,096	
	Male	-	-	-	-
	Female	10,293	100.0	20,096	100.0
	Mean age		62.5		
Lung	Total	6,537		12,514	
	Male	4,035	61.73	7,653	61.16
	Female	2,502	38.27	4,861	38.84
	Mean age		71.0		
Prostate	Total	7,531		14,329	
	Male	7,531	100.0	14,329	100.0
	Female	-	-	-	-
	Mean age		72.5		

3.3.2 Covariates

Table 3.2 describes covariates with all cancer types grouped together. Some large differences in between different cancer types mean that the breakdown by cancer type (Table 3.3) is potentially more informative. As can be seen from Table 3.2, smoking was associated with an increased risk of cancer (OR=1.47 CI=1.42–1.53). This risk is almost entirely attributable to lung cancer, with an odds ratio of 7.4 (CI=6.74–8.12) in smokers compared to non smokers. There is a slight increase in risk of cancer for alcohol users (OR=1.09 CI=1.05–1.14), which was mostly due to breast (OR= 1.11 CI=1.04–1.18) and colorectal cancers (OR=1.12 CI=1.02–1.23). There is an apparent decrease in cancer risk as BMI increases. This effect is mainly found in lung cancer patients, where the decrease is caused by confounding by smoking status, and is not statistically significant if only non-smokers are considered. NSAID use is not significantly different when all cancers are considered together, but does show a significant reduction in colorectal cancer (OR=0.93, CI=0.87–0.99), as has been reported previously (Cuzick et al. 2009).

Table 3.2 Covariates- all cancers

Exposure	Status	Case	Control	OR	95% CI		
Smoking status	No	15,369	32,153	1			
	Ex	5,911	10,263	1.23	1.19	1.28	←
	Yes	7,978	11,615	1.47	1.42	1.53	←
	Missing	2,695	7,560	0.69	0.65	0.72	←
Alcohol use	No	4,778	9,542	1			
	Ex	348	572	1.24	1.08	1.43	←
	Yes	21,028	38,670	1.09	1.05	1.14	←
	Missing	5,799	12,807	0.87	0.83	0.91	←
Mean BMI	Normal	10,713	19,466	1			
	Underweight	701	1,020	1.26	1.14	1.39	←
	Overweight	10,086	19,005	0.96	0.93	1.00	←
	Obese	3,191	6,240	0.93	0.89	0.98	←
	Morbidly obese	961	2,008	0.88	0.81	0.95	←
	Missing	6,301	13,852	0.79	0.76	0.82	←
NSAID use	No	21,122	41,006	1			
	Yes	10,831	20,585	1.02	0.99	1.05	
Statin use	No	26,957	51,933	1			
	Yes	4,996	9,658	1.00	0.96	1.04	
Depression	No	23,890	47,458	1			
	Yes	8,063	14,133	1.15	1.11	1.19	←

Table 3.3 Covariates- by cancer type

Cancer type	Exposure	Status	Case	Control	OR	95% CI		
Brain (excluding Glioma)	Smoking status	No	339	621	1			
		Ex	76	159	0.89	0.65	1.22	
		Yes	135	240	1.02	0.79	1.32	
		Missing	49	144	0.57	0.39	0.83	←
	Alcohol use	No	104	180	1			
		Ex	9	11	1.34	0.53	3.35	
		Yes	364	708	0.86	0.65	1.15	
		Missing	122	265	0.74	0.52	1.05	
	BMI	Normal	15	16	1			
		Underweight	186	402	0.46	0.21	1.00	←
		Overweight	176	295	0.61	0.28	1.32	
		Obese	74	115	0.66	0.29	1.49	
		Morbidly obese	27	50	0.57	0.24	1.39	
		Missing	121	286	0.40	0.18	0.87	←
	NSAID use	No	397	805	1			
		Yes	202	359	1.16	0.93	1.46	
	Statin use	No	527	996	1			
		Yes	72	168	0.79	0.57	1.08	
	Depression	No	414	884	1			
		Yes	185	280	1.43	1.15	1.79	←
Glioma	Smoking status	No	415	737	1			
		Ex	105	196	0.96	0.73	1.27	
		Yes	168	343	0.86	0.69	1.08	
		Missing	85	226	0.60	0.44	0.82	←
	Alcohol use	No	88	199	1			
		Ex	3	8	0.91	0.24	3.53	
		Yes	532	945	1.28	0.97	1.70	
		Missing	150	350	0.91	0.65	1.27	
	BMI	Normal	11	28	1			
		Underweight	253	443	1.49	0.72	3.08	
		Overweight	251	456	1.43	0.69	2.96	
		Obese	75	155	1.26	0.59	2.71	
		Morbidly obese	23	52	1.17	0.49	2.78	
		Missing	160	368	1.07	0.51	2.25	
	NSAID use	No	601	1,132	1			
		Yes	172	370	0.86	0.69	1.08	
	Statin use	No	675	1,319	1			
		Yes	98	183	1.04	0.79	1.36	
	Depression	No	597	1,190	1			
		Yes	176	312	1.14	0.92	1.42	

Table 3.3 Covariates- by cancer type (continued)

Cancer type	Exposure	Status	Case	Control	OR	95% CI		
Breast	Smoking status	No	6,218	11,911	1			
		Ex	1,181	2,172	1.05	0.97	1.14	
		Yes	2,001	3,825	1.00	0.94	1.07	
		Missing	893	2,188	0.74	0.67	0.81	←
	Alcohol use	No	1,845	3,805	1			
		Ex	75	121	1.30	0.97	1.75	
		Yes	6,533	12,289	1.11	1.04	1.18	←
		Missing	1,840	3,881	0.96	0.88	1.04	
	BMI	Normal	202	452	1			
		Underweight	3787	7,327	1.16	0.97	1.37	
		Overweight	2779	5,273	1.18	0.99	1.40	
		Obese	1142	2,093	1.22	1.02	1.47	←
		Morbidly obese	484	917	1.18	0.97	1.44	
		Missing	1899	4,034	1.02	0.86	1.22	
	NSAID use	No	7,478	14,582	1			
		Yes	2,815	5,514	1.00	0.94	1.05	
	Statin use	No	9106	17,842	1			
		Yes	1187	2,254	1.01	0.96	1.05	
	Depression	No	6927	13,978	1			
		Yes	3366	6,118	1.11	1.06	1.17	←
Colorectal	Smoking status	No	3,236	6,202	1			
		Ex	1,225	2,139	1.11	1.02	1.21	←
		Yes	1,162	2,131	1.05	0.96	1.14	
		Missing	609	1,538	0.71	0.64	0.80	
	Alcohol use	No	923	1,868	1			
		Ex	62	116	1.12	0.81	1.54	
		Yes	4,056	7,419	1.12	1.02	1.23	←
		Missing	1,191	2,607	0.89	0.80	1.00	←
	BMI	Normal	121	191	1			
		Underweight	1841	3,607	0.81	0.64	1.03	
		Overweight	2074	3,761	0.88	0.69	1.11	
		Obese	694	1,194	0.93	0.72	1.19	
		Morbidly obese	184	365	0.81	0.60	1.09	
		Missing	1318	2,892	0.70	0.55	0.89	←
	NSAID use	No	4,087	7,693	1			
		Yes	2,145	4,317	0.93	0.87	0.99	←
	Statin use	No	5126	9,869	1			
		Yes	1106	2,141	1.00	0.92	1.08	
	Depression	No	4924	9,444	1			
		Yes	1308	2,566	0.98	0.91	1.06	

Table 3.3 Covariates- by cancer type (continued)

Cancer type	Exposure	Status	Case	Control	OR	95% CI		
Lung	Smoking status	No	1,286	6,096	1			
		Ex	1,497	2,318	3.45	3.13	3.81	←
		Yes	3,231	2,446	7.40	6.75	8.12	←
		Missing	523	1,654	1.36	1.19	1.55	←
	Alcohol use	No	999	1,943	1			
		Ex	115	145	1.59	1.23	2.06	←
		Yes	4,105	7,758	1.03	0.94	1.13	
		Missing	1,318	2,668	0.94	0.84	1.04	
	BMI	Normal	274	199	1			
		Underweight	2369	3,671	0.46	0.38	0.56	←
		Overweight	1772	4,058	0.31	0.25	0.37	←
		Obese	499	1,299	0.27	0.22	0.34	←
		Morbidly obese	134	364	0.26	0.20	0.35	←
		Missing	1489	2,923	0.36	0.29	0.43	←
	NSAID use	No	3,958	7,957	1			
		Yes	2,579	4,557	1.15	1.08	1.23	←
	Statin use	No	5430	10,403	1			
		Yes	1107	2,111	1.01	0.93	1.09	
	Depression	No	4923	9,870	1			
		Yes	1614	2,644	1.24	1.16	1.34	←
Prostate	Smoking status	No	3,878	6,598	1			
		Ex	1,833	3,284	0.97	0.90	1.04	
		Yes	1,283	2,635	0.83	0.77	0.90	←
		Missing	537	1,812	0.46	0.41	0.51	←
	Alcohol use	No	823	1,550	1			
		Ex	82	172	0.92	0.70	1.21	
		Yes	5,447	9,568	1.09	0.99	1.20	
		Missing	1,179	3,039	0.69	0.61	0.77	←
	BMI	Normal	78	137	1			
		Underweight	2279	4,019	0.99	0.74	1.31	
		Overweight	3041	5,168	1.03	0.78	1.37	
		Obese	707	1,388	0.89	0.66	1.20	
		Morbidly obese	109	263	0.73	0.51	1.05	
		Missing	1317	3,354	0.64	0.48	0.85	←
	NSAID use	No	4,610	8,853	1			
		Yes	2,921	5,476	1.02	0.96	1.09	
	Statin use	No	6105	11,519	1			
		Yes	1426	2,810	0.96	0.90	1.04	
	Depression	No	6117	12,110	1			
		Yes	1414	2,219	1.27	1.18	1.37	←

3.3.3 Binary exposure

The initial definition of a tricyclic user was someone with six or more prescriptions. This gave the results seen in Table 3.4. A significant reduction in tricyclic usage in colorectal cancer patients is observed (multivariate OR=0.87 CI= 0.77–0.99). While tricyclic use is also lower in glioma cases than controls this effect is not significant (OR=0.70 CI=0.45–1.08). Patients with other cancers of the nervous system had non-significantly higher tricyclic use than controls. For most cancer types there was little evidence found of confounding of the results by the available potential confounders, except for lung cancer patients, where smoking status had a large confounding effect. The significant increase in tricyclic use observed in the univariate model (OR=1.34 CI=1.20–1.49) is reduced when adjusted for confounders (OR=1.15 CI=1.01–1.30). This change is mostly attributable to confounding by smoking status, as adjusting only for this gives an odds ratio of 1.16 (CI=1.05-1.28). Other cancer types show little variation in drug usage, with odds ratios very close to unity.

Table 3.4 Binary analysis- initial results

				Univariate				Multivariate*			
Cancer type	**Exposed**	**Case**	**Control**	**OR**	**95% CI**			**OR**	**95% CI**		
Glioma	No	735	1,390	1				1			
	Yes	38	112	0.70	0·46	1·08		0·70	0·45	1·08	
Colorectal	No	5,823	11,105	1				1			
	Yes	409	905	0·87	0·77	0·98	←	0·87	0·77	0·99	←
Brain	No	1,267	2,444	1				1			
(excl glioma)	Yes	93	198	1·22	0·86	1·74		1·17	0·82	1·67	
Breast	No	9,321	18,167	1				1			
	Yes	972	1,929	0·98	0·91	1·07		0·98	0·90	1·07	
Lung	No	5,929	11,607	1				1			
	Yes	608	907	1·34	1·20	1·49	←	1·15	1·01	1·30	←
Prostate	No	7,172	13,638	1				1			
	Yes	359	691	0·99	0·87	1·13		1·00	0·88	1·15	
All	No	29,512	56,961	1				1			
	Yes	2,441	4,630	1·03	0·97	1·08		0·99	0·94	1·04	

*All cancer types adjusted for smoking status, BMI, alcohol use and diagnosed depression. Colorectal cancer is also adjusted for NSAID use.

It was suggested that the original definition of a user being someone with six or more prescriptions might introduce a disease severity bias. This led to a change in the definition such that patients with two or more prescriptions were users. It was also decided to add diagnosed depression into the multivariate model (see section 3.3.6). As a result of these changes the binary analysis results changed somewhat (Table 3.5). This analysis still demonstrates a significant reduction in tricyclic usage in colorectal cancer patients compared to controls (multivariate OR=0.84 CI=0.75–0.94). Tricyclic use is now significantly lower in glioma patients compared to controls, and has a larger effect estimate (OR=0.59 CI=0.42–0.81). Other brain tumours no longer exhibited higher tricyclic use in cases, while other results remained largely similar to the previous analysis.

Table 3.5 Binary analysis- refined results

				Univariate				Multivariate*			
Cancer type	**Exposed**	**Case**	**Control**	**OR**	**95% CI**			**OR**	**95% CI**		
Glioma	No	706	1,317	1				1			
	Yes	67	185	0.66	0.49	0.89	←	0.59	0.42	0.81	←
Colorectal	No	5,574	10,543	1				1			
	Yes	658	1,467	0.85	0.77	0.94	←	0.84	0.75	0.94	←
Brain	No	505	1,013	1				1			
(excl glioma)	Yes	94	151	1.26	0.95	1.67		1.00	0.72	1.38	
Breast	No	8,651	16,834	1				1			
	Yes	1,642	3,262	0.98	0.92	1.05		0.97	0.91	1.04	
Lung	No	5,555	10,992	1				1			
	Yes	982	1,522	1.30	1.19	1.42	←	1.14	1.02	1.28	←
Prostate	No	6,861	13,112	1				1			
	Yes	670	1,217	1.06	0.96	1.17		0.94	0.84	1.04	
All	No	27,841	53,790	1				1			
	Yes	4,112	7,801	1.03	0.99	1.07		0.93	0.89	0.97	

*All cancer types adjusted for smoking status, BMI, alcohol use and diagnosed depression. Colorectal cancer is also adjusted for NSAID use.

3.3.4 Dose response

When tricyclic exposure is coded as an ordered categorical variable with categories created according to the numbers of prescriptions used, an increasing protective effect with higher prescription numbers was observed for glioma, (test for trend *P*= 0.006) (Table 3.6). Colorectal cancer showed a slight trend towards lower occurrence in higher prescription categories, but there was no statistically significant trend (*P*=0.104). For other cancer types, no statistically significant trends were present.

Table 3.6 Number of tricyclic prescriptions

Cancer type	Number of prescriptions	OR	95% CI		
Glioma	0	1			
	1-10	0.88	0.65	1.19	
	11-50	0.51	0.29	0.89	←
	>50	0.46	0.20	1.08	
Colorectal	0	1			
	1-10	0.93	0.84	1.03	
	11-50	0.97	0.82	1.15	
	>50	0.85	0.67	1.09	
Brain	0	1			
	1-10	1.14	0.85	1.54	
	11-50	1.51	0.94	2.44	
	>50	0.87	0.41	1.82	
Breast	0	1			
	1-10	0.97	0.91	1.04	
	11-50	0.99	0.88	1.11	
	>50	1.10	0.94	1.30	
Lung	0	1			
	1-10	1.18	1.06	1.32	←
	11-50	1.07	0.90	1.28	
	>50	1.17	0.92	1.48	
Prostate	0	1			
	1-10	1.07	0.97	1.18	
	11-50	1.01	0.84	1.21	
	>50	0.94	0.71	1.25	
All	0	1			
	1-10	1.01	0.96	1.05	
	11-50	1.00	0.93	1.07	
	>50	1.02	0.92	1.13	

All cancer types adjusted for smoking status, BMI and alcohol use. Colorectal cancer is also adjusted for NSAID use.

When tricyclic exposure is divided into 'low' and 'high' dose exposure (Table 3.7), odds of high dose users developing cancer are lower for both glioma (OR=0.49 CI=0.30–0.78) and colorectal cancer (OR=0.79 CI=0.67–0.93). Highly significant trends validate these findings further for glioma (*P*=0.0005) and colorectal cancer (*P*=0.0010). Other cancer types were considered in the same way, though no notable or statistically significant trends were present.

Table 3.7 Dose response according to mean corrected dose

Cancer	Exposure Status	Case	Control	OR	95% CI			p-trend	
Glioma	Unexposed	707	1,323	1					
	Low dose	38	97	0.67	0.45	1.01			
	High Dose	28	82	0.49	0.30	0.78	←	0.0005	←
Colorectal	Unexposed	5,592	10,595	1					
	Low dose	382	821	0.87	0.76	1.00	←		
	High dose	258	594	0.79	0.67	0.93	←	0.0010	←
Brain	Unexposed	619	1.218	1					
	Low dose	68	85	1.54	1.08	2.19	←		
	High Dose	37	103	0.70	0.47	1.04		0.0780	
Breast	Unexposed	8,651	16,834	1					
	Low dose	875	1,747	0.97	0.89	1.05			
	High dose	767	1,515	0.98	0.89	1.07		0.6993	
Lung	Unexposed	5,555	10,992	1					
	Low dose	526	893	1.13	0.97	1.25			
	High Dose	456	629	1.16	0.98	1.35		0.0811	
Prostate	Unexposed	6,861	13,112	1					
	Low dose	385	699	1.04	0.91	1.18			
	High dose	285	518	1.07	0.92	1.24		0.6132	

All cancer types adjusted for smoking status, BMI, alcohol use and diagnosed depression. Colorectal cancer is also adjusted for NSAID use. High and low dose categories are created according to whether a patient's corrected dose was above or below the mean.

3.3.5 Timing/duration of exposure

I then went on investigate the relationship between the timing of the first prescription of tricyclics and cancer risk. Glioma exhibits a strong, significant trend (*P*= 0.006) towards reduced cancer incidence with tricyclic treatment beginning earlier (Table 3.8). A similar trend is shown with colorectal cancer, with lower cancer incidence observed with earlier treatment with tricyclics. Colorectal cancer incidence is almost 25% less in the earliest treatment category and the test for trend confirms this (*P*= 0.003). As with the dose response analysis, the data from the other cancer types were analysed in the same way, but this analysis did not demonstrate any statistically significant trends.

Table 3.8 Timing of exposure

Cancer type	Time of exposure (years)	OR	95% CI		
Glioma	Never	1			
	1-5	1.03	0.65	1.62	
	5-10	0.82	0.54	1.25	
	>10	0.57	0.32	1.02	
Colorectal	Never	1			
	1-5	1.11	0.96	1.27	
	5-10	0.93	0.82	1.06	
	>10	0.76	0.66	0.89	←
Brain	Never	1			
	1-5	1.15	0.79	1.68	
	5-10	1.01	0.71	1.44	
	>10	0.91	0.61	1.38	
Breast	Never	1			
	1-5	0.97	0.88	1.07	
	5-10	0.97	0.89	1.05	
	>10	1.03	0.93	1.13	
Lung	Never	1			
	1-5	1.17	1.01	1.36	←
	5-10	1.25	1.09	1.43	←
	>10	1.02	0.87	1.19	
Prostate	Never	1			
	1-5	0.97	0.84	1.11	
	5-10	1.12	0.99	1.28	
	>10	1.04	0.89	1.21	
All	Never	1			
	2-5	1.03	0.97	1.09	
	5-10	1.02	0.97	1.08	
	>10	0.96	0.90	1.02	

All cancer types adjusted for smoking status, BMI and alcohol use. Colorectal cancer is also adjusted for NSAID use.

It was decided that the above analysis did not provide a sufficient indication of the length and consistency of exposure. Categorising by the number of days the drug was prescribed for seemed a better way of doing this. When this analysis was carried out (Table 3.9), long term tricyclic use is significantly lower in glioma cases (OR= 0.36 CI= 0.19–0.69) and colorectal cancer cases (OR= 0.82 CI= 0.68-0.97). Highly significant trends were observed for glioma (*P*= 0.0005) and colorectal cancer (*P*= 0.0086). No notable or statistically significant trends were present for other cancer types.

Table 3.9 Duration of exposure

Cancer	Total days of exposure	Case	Control	OR	95% CI			p-trend	
Glioma	Unexposed	399	1,051	1					
	1-117	22	71	0.65	0.37	1.13			
	>117	14	75	0.36	0.19	0.69	←	0.0005	←
Colorectal	Unexposed	3,598	7,752	1					
	1-117	345	747	0.84	0.70	1.01			
	>117	305	785	0.82	0.68	0.97	←	0.0086	←
Brain	Unexposed	699	1,820	1					
	1-117	53	133	0.81	0.55	1.18			
	>117	50	132	0.77	0.52	1.14		0.2850	
Breast	Unexposed	6,751	13,383	1					
	1-117	646	1,318	0.91	0.82	1.02			
	>117	632	1,267	0.89	0.80	1.01		0.0854	
Lung	Unexposed	2,711	8,577	1					
	1-117	273	581	1.13	0.93	1.39			
	>117	284	618	1.15	0.94	1.41		0.2449	
Prostate	Unexposed	5,185	10,044	1					
	1-117	261	483	0.91	0.77	1.08			
	>117	231	422	0.89	0.74	1.07		0.3211	

All cancer types adjusted for smoking status, BMI, alcohol use and diagnosed depression. Colorectal cancer is also adjusted for NSAID use.

3.3.6 Confounding by indication

When SSRI use was examined in the same way as tricyclics were in the binary analysis, no significant multivariate results are found (Table 3.10). However, the results do exhibit a similar pattern to the tricyclic analysis, which warrants further investigation.

Table 3.10 SSRI use- binary analysis

				Univariate				Multivariate*		
Cancer type	Exposed	Case	Control	OR	95% CI			OR	95% CI	
Glioma	No	729	1,391	1				1		
	Yes	44	111	0.77	0.53	1.10		0.77	0.53	1.11
Colorectal	No	5,808	11,118	1				1		
	Yes	424	892	0.93	0.82	1.05		0.92	0.82	1.05
Brain	No	539	1,064	1				1		
(excl glioma)	Yes	60	100	1.21	0.86	1.70		1.14	0.81	1.62
Breast	No	9,126	17,840	1				1		
	Yes	1,167	2,256	1.02	0.95	1.10		1.02	0.94	1.10
Lung	No	5,982	11,640	1				1		
	Yes	555	874	1.27	1.13	1.42	←	1.09	0.96	1.24
Prostate	No	7,103	13,550	1				1		
	Yes	428	779	1.06	0.94	1.20		1.04	0.92	1.18
All	No	29,275	56,581	1				1		
	Yes	2,678	5,010	1.05	1.00	1.11	←	1.01	0.96	1.06

*All cancer types adjusted for smoking status, BMI, alcohol use and diagnosed depression. Colorectal cancer is also adjusted for NSAID use.

Dividing the exposures into those given only tricyclics, only SSRIs and those given both drugs (Table 3.11) appears to negate most of the apparent effects in SSRIs, while a lot of the effects for tricyclics remain. For glioma, SSRI use without tricyclic use showed little deviation between cases and controls (OR= 0.96 CI= 0.61–1.53). This is in contrast to tricyclic use with (OR= 0.50 CI= 0.27–0.92) and without (OR= 0.74 CI= 0.52–1.06) SSRI use. Colorectal cancer was similar to glioma in terms of exclusive SSRI use (OR= 0.95 CI= 0.81–1.12), and showed a similar pattern to the above binary analysis for tricyclic use with SSRI use (OR= 0.85 CI= 0.70–1.02), and exclusive tricyclic use (OR= 0.85 CI= 0.76–0.95). Patients treated with both drugs exhibiting a stronger effect size for glioma could be indicative of confounding by indication, but this is dissected further in the investigation of the effect of depression.

Table 3.11 Tricycic/SSRI exposure stratification (multivariate)

Cancer type	Exposure	OR	95% CI		
Glioma	Unexposed	Reference			
	Tricyclic only	0.74	0.52	1.06	
	SSRI only	0.96	0.61	1.53	
	Both	0.50	0.27	0.92	←
Colorectal	Unexposed	Reference			
	Tricyclic only	0.85	0.76	0.95	←
	SSRI only	0.95	0.81	1.12	
	Both	0.85	0.70	1.02	
Brain	Unexposed	Reference			
	Tricyclic only	1.11	0.79	1.57	
	SSRI only	1.00	0.61	1.64	
	Both	1.33	0.83	2.14	
Breast	Unexposed	Reference			
	Tricyclic only	0.97	0.89	1.04	
	SSRI only	1.02	0.92	1.12	
	Both	1.00	0.90	1.12	
Lung	Unexposed	Reference			
	Tricyclic only	1.13	1.01	1.27	←
	SSRI only	1.08	0.91	1.28	
	Both	1.14	0.95	1.38	
Prostate	Unexposed	Reference			
	Tricyclic only	1.03	0.92	1.16	
	SSRI only	1.03	0.88	1.21	
	Both	1.07	0.88	1.29	
All	Unexposed	Reference			
	Tricyclic only	0.92	0.87	0.97	←
	SSRI only	0.92	0.86	0.99	←
	Both	0.90	0.83	0.98	←

All cancer types adjusted for smoking status, BMI, alcohol use and diagnosed depression. Colorectal cancer is also adjusted for NSAID use.

Due to the long term nature of diseases such as depression it is not possible to directly link each prescription to an indication. It was however possible to determine whether each patient had a diagnosis of depression at any point during their registration, and this seemed to be the best proxy for antidepressant indication. If depression is added as a covariate in the regression model the size of effect appears to increase the size of effect of tricyclics on glioma. This is reflected in the stratified analysis and interaction terms (see table below). Depression appears to have little effect on colorectal cancer.

Table 3.12 Effect of depression

Cancer type	Analysis	Exposure	Odds	95% CI		
Glioma	Depression as a covariate	Unexposed	Reference			
		Exposed	0.59	0.42	0.81	←
	Stratified- Depression	Unexposed	Reference			
		Exposed	0.35	0.15	0.81	←
	Stratified- No Depression	Unexposed	Reference			
		Exposed	0.90	0.52	1.54	
	Interaction terms	Unexposed	Reference			
		Tricyclic	0.93	0.57	1.50	
		Depression	1.50	1.16	1.95	
		Tricyclic x Depression	0.46	0.24	0.87	←
Colorectal	Depression as a covariate	Unexposed	Reference			
		Exposed	0.84	0.75	0.94	←
	Stratified- Depression	Unexposed	Reference			
		Exposed	0.94	0.73	1.22	
	Stratified- No Depression	Unexposed	Reference			
		Exposed	0.98	0.81	1.19	
	Interaction terms	Unexposed	Reference			
		Tricyclic	0.94	0.79	1.12	
		Depression	1.07	0.97	1.17	
		Tricyclic x Depression	0.83	0.66	1.03	

Although it is still possible that confounding by indication may explain these data, it appears unlikely that depression would reduce the risk of any cancer. It seems more likely to us that depression is a proxy for high dose tricyclics (Table 3.13). Depression tends to require higher doses than other indications such as pain management and as is demonstrated in the dose analysis, tricyclics appear to be more effective at higher dose.

Table 3.13 Depression vs dose

Depression	**Tricyclic dose**			
	Low dose (frequency)	%	High dose (frequency)	%
No	2584	75.71	829	24.29
Yes	4008	47.15	4492	52.85

3.4 Discussion

3.4.1 Summary of findings

The data presented in this study shows that tricyclic use in man may be associated with a subsequent reduction in the risk of developing glioma and colorectal cancer. These protective effects appear to be specific to these particular cancers, as such protection was not observed for the other cancer types studied, although a protective effect in cancer types not included in the study cannot be ruled out. The data also indicate that these apparent protective effects are greatest for patients receiving high dose prescriptions over a long period of time.

There did appear to be an increase in risk of lung cancer development in tricyclic users. This increase in risk is observed to be highest in the unadjusted model, and is more than halved when the various covariates are taken into account. Smoking is thought to be the primary cause of this confounding and given that there is missing smoking data, coupled with the potential for misclassification of smoking status by GPs, it is highly likely that a large part of the remaining observed effect (a marginally significant 15% increase in risk) is due to residual confounding by smoking. The lack of a consistent tricyclic dose or duration based trend also suggests that there is no true association between lung cancer and tricyclic use.

3.4.2 Method refinements

As this was the first of the studies carried out within this thesis, there were a number of substantial refinements to the methods during the study. The most obvious of these are the changes to the dose and duration analyses.

Originally I used the number of prescriptions that each patient received as a proxy for dose. This was a somewhat crude method, and there was also an element of exposure duration in this analysis. To better determine the true effect of dose it was decided that the mean dose per prescribed day would be used. This was not a perfect method of determining dose, due to the need to standardise the dosing across multiple drug types. However, it does still provide a good measure of what dose each patient received, and produced a good dose response correlation.

For the analysis of duration, the original method of using the time of first exposure was again somewhat crude, as it did not account for the consistency of exposure between first prescription and diagnosis. The revised method instead used the total number of days for which tricyclics were prescribed. While this method did not describe the total time period over which a patient was exposed to tricyclics, it did offer a much better representation of the number of days exposed for.

Though it seemed logically unlikely, confounding by indication was still a possible alternative explanation for the results found. However, the investigation of this matter by looking at the effect of SSRIs and diagnosis of depression confirms that confounding by indication is not a likely explanation.

The fairly substantial changes to the methods seen in this chapter, such as the confounding by indication investigation, could have led to the results changing in either direction. However, despite these various changes, the message delivered by the results throughout was consistent, with the odds ratio for glioma and colorectal cancer showing a fairly consistent protective effect for tricyclic use in terms of size and direction. The other cancers consistently did not exhibit this protective effect.

3.4.3 Strengths and weaknesses

The data presented here have a number of important strengths compared to previous reports. The use of routinely collected general practice records (from the GPRD) ensures that there is no opportunity for recall bias to effect the ascertainment of exposures. In addition, by selecting all relevant malignancies within the population and a random sample of the suitable controls, the possibility of selection bias was dramatically reduced. However, the selection of data does have some weaknesses. Though the numerous validation studies of a variety of diagnoses in this dataset suggest that the outcomes are likely to be accurately coded (Jick et al. 1991; Fombonne et al. 2004; Herrett et al. 2010), and the prospective electronic recording of prescription data suggests the same for the primary exposure, it is difficult to be equally confident about the recording of all potential confounders. As can be seen from Table 2 there is much missing data with respect to smoking, obesity and alcohol. There is clearly therefore a potential for residual confounding by these factors. However I believe that with the exception of lung cancer where this is clearly an issue (and residual confounding by smoking might account for the positive association with tricyclics), the close similarity between univariate and multivariate models suggests that any residual confounding from these additional factors will be minor. A potentially greater issue is that the study lacks any data on other potential confounders such as diet and exercise, and therefore their impact on the results cannot be assessed.

Another strength of the study is that I have been able to study several cancer types and to demonstrate that the protective antineoplastic effect of tricyclics appears to be specific to certain malignancies. I have also been able, due to the size of this study, to subdivide exposure further and hence demonstrate that longer term use and higher doses of tricyclics appear to give greater protection from developing glioma and colorectal cancers. As the proposed anti-cancer mechanism of action is a mitochondrial one (Daley et al. 2005)

and independent of the psychoactive mechanism of action, there is good reason to believe that these findings are generalisable, and not restricted only to a “depressed” population.

3.4.4 Confounding by indication

Confounding by indication is an important factor to investigate in this study. In the binary analysis of SSRIs, they display a similar pattern to tricyclics, this may be due to SSRI use being predictive of tricyclic use. If patients using SSRIs exclusively (i.e. no tricyclics) are considered, most of the effect disappears. The multivariate results were adjusted for diagnosis of depression, and this adjustment increased the apparent protective effect of tricyclics. From a biological plausibility point of view, depression seems more likely to increase cancer risk than decrease it. This has been suggested in a variety of studies, and could possibly be mediated through depression causing suppression of the immune system (reviewed in (Reiche et al. 2004)). It is therefore likely that depression is a proxy for high dose tricyclics (as depression is usually treated with a higher dose than other tricyclic indications, such as pain). This is supported by a relationship between dose and depression (where those without depression have a higher proportion of low dose and *vice versa*).

3.4.5 Comparison with previous literature

As mentioned above there have been previous epidemiological studies in this area. These have examined the incidence of colorectal cancer (Xu et al. 2006), prostate cancer (Tamim et al. 2008), breast cancer (Cotterchio et al. 2000; Gonzalez-Perez et al. 2005; Fulton-Kehoe et al. 2006; Wernli et al. 2009) and lung cancer (Toh et al. 2007a) in relation to tricyclic use. However, little consistent, significant evidence has been found to link tricyclics with changes in cancer incidence. Of perhaps greatest interest here are the studies looking in more detail at the malignancies in which relationships have been shown.

In their study on colorectal cancer Xu et al (2006) hypothesized that tricyclics may be genotoxic, and would therefore increase cancer risk. However, their results suggest a non-significant protective effect, while another recent study using a different type of data source (Coogan et al. 2009) confirms these findings. The Xu et al study used data from the Saskatchewan Cancer Agency registry, which contains broadly similar data to the GPRD. In the study there were approximately half the number of cases than in the present study, which pay in part explain the lack of significant findings, despite the similar effect size. Additionally, the present study uses slightly more extensive analysis, in that dose could be investigated, where as It was not by Xu et al. The prostate cancer study (Tamim et al. 2008) also uses the Saskatchewan database. While this study does find a small increase in risk of prostate cancer in tricyclic users, the authors attribute this to detection bias. This is due to the apparently short latency period between exposure and diagnosis, and therefore the lack of etiological plausibility.

Coogan et al (2009) used survey based data, which were collected at various times and locations. While this approach can provide richer and more specific data, it is also more labour intensive (as it is not routinely collected). Hence this study has a smaller sample size than in this thesis, which is likely to have contributed to the lack of significant findings. The findings in this thesis for colorectal cancer fit well with these previous data, with the added benefit of the study's ability to show statistically significant protection. This is a function of the greater numbers of cases and case controls used in the study.

The previous lung cancer study (Toh et al. 2007a) showed an apparent increase in risk of lung cancer with tricyclic use, which is largely mitigated by adjusting for confounders (including smoking status). This is remarkably consistent with the data presented in this thesis. Another UK primary care database, The Health Improvement Network (THIN) (which shares many GPs with the GPRD) was used as a data source in this study. Despite smoking

status being more comprehensively classified in this study, there was still substantial missing data on this clearly important confounding variable.

Studies which looked at breast cancer are slightly more numerous, perhaps reflecting the greater incidence of breast cancer. Of these, the study by Cotterchio et al (2000) is the only study to find an significant change (increase) in breast cancer risk. These results are marginally significant, based on a small sample size (~700 cases) and the author's conclusions have been criticised for being somewhat overzealous (Lawlor 2000). The other studies (Gonzalez-Perez et al. 2005; Fulton-Kehoe et al. 2006; Wernli et al. 2009) are based on larger datasets of 3-4 thousand patients and do not find any associations between antidepressant use and breast cancer risk.

3.4.6 Interpretation

How then should these findings be interpreted? I have found a significant reduction in incidence of colorectal cancer and glioma in a manner consistent with previous laboratory evidence (Daley et al. 2005) and not inconsistent with other epidemiological studies. The findings show specificity of protection against those malignancies I originally hypothesised might be affected and show a dose response relationship and a clear temporal relationship. It remains credible that the associations I have found may be causal, however the modest size of the effect demonstrated limits the potential of these drugs as a chemopreventative agent in the general population. Groups at increased risk of colorectal cancer (e.g. those with a familial or other genetic predisposition to the disease) might still represent a group in whom an RCT could be appropriate as they would have the best chance of benefitting from chemoprevention. Even here though, one would need to balance potential benefits against possible side effects. As glioma is a rare cancer with ill-defined high risk groups, prescribing chemopreventative drugs is of limited value in the general population. As an

illustration of this, I estimate that approximately 40,000 people would need to be treated (for >117 days) in order to prevent one glioma.

If the antineoplastic effects of this group of drugs are to be therapeutically useful therefore, it is likely to be either after the identification of an individual compound within the group which is the most potent, or in post diagnosis treatment of both colorectal cancer and glioma. As an illustration of the potential of the latter, aspirin, a recognized prophylactic for colorectal cancer has recently been demonstrated to reduce colorectal cancer specific mortality when used after diagnosis (Chan et al. 2009; Zell et al. 2009). The effect demonstrated here is of a magnitude similar to that achieved previously only by far more toxic compounds and it would therefore certainly be useful to discover whether tricyclics have similar effects on colorectal cancer and glioma.

This is the aim of the next study.

4 Tricyclic antidepressants and cancer survival

A cohort study using the GPRD

4.1 Introduction

The reduction in incidence of glioma and colorectal cancer caused by tricyclic antidepressants described in the previous chapter is intriguing and further hints at their anti-cancer action. However, their immediate use as an anti-cancer agent is more likely to be in treatment, rather than chemoprevention, as explained at the end of Chapter 3. The premise of this chapter is therefore to attempt to determine whether the previously suggested cancer prevention action of tricyclics translates into a reduction in mortality in cancer patients using these drugs.

4.1.1 Conventional treatment for glioma/colorectal cancer

Conventional chemotherapeutic agents often have unpleasant side effects and despite advances over recent years often produce benefits which are limited for many patients.

For both glioma and colorectal cancer, curative treatment can sometimes be achieved through surgery alone. This is highly dependent on the stage of the cancer, with most localised colorectal tumours being treated in this way, while later stages are likely to require chemotherapy and/or radiotherapy. First line chemotherapy for colorectal cancer often consists of either 5-fluorouracil/leucovorin or oxaliplatin. These are also commonly used for adjuvant treatment. Also, recently the use of bevacizumab in combination with one of these drugs has become more common in metastatic disease. Radiotherapy is not commonly used for first line treatment of colon cancer, but is often used as a neoadjuvant in rectal cancer, or for palliative treatment.

For later stage glioma, treatment options are more limited. Combinations of radiation, surgery and chemotherapy using temozolomide are often used. Despite this, prognosis for grade IV gliomas such as glioblastoma multiforme is bleak, with the main aim of treatment in these cases to extend life and palliate symptoms rather than cure.

The search for less toxic and more efficacious drugs is therefore vital to patients.

4.1.2 Tricyclics and cancer

As this has been covered in detail in Chapter 3, only a brief revision of previous evidence of the anti-cancer effects of tricyclics is included here.

Tricyclic antidepressants, conventionally used in the treatment of psychological disorders, such as depression, anxiety, insomnia and some types of chronic pain, may have an anti-cancer action. Laboratory evidence has demonstrated anticancer effects in several tricyclics, including chlorimipramine (clomipramine), imipramine, citalopram, amitriptyline, and desipramine (Xia et al. 1999; Arimochi et al. 2006). This includes *in vitro* data which suggest tricyclics can have cytotoxic actions in various cancer cell lines including glioma cells (Xia et al. 1999; Daley et al. 2005; Levkovitz et al. 2005) and colorectal cancer cells (Arimochi et al. 2006). The mechanism for this anticancer action may be inhibition of mitochondrial complex III activity, leading to a decrease in mitochondrial membrane potential and apoptosis (Weinbach et al. 1986; Daley et al. 2005; Higgins et al. 2010). Animal studies substantiate this anticancer action in various cancer models, such as sarcoma, lymphocytic leukaemia and leukaemia grown as a solid tumour (Tsuruo et al. 1983; Merry et al. 1991; Pommerenke et al. 1995). Glioma is the most studied of the cancer types in relation to the tricyclics' anticancer action and research in this area extends to preliminary clinical studies in humans using chlorimipramine therapeutically (Beaney et al. 2005).

4.1.3 Rationale for study

The previous chapter demonstrated that tricyclics appear to reduce the incidence of both colorectal cancer and glioma in vivo (Walker et al. 2011). This study showed that glioma incidence was reduced by around 50% with higher dose tricyclic use, while colorectal

cancer incidence was reduced by a more modest but still highly significant amount. The need however to treat large numbers of people when using drugs as chemotherapeutics means that they cannot realistically be so used at present. The reason for this is that their side effect profile would outweigh any benefit from reduction in cancer incidence if used in the general population. My previous results do however encourage the search for evidence of the efficacy of tricyclics as an adjuvant treatment in glioma and colorectal cancer patients, which could be of immediate benefit to patients. This is because the side effects of tricyclics are very mild in comparison to chemotherapeutics.

The BNF (edition 60), is very clear about the risks of cytotoxic drug use. It states that in addition to their anti-cancer action, they have the potential to damage normal tissue and are teratogenic. In addition to this it has stringent guidelines for their handling, including: Use of trained personnel, protective clothing, eye protection and monitoring of staff exposure to the drugs. Side effects common to most cytotoxic drugs are listed as:

- **Severe tissue necrosis** if leakage into an extravascular compartment occurs- common if incorrectly administered.
- **Oral mucositis** is common with fluorouracil, methotrexate, and the anthracyclines.
- **Tumour lysis syndrome** is a condition caused by rapid destruction/necrosis of cancer tissue. This can cause imbalances in various electrolytes in the blood, renal damage and arrhythmias.
- **Nausea and vomiting** is common with most cytotoxics, especially cisplatin, dacarbazine, and high doses of cyclophosphamide.
- **Bone marrow suppression** is caused by all cytotoxic drugs apart from vincristine and bleomycin. Due to increased risk of infection, and other conditions such as anaemia, it is a common limiting factor in treatment, as it often requires reduction or delaying of treatment to allow blood cell counts to recover.

- **Alopecia** is a well known side effect that occurs with some drugs, though it is generally reversible.
- **Reproductive function** can be affected, sometimes including permanent male sterility, premature menopause. Also due to their teratogenic properties, use in pregnancy is not recommended.
- **Venous thromboembalism** risk is increased by cytotoxics (beyond the increase in risk already seen in cancer patients).

While tricyclic antidepressants do have a number of side effects associated with them, these are generally less serious and/or less common than for cyctotoxic drugs. Additionally, some tolerance to these side effects can develop:

- **Risk in overdose** due to their cardiovascular and epileptogenic effects is an important consideration when determining dosage.

- **Arrhythmias and heart block** can occur occasionally, particularly in patients with cardiovascular disease.

- **CNS side effects,** including anxiety, dizziness, agitation, confusion, sleep disturbances, irritability, and paraesthesia are quite common.

- **Antimuscarinic side-effects** including dry mouth, blurred vision, constipation, and urinary retention.

- **Endocrine effects** including breast enlargement, galactorrhoea, gynaecomastia and sexual dysfunction may occur.

- **Other side effects** can include nausea, vomiting and suicidal behaviour in some patients.

Given the apparent anti-cancer effects of tricyclics and their great advantages in terms of toxicity, I therefore carried out a cohort study using the General Practice Research Database, with the hypothesis that tricyclic use would improve survival in patients with glioma or colorectal cancer.

4.2 Methods

4.2.1 Study design

A prospective cohort study was carried out using the GPRD to determine the relationship between tricyclic drug usage and survival, post cancer diagnosis.

4.2.2 Subjects

Any person with a recorded diagnosis of colorectal cancer or glioma within the GPRD was selected occurring at least one year after their entry to the database. Patient data were collected from the beginning of the GPRD database (1987), up to the last available data in the database (2010). Patients with a previous diagnosis of the cancer being studied (either glioma or colorectal cancer) were excluded from the cohort, as were patients contributing less than 6 months of data to the GPRD.

4.2.3 Outcome and exposures

The outcome to be observed was all cause mortality. Date of patient death was determined from the existence of one of two records; either a patient with a "Transfer out reason" specified as "death"; or by a "Statement Of Death" (SoD) code (a "Clinical" or "Referral" event with a Read/OXMIS code indicating a death). Where both records existed, the date of death was determined preferentially from the SoD code. The survival time was determined to be the time between diagnosis and the death date determined by the above method. Follow up time for patients not dying in the study was determined either from the date that the patient transferred out from the GP or by the last data collection date for the GP.

The primary exposure was the use of tricyclic antidepressants (section 4.3.3 of the British National Formulary (BNF)). To be exposed, a patient must have had a repeat prescription (≥2) within the period being examined for exposure. In order to minimise reverse causality (i.e. patients who die soon after diagnosis being less likely to receive a prescription) a fixed period of 6 months post-diagnosis was used to determine drug exposure and patients who died or were censored within this period were excluded from the analysis. In addition, a 3 month period post diagnosis was examined for drug exposure, to explore any early effects on mortality.

Pre-diagnosis exposure was considered to determine whether it influenced the effect of post-diagnosis exposure. This was done in two ways; 1) by adjusting for pre-diagnosis use in the Cox proportional hazards model, and 2) by stratifying the analysis according to whether patients received pre-diagnosis tricyclics.

Potential associations were examined further by investigating the dose used. To allow comparison of the effect of high and low doses across all the tricyclics. I standardized the definition of high dose and low dose between drugs relative to the maximum recommended doses for each drug (determined from the BNF). These standardized doses were then used to calculate the mean dose across all prescriptions for each patient individually. Patients with tricyclic use were divided into 'high' dose or 'low' dose groups based on the median corrected dose of 0.31 times maximum recommended dose.

4.2.4 Other covariates

I extracted data on gender, age, smoking status, alcohol use, body mass index (BMI), diagnosis of depression and comorbidity (coded as the Charlson Index (Charlson et al. 1987)). Of these potential confounders, gender, age and smoking status were retained in multivariate models as *a priori* predictors of mortality risk. Other covariates were only

retained in the multivariate model if they produced a 10% or greater change in the measured size of effect.

4.2.5 Statistical methods

I used Cox proportional hazards modelling to assess the effect of tricyclic antidepressants on mortality risk, adjusting for multiple potential confounding variables as described above. Results are presented as hazard ratios (HR), with accompanying 95% confidence intervals (CIs).

Validity of the proportional hazards assumption was tested using a log-log plot. If the proportional hazards assumption was found to be violated, this non proportionality was then further characterised. The approximate time period that a change in effects occurred was determined by using observed/predicted survival curves and observing where the observed curve deviated from the predicted. No serious violation of the proportional hazards assumption was found during this study, indicating that the effect size (hazard ratio) did not vary according to time since diagnosis of the cancer.

All data handling and analysis was done using Stata v11.1 SE (Statacorp, 4905 Lakeway Drive, College Station, Texas 77845 USA).

Further details of how and why the various statistical methods in this chapter (and chapter 5) were used can be found in Appendix III.

4.3 Results

4.3.1 Study population/ covariates

2592 patients with glioma and 22,524 patients with colorectal cancer were identified. Of these patients, 1227 (47.3%) glioma and 6004 (26.7%) colorectal cancer patients were excluded from most of the study, as their death or loss to follow up occurred during the 6 month post diagnosis period being examined for exposure. Analysis was carried out on the remaining cohort, where there were 679 deaths in the glioma group and 6,947 deaths in the colorectal cancer group. Median time of post diagnosis follow-up for those remaining alive to the end of follow-up was 3.2 years for glioma and 3.7 years for colorectal cancer. In total, 4.2% for glioma patients and 4.1% for colorectal cancer patients received tricyclic prescriptions (≥2 prescriptions between diagnosis and 6 months post diagnosis, as defined in methods). The patient population is summarised, along with other covariates used in the study in Table 4.1.

Table 4.1 Population/covariates

Cancer type			Tricyclic user	%	Tricyclic nonuser	%
Glioma	All patients		57		1307	
	Women		39	68.4	547	41.8
	Mean age (SD)		56.7	(14.4)	45.5	(20.7)
	Mean BMI		26.5	(4.2)	26.1	(4.9)
	Smoking status	No	29	50.9	661	50.6
		Ex	10	17.5	205	15.7
		Yes	13	22.8	227	17.4
		Missing	5	8.8	214	16.4
	Alcohol use	No	9	15.8	166	12.7
		Ex	1	1.8	25	1.9
		Yes	36	63.2	753	57.6
		Missing	11	19.3	363	27.8
	Mean Charlson Index		11.6	(6.2)	10.0	(7.7)
	Diagnosed depression		33	57.9	272	20.8
Colorectal	All patients		669		15 850	
	Women		425	63.5	7011	44.2
	Mean age		70.6	(11.4)	69.7	(11.6)
	Mean BMI		26.5	(4.9)	26.4	(4.5)
	Smoking status	No	331	49.5	8357	52.7
		Ex	180	26.9	4445	28.0
		Yes	128	19.1	2280	14.4
		Missing	30	4.5	768	4.9
	Alcohol use	No	150	22.4	2438	15.4
		Ex	26	3.9	273	1.7
		Yes	404	60.4	10 919	68.9
		Missing	89	13.3	2220	14.0
	Mean Charlson Index		12.7	(8.3)	10.4	(7.5)
	Diagnosed depression		401	59.9	3176	20.0

Numbers in table represent N (%) for categorical variables and Mean (SD) for continuous variable

4.3.2 Binary analysis- glioma

I found that post diagnosis tricyclic use (Table 4.2) was associated with a non-significant decrease in the hazard ratio among glioma patients (multivariate HR=0.83 95% confidence interval (CI) =0.53-1.28). This is similar when 3 months post diagnosis are used to determine exposure, rather than 6 months (HR=0.87 CI=0.57-1.33). This effect is due entirely to those not exposed to tricyclics before diagnosis. The size of effect was larger in this group, though still non-significant (HR=0.54, CI=0.25-1.14). In contrast to this, there was no substantial difference in mortality risk for those exposed to tricyclics pre diagnosis. If pre diagnosis tricyclic use is considered exclusively, there was no association with survival in glioma patients (multivariate HR=1.05 CI=0.88-1.25). This analysis using pre diagnosis exposure included the patients excluded in the rest of the study. Age was found to be an important confounding factor in this analysis, as tricyclic users tend to be older than non-users. For this reason, only age adjusted results are displayed in the table. If an entirely univariate Cox regression is carried out, the hazard ratio is 1.21 (CI=0.85-1.73).

Table 4.2 Binary Cox regression- glioma

Prediagnosis drug exposure group	Post diagnosis Exposure	Patient status at end of follow-up		Age adjusted			Age and prediagnosis drug use adjusted			Multivariate*		
		Alive	Dead	HR	95%	CI	HR	95%	CI	HR	95%	CI
All participants	Unexposed	660	647	1			1			1		
	Exposed	25	32	0.81	0.57	1.16	0.87	0.59	1.28	0.83	0.53	1.28
Tricyclic nonusers	Unexposed	611	611	1			-	-	-	1		
	Exposed	10	11	0.68	0.38	1.24	-	-	-	0.54	0.25	1.14
Tricyclic users	Unexposed	49	36	1			-	-	-	1		
	Exposed	15	21	1.01	0.58	1.78	-	-	-	1.07	0.53	2.17

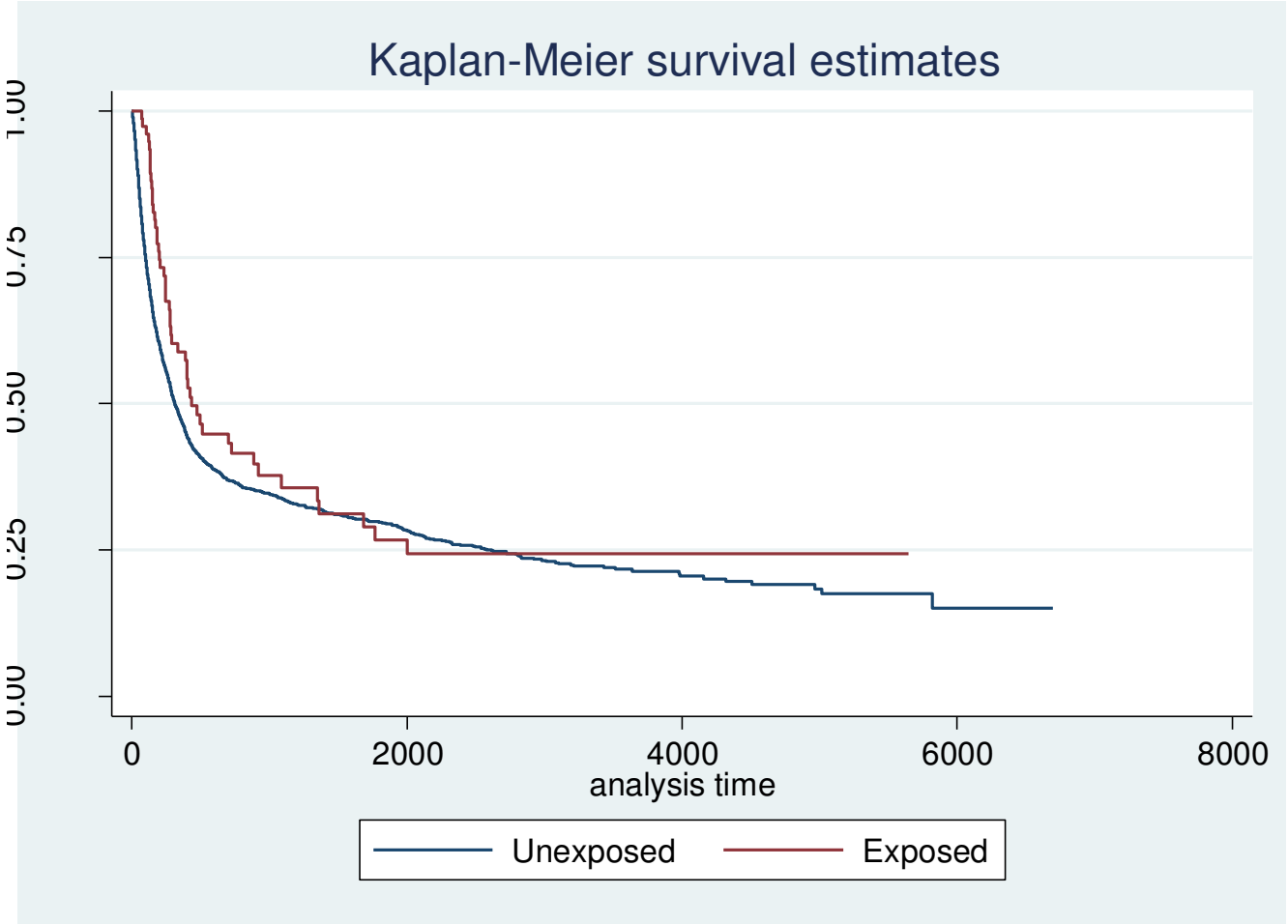
*adjusted for age, gender, depression, Charlson index, BMI and smoking

4.3.3 Kaplan-Meier curves- glioma

Drawing a KM curve without excluding patients who died or were censored during the period examined for exposure (Figure 4.1) gives the impression that there is reduced mortality in drug users in the unadjusted analysis. This is corrected in Figure 4.2 by excluding these patients, and these curves reflect the unadjusted Cox regression, where a small increase in mortality in drug users is evident.

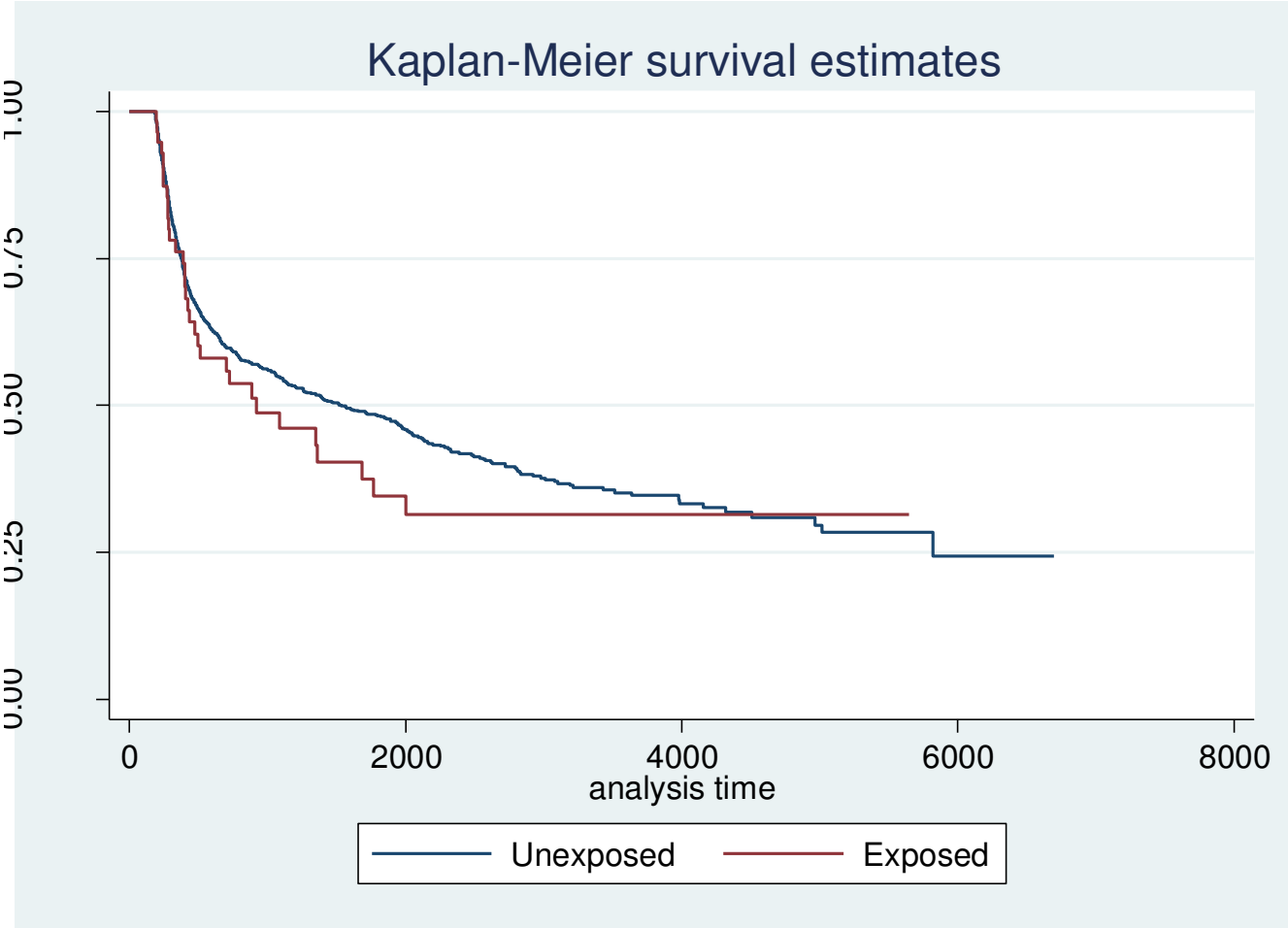
To better reflect the multivariate Cox regression, the KM curves were adjusted for the same covariates as in this analysis (Figure 4.3). This shows that there is a reduction in mortality for drug users.

Figure 4.1 Glioma binary unadjusted analysis- without exposure time exclusion



Survival curves for glioma. Exposed patients received ≥2 prescriptions in the 6 months following cancer diagnosis. This graph includes patients who died or were lost to follow up in the first 6 months after diagnosis. This introduced a bias in the initial part of the curve.

Figure 4.2 Glioma binary unadjusted analysis- with exposure time exclusion



Survival curves for glioma. Exposed patients received ≥2 prescriptions in the 6 months following cancer diagnosis. Patients dying or being lost to follow up in the 6 months post diagnosis were excluded from this graph.

Figure 4.3 Glioma binary multivariate analysis

This multivariate survival curve depicts the same patients as in Figure 4.2, but was adjusted for age, gender, depression, Charlson index, BMI and smoking.

4.3.4 Dose response- glioma

These data were further investigated by looking at the dose of tricyclics used (Table 4.3). For glioma, there was no apparent dose response effect, as there was not a trend towards greater effect size at higher doses. The greatest effect size was seen in the 'low' dose category, with a non-significant hazard ratio of 0.75 (CI=0.42-1.33).

Table 4.3 Dose response- glioma

Post diagnosis	Patient status at end of follow-up		Age adjusted			Multivariate*		
Exposure	Alive	Dead	HR	95%	CI	HR	95%	CI
Unexposed	668	661	1			1		
Low dose	11	8	0.69	0.41	1.17	0.75	0.42	1.33
High dose	6	10	0.95	0.59	1.52	0.88	0.51	1.54

*adjusted for age, gender, depression, Charlson index, BMI and smoking

4.3.5 Binary analysis- colorectal cancer

For colorectal cancer, post diagnosis tricyclic exposure was found to be associated with a significant increase in the hazard ratio (HR=1.40, CI=1.22-1.60). Once again when 3 months post diagnosis are used to determine exposure the effect is similar (HR=1.30 CI=1.11-1.51). This effect was only observed in those beginning tricyclic use after diagnosis having not used them prior to diagnosis (HR=2.02, CI=1.63-2.49). In concurrence with the glioma findings, no effects were observed when pre-diagnosis exposure only was considered (HR=1.01, CI=0.95-1.08). An entirely unadjusted Cox regression gives a hazard ratio of 1.32 (CI=1.18-1.47).

Table 4.4 Binary Cox regression- colorectal cancer

Prediagnosis drug exposure group	Post diagnosis Exposure	Patient status at end of follow-up		Age adjusted			Age and prediagnosis drug use adjusted			Multivariate*		
		Alive	Dead	HR	95%	CI	HR	95%	CI	HR	95%	CI
All participants	Unexposed	9,237	6,613	1			1			1		
	Exposed	335	334	1.29	1.15	1.44	1.39	1.22	1.57	1.40	1.22	1.60
Tricyclic nonusers	Unexposed	8,338	6,075	1			-	-	-	1		
	Exposed	70	107	1.93	1.59	2.33	-	-	-	2.02	1.63	2.49
Tricyclic users	Unexposed	899	538	1			-	-	-	1		
	Exposed	265	227	1.16	0.99	1.35	-	-	-	1.15	0.97	1.36

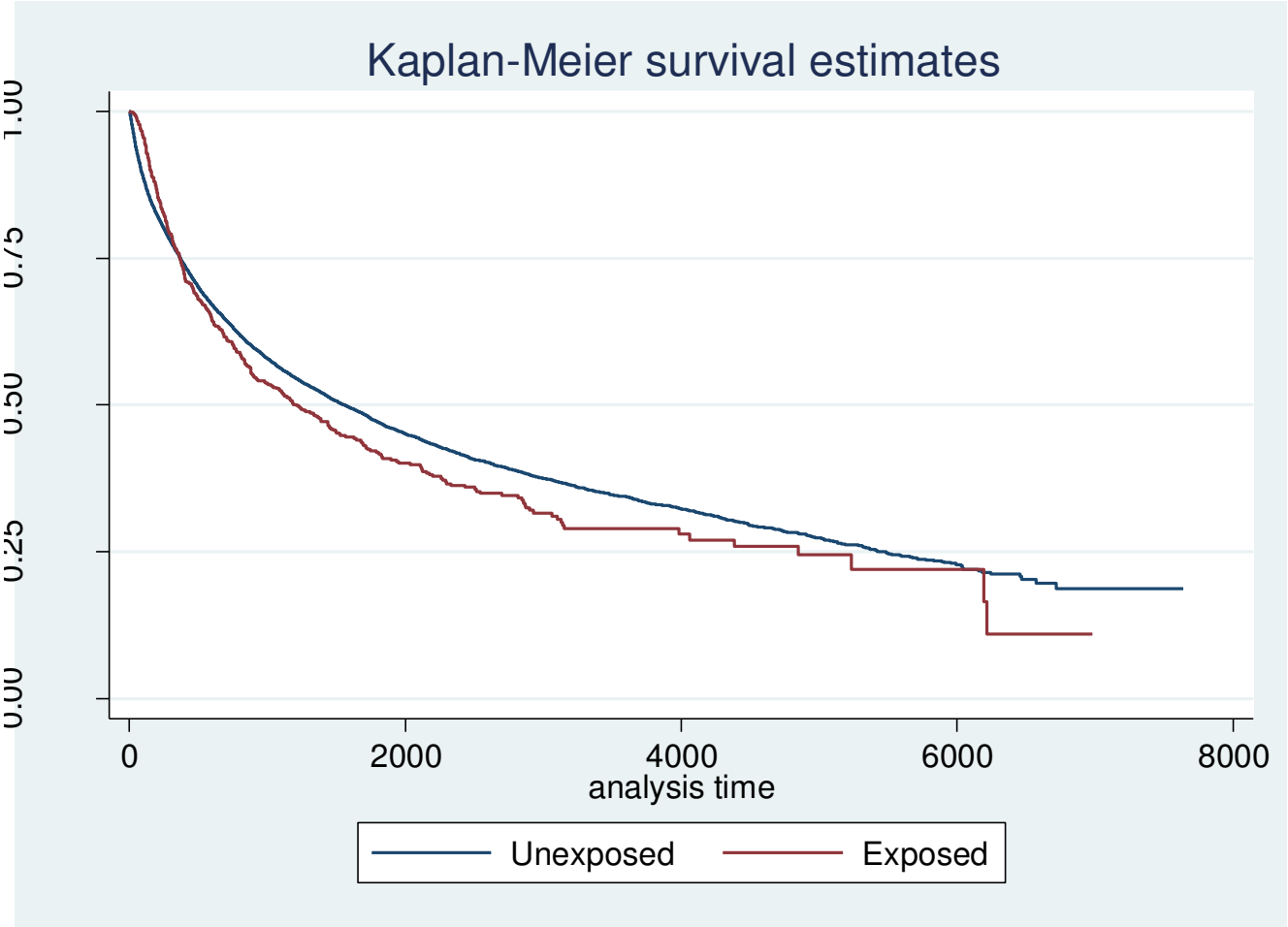
*adjusted for age, gender, depression, Charlson index, BMI and smoking

4.3.6 Kaplan –Meier curves- colorectal cancer

In a similar manner to the glioma curves without excluding patients who died or were censored during the period examined for exposure, there is an initial improvement in drug user mortality (Figure 4.4**Error! Reference source not found.**). This is corrected by excluding these patients (Figure 4.5) and shows an increase in mortality for tricyclic users.

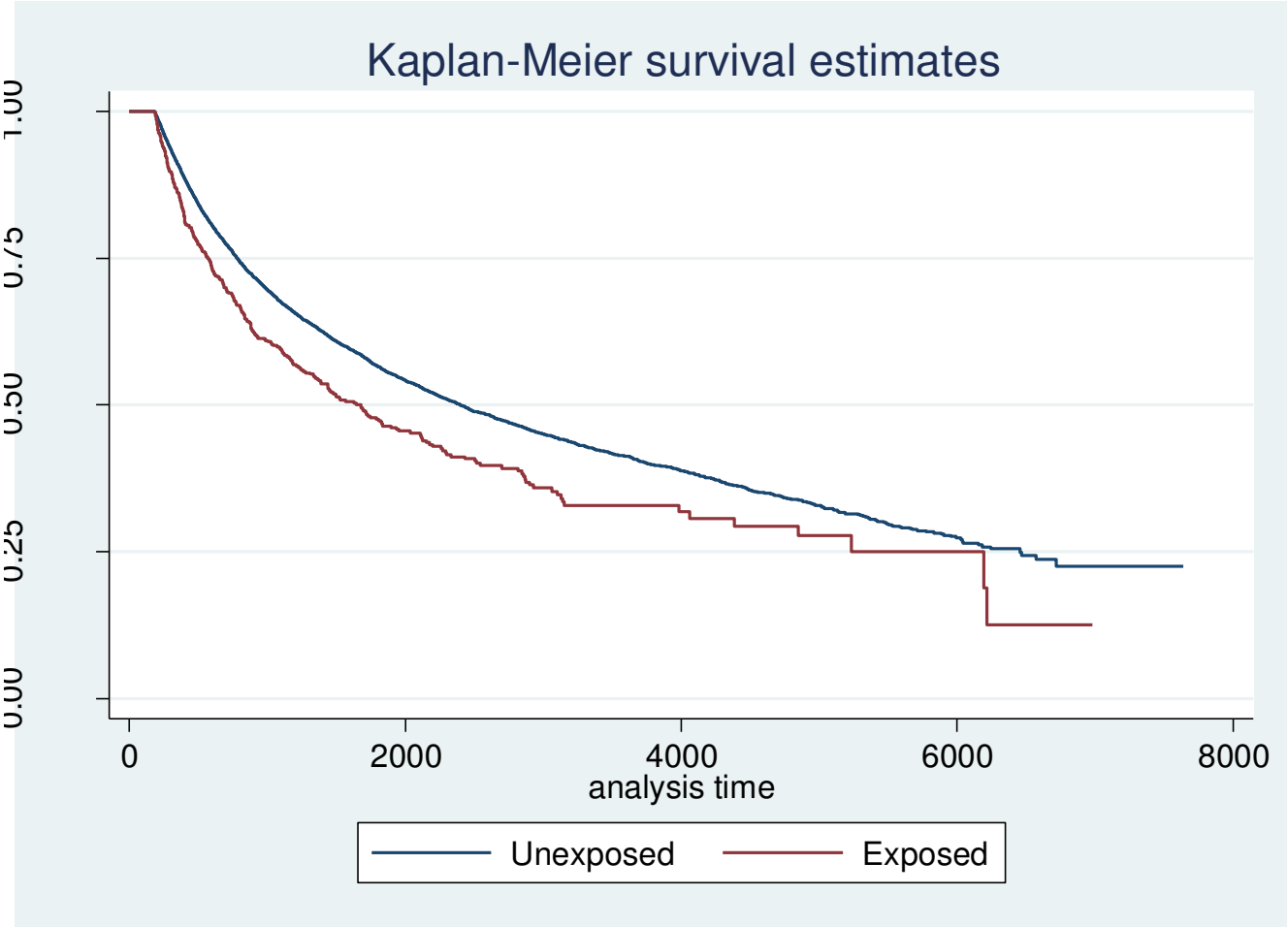
Adjustment for multiple confounders increases mortality in the KM curve (Figure 4.6), which is in line with the multivariate Cox regression.

Figure 4.4 Colorectal binary unadjusted analysis- no exposure time exclusion



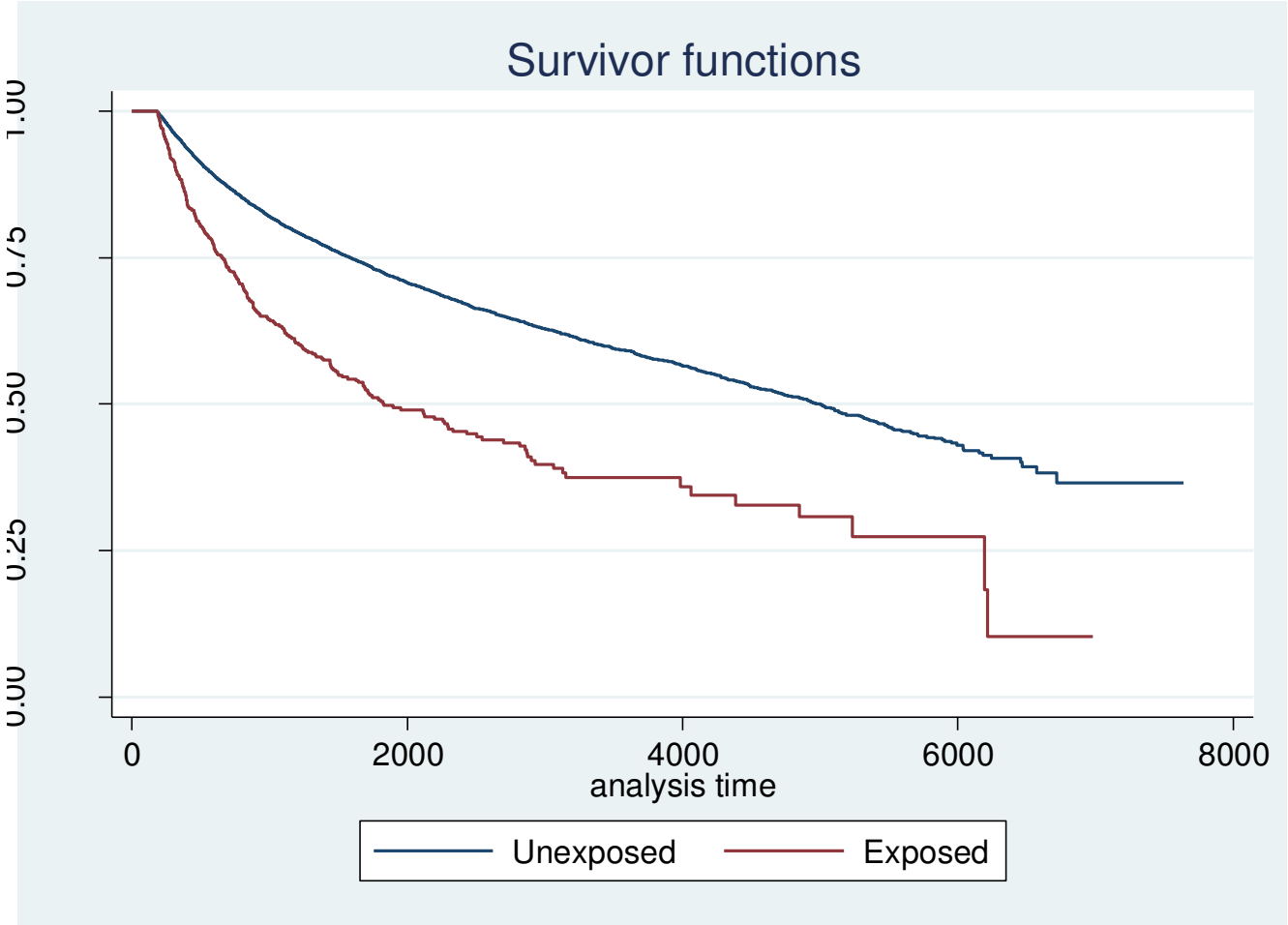
Survival curves for colorectal cancer. Exposed patients received ≥2 prescriptions in the 6 months following cancer diagnosis. This graph includes patients who died or were lost to follow up in the first 6 months after diagnosis. This introduced a bias in the initial part of the curve.

Figure 4.5 Colorectal binary unadjusted analysis- with exposure time exclusion



Survival curves for colorectal cancer. Exposed patients received ≥2 prescriptions in the 6 months following cancer diagnosis. Patients dying or being lost to follow up in the 6 months post diagnosis were excluded from this graph.

Figure 4.6 Colorectal cancer binary multivariate analysis



This multivariate survival curve depicts the same patients as in Figure 4.2, but was adjusted for age, gender, depression, Charlson index, BMI and smoking.

4.3.7 Dose response- colorectal cancer

Similarly, with colorectal cancer, there was no consistent dose response trend for the deleterious effect observed. Once again, the 'low' dose category exhibited the largest size of effect (HR= 1.55, CI=1.31-1.83).

Table 4.5 Dose response- colorectal cancer

Post diagnosis	**Patient status at end of follow-up**		**Age adjusted**				**Multivariate***			
Exposure	Alive	Dead	HR	95%	CI		HR	95%	CI	
Unexposed	9,367	6,766	1				1			
Low dose	90	79	1.49	1.28	1.74	←	1.55	1.31	1.83	←
High dose	115	101	1.13	0.97	1.32		1.15	0.98	1.36	

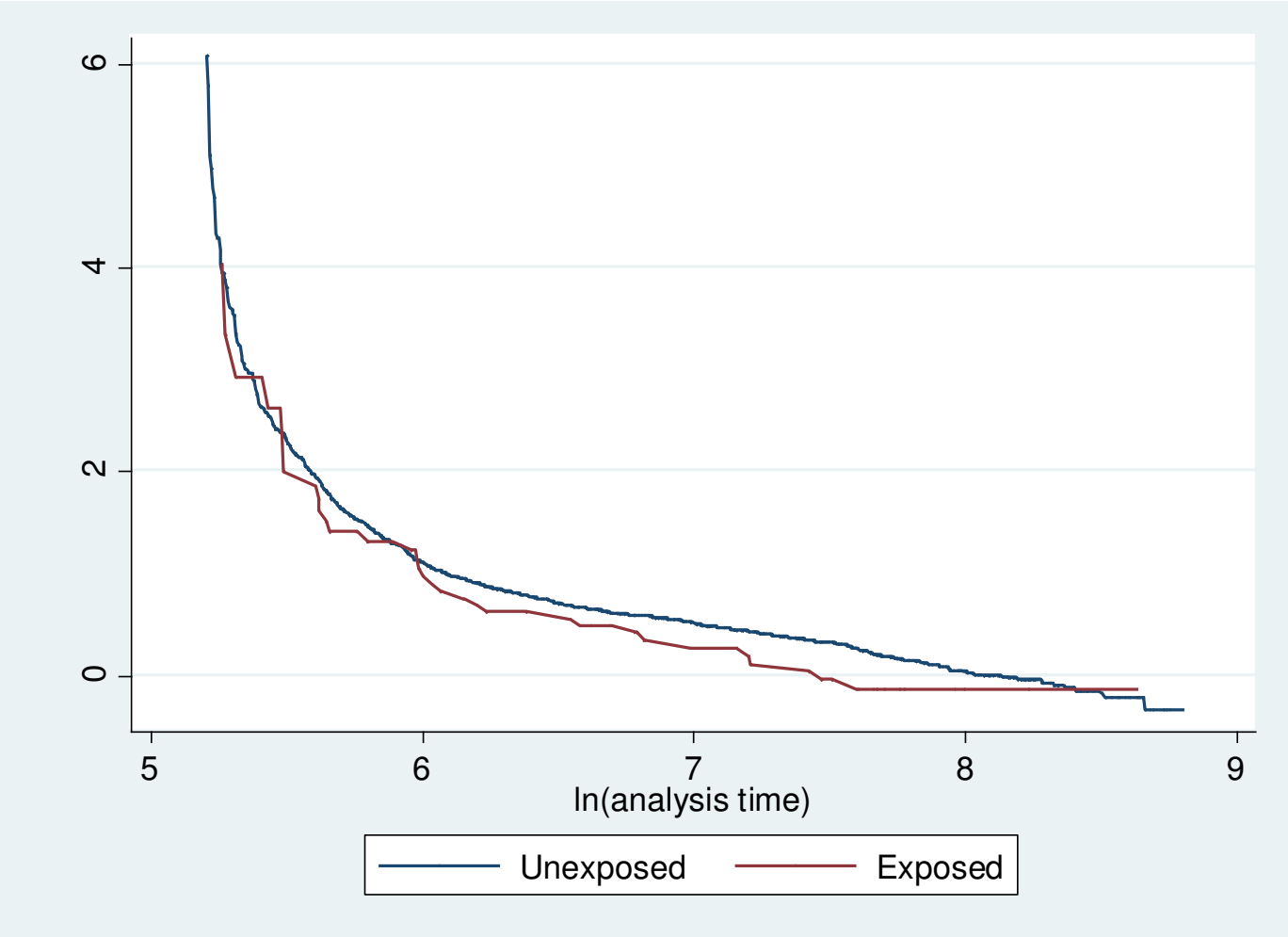
*Adjusted for age, gender, depression, Charlson index, BMI and smoking.

4.3.8 Proportional hazards assumption

The log-log plots for both glioma (Figure 4.7) and colorectal cancer (Figure 4.9) indicate that the proportional hazards assumption holds reasonably. Although the lines for tricyclic users and non users are quite close, they are reasonably parallel along their length. There is some fluctuation in the tricyclic using glioma patients, but this is likely due to small numbers rather than differential effects.

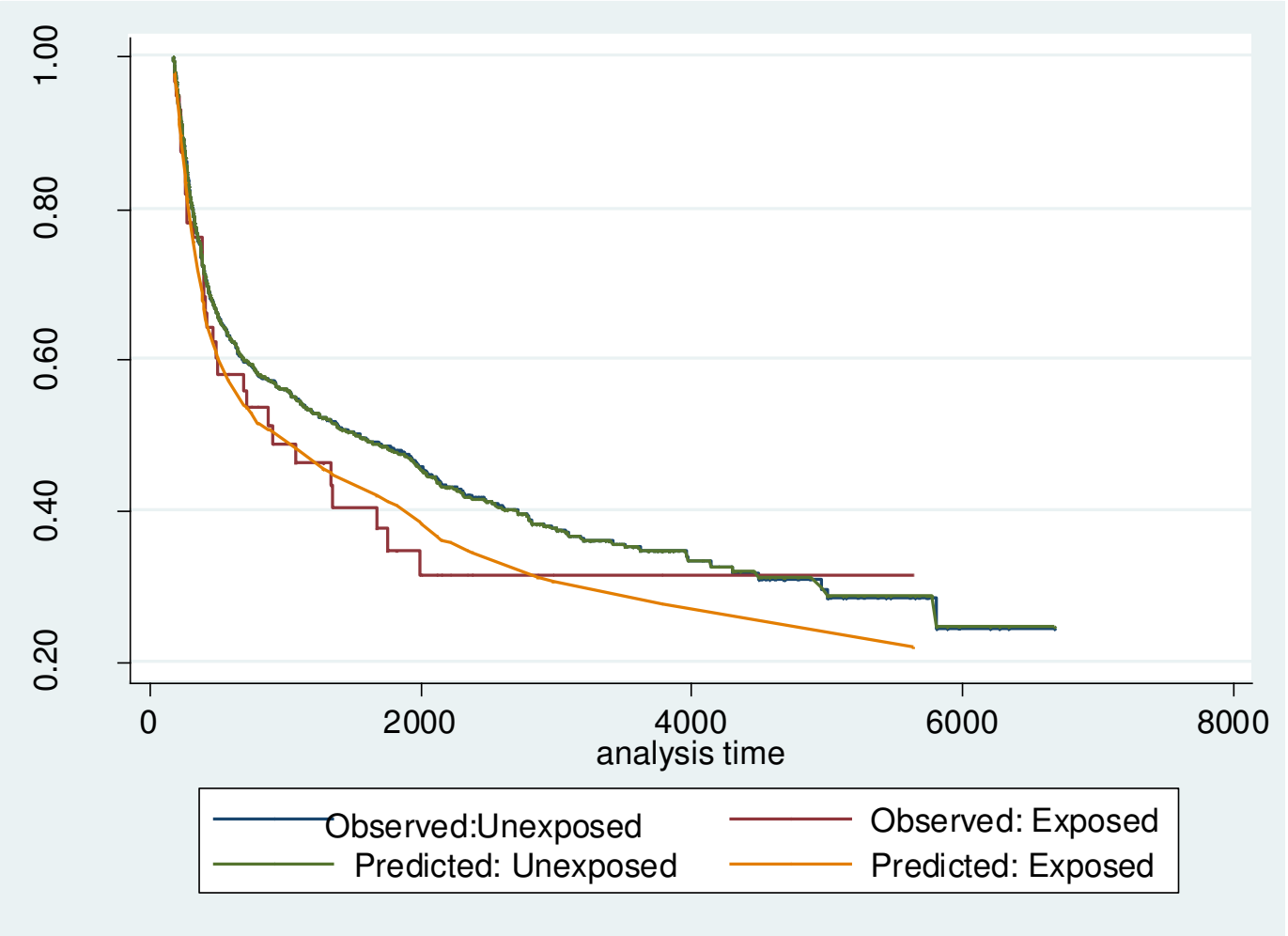
The observed/predicted KM curves for both glioma (Figure 4.8) and colorectal cancer (Figure 4.10) give further indication of the characteristics of how the hazards change over time. Large deviations from the predicted line could be used to determine where changes in mortality hazard occur. However, given the findings from the log-log plot, and that the observed lines follow the predicted reasonably closely, it seems the proportional hazards assumption is reasonably valid.

Figure 4.7 Glioma log-log plot



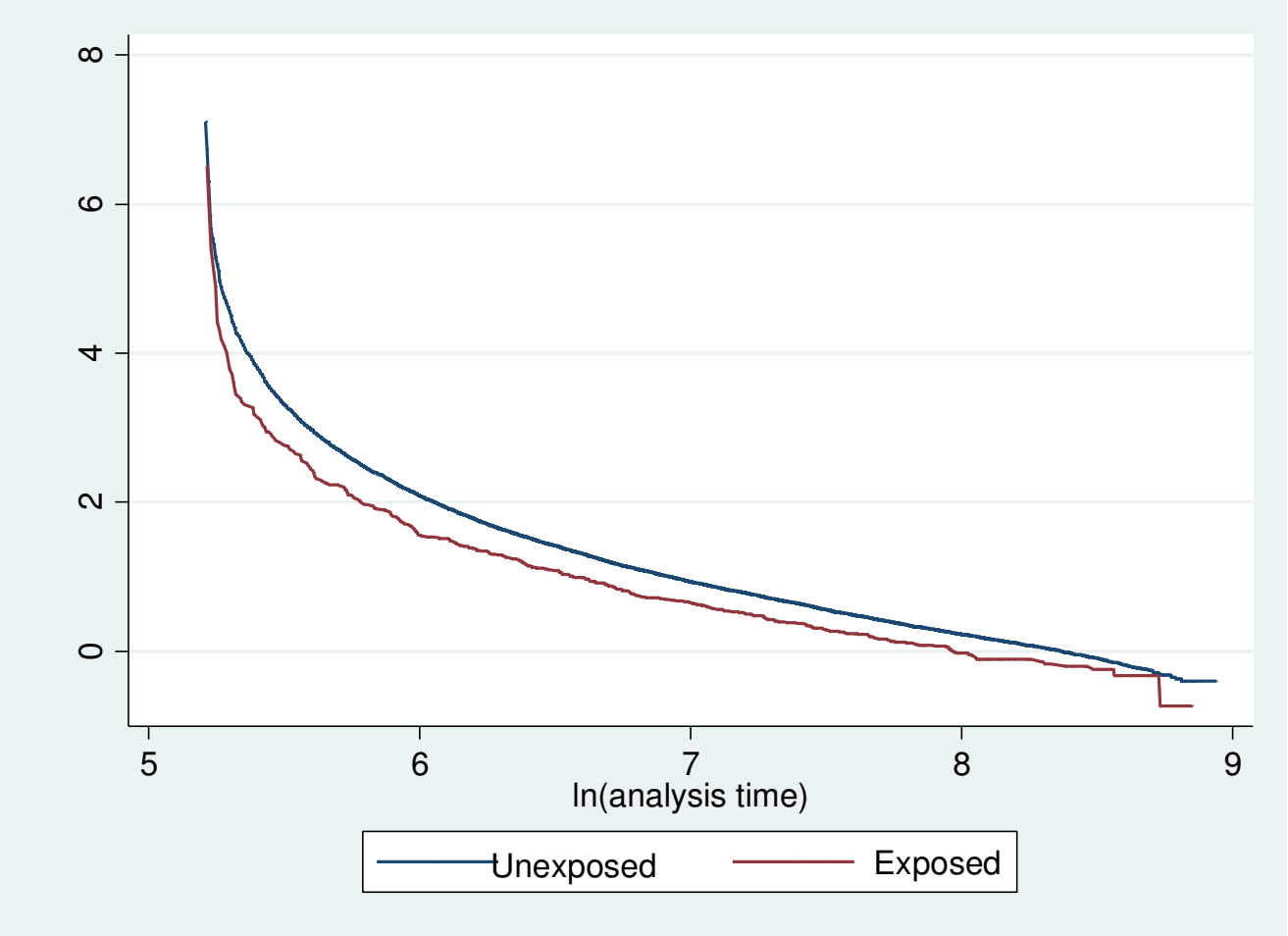
Assessment of the proportional hazards assumption for tricyclic antidepressant exposure in glioma mortality.

Figure 4.8 Glioma- observed vs predicted hazards



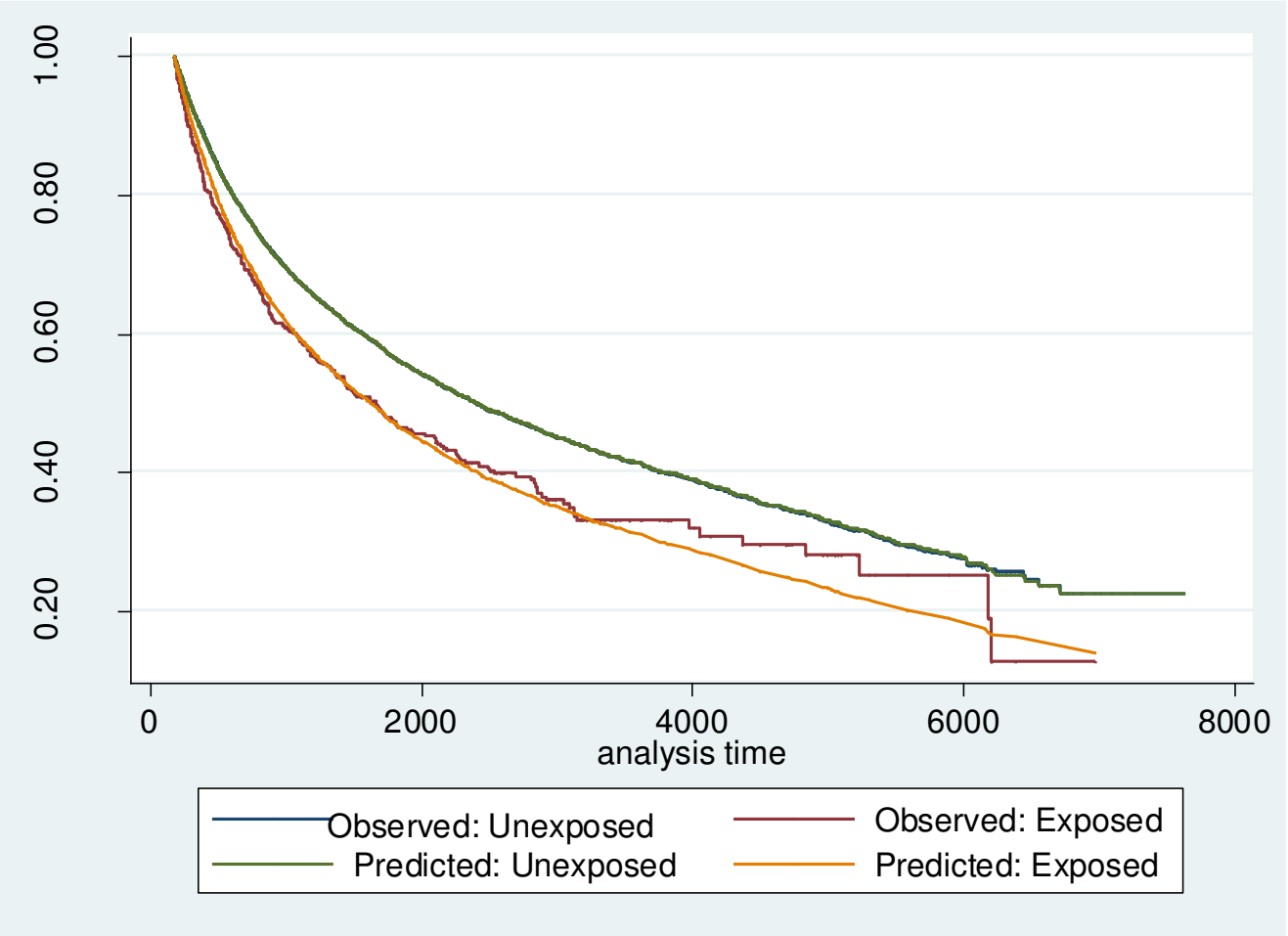
Determination of how observed effects on mortality deviate from effects predicted in the regression model.

Figure 4.9 Colorectal cancer log-log plot



Assessment of the proportional hazards assumption for tricyclic antidepressant exposure in colorectal cancer mortality.

Figure 4.10 Colorectal cancer- observed vs predicted hazards



Determination of how observed effects on mortality deviate from effects predicted in the regression model.

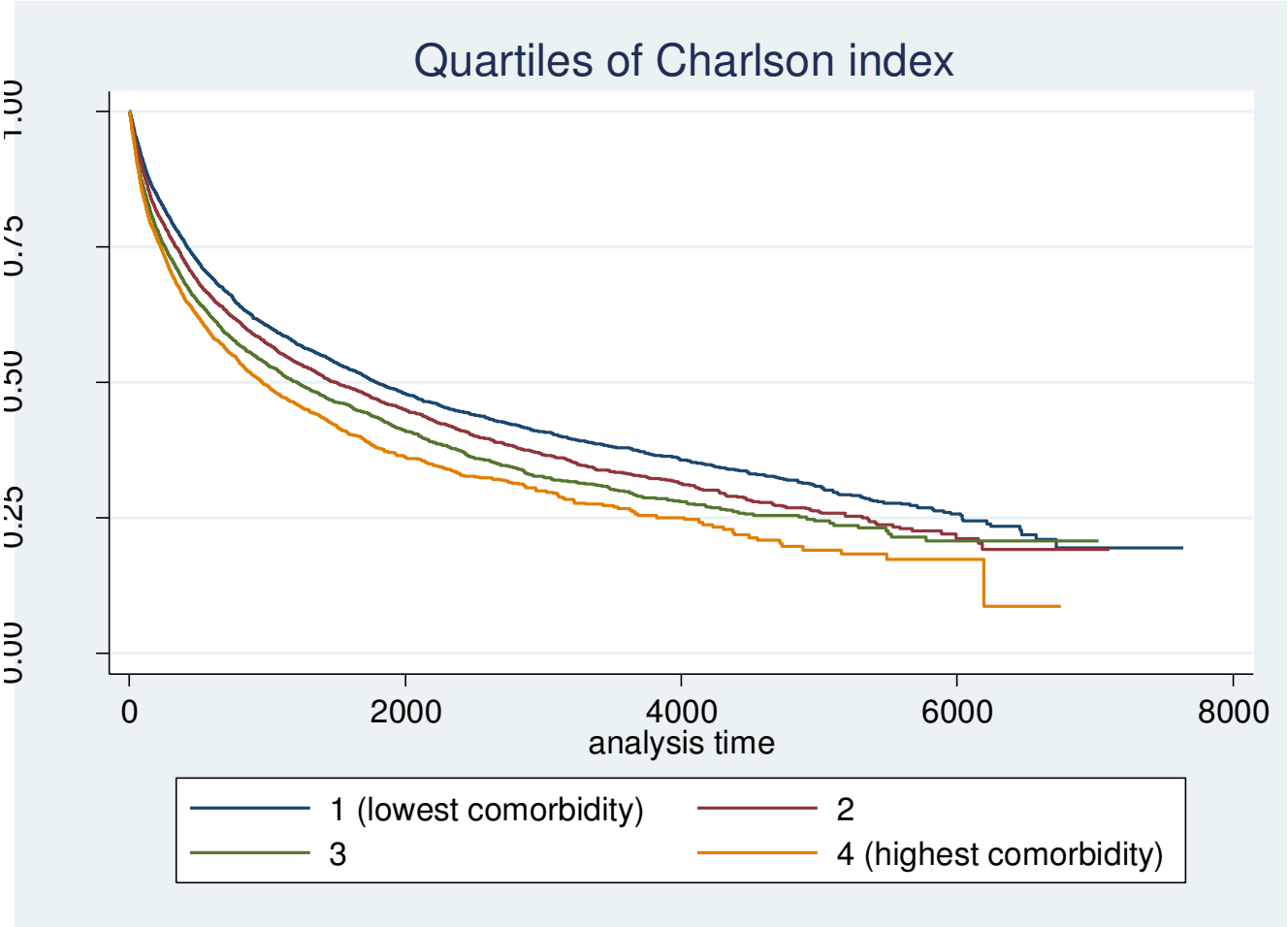
4.4 Charlson Index coding assessment

In order to assess whether the Charlson Index was a valid method of adjusting for comorbidity, the patient values for Charlson were divided into quartiles. This meant that both Cox regression could be performed and KM curves could be created. Both the Cox regression (Table 4.6) and KM curve (Figure 4.11) showed a statistically significant trend (p value for trend <0.001) trend towards higher comorbidity leading to increased mortality.

Table 4.6 Charlson Index validation

Quartile of Charlson Index	OR	95% CI		
1 (lowest comorbidity)	1			
2	1.12	1.07	1.18	←
3	1.26	1.20	1.33	←
4 (highest comorbidity)	1.42	1.34	1.49	←

Figure 4.11 Charlson index validation



Survival curves to assess the effects of comorbidity, coded as the Charlson Index, on mortality in both glioma and colorectal cancer patients. Patients were divided into quintiles according to their Charlson Index score.

4.5 Discussion

4.5.1 Summary of findings

This study found that there was no observed reduction in mortality among colorectal cancer patients treated with tricyclics. In this group there was a statistically significant increase in mortality risk, which was only evident in patients beginning tricyclic treatment post diagnosis. The effect was also confined only to 'low' dose tricyclic group. As low dose tricyclics are commonly used for chronic pain management, these factors combined might suggest that the observed detrimental effect is related to pain management (which may be a proxy for poor prognosis if for example pain is caused by bony metastases), rather than a true decrease in survival time caused by tricyclics.

Despite often observing hazard ratios below one, a statistically significant reduction in mortality for glioma patients treated with tricyclics was not observed. In addition, any observed effects were not backed up by a trend in the dose response analysis, though due to stratification patient numbers are small here. It is possible therefore that tricyclic antidepressant use does not confer a benefit in reducing mortality to glioma patients. It is also possible that these findings may be a type II error, which would mean that there is an association, but it was not found due to some factor such as lack of power. If this were the case, it seems from these findings that some groups are more likely to benefit than others. The majority of the observed effects were in patients who were not previously exposed to tricyclics before diagnosis. So this would seem to be a subset of patients which is more likely to benefit from tricyclics if there is a real effect on mortality. This is consistent with the hypothesis previously stated by Chan et al (Chan et al. 2009), in relation to aspirin use and colorectal cancer, that one would expect those tumours susceptible to the anti-neoplastic effects of an agent not to reach diagnosis in subjects taking the drug, as the drug's chemopreventative action would prevent the cancer developing.

4.5.2 Strengths/weaknesses

As no consistent significant effects were found and my previous data showing that tricyclic antidepressants are chemopreventative effect in glioma and colorectal cancer (Walker et al. 2011), study power might be the most obvious concern. Power calculations carried out whilst designing the study estimated that for glioma, the study would have 80% power to detect a hazard ratio of 0.67. This hazard ratio was not observed within the study, but the confidence intervals do not rule out a much greater effect size. For colorectal cancer, where there are around ten times more patients, it is quite certain that the study had sufficient power to detect any important changes.

Our data have certain important strengths. The use of routinely collected general practice records (from the GPRD) ensured that there was little opportunity for recall bias to effect the ascertainment of exposures. In addition, by selecting all relevant malignancies within the population, the possibility of selection bias was greatly reduced. However, the data quality and completeness of all potential confounders may not be of the same standard.

There is also some missing data with respect to smoking, obesity and alcohol, and therefore some potential for residual confounding by these factors. However, these factors, even when combined had a relatively small confounding effect and so it is likely that any residual confounding by these factors would be minor. A potentially greater issue is that there is no data on factors such as cancer stage and histological grade, though adjusting for these factors had a limited effect on mortality in a similar study (Chan et al. 2009). In addition, adjusting for factors such as comorbidity may have reduced the extent of this. There may be concern that excluding patients who die or are censored during the therapy observation period (up to 6 months after diagnosis) may cause some early effects to be overlooked. However the reduced opportunity for exposure among those dying quickly means that any beneficial association found would be more likely to be due to reverse causality were they

included. My efforts to assess this by using a shorter 3 month period to determine exposure produced results that were broadly the same.

4.5.3 Comparison with previous literature

Previous laboratory evidence suggests a substantial anti-cancer effect for many of the tricyclics both *in vitro* (Pilkington et al. 2008) and *in vivo* (Tsuruo et al. 1983; Merry et al. 1991; Pommerenke et al. 1995). While these are important in establishing a possible anti-cancer effect, it is difficult to directly compare them to any study in humans. The *in vitro* studies for example are essentially qualitative in nature, and in many cases attempt to determine the mechanism of action (Daley et al. 2005; Arimochi et al. 2006). These are of course all important steps in determining efficacy of a drug, but it is all too common for a drug which shows promise in the laboratory to meet with a lack of efficacy in the clinic (for example Sparano et al (2004)).

In addition, the previous study examining cancer incidence (Chapter 3), demonstrates a statistically significant chemopreventive effect in humans (Walker et al. 2011). This current study is the first known large-scale study to investigate the effect of tricyclics on cancer survival. It is thought however that there are around 350 primary brain tumour patients in the UK who have at some point been treated with the tricyclic chlorimipramine (Higgins et al. 2010). In example of such treatment, 27 malignant patients were treated with up to 150mg of chlorimipramine over a 4.5 year period (Beaney et al. 2005). This small scale study showed promising results but also hints at another important consideration. The dose used in this study is substantially higher than the typical dose of chlorimipramine used in the patient cohort (median= 50mg). Therefore, it may well be that therapeutic benefit can only be found at doses which are relatively rarely used in the general population, or in patients with lower grade or smaller tumours. It is clear then that this, combined with the relatively low power available (despite the very large size of the GPRD), means that it is

very difficult in this kind of study to reliably determine the effects that these drugs would have if used clinically for cancer treatment.

4.5.4 Interpretation

The obvious conclusion then is that further and larger scale interventional clinical work is required to truly reveal the potential of tricyclics in cancer therapy. From this and previous work, it would appear that glioma is the best candidate cancer for such clinical studies. Carrying out a simple calculation to determine the likely minimum size of a trial in glioma patients indicates that at least 130 patients would be required to achieve 90% power with a sensitivity of 5%. This is based on the hazard ratio of 0.83, obtained in the multivariate binary Cox regression for all participants. This figure would perhaps be lower for some sub-groups, such as patients not using tricyclics before diagnosis. However, the evidence for these sub-groups being different to the population as a whole is arguably not sufficient to warrant a trial looking exclusively at such sub groups. It may however be useful to carry out a sub-group analysis within a more general trial. This trial seems like a realistic goal for glioma. For colorectal cancer however, it now seems unlikely that there are any real beneficial effects on mortality and therefore a clinical trial is not likely to be useful.

5 Non-steroidal anti-inflammatory drugs and cancer survival

A cohort study using the GPRD

5.1 Introduction

Although some targeted agents, such as bevacizumab, are beginning to be used in colorectal cancer chemotherapy (Welch et al. 2010), conventional chemotherapeutic drugs (for example 5-fluorouracil) are still the mainstay of chemotherapy treatment. Such drugs are renowned for their unpleasant side effects, so the search for alternative drugs that have a lower side effect profile, or could be used to increase effectiveness of conventional drugs could be potentially very rewarding.

5.1.1 COX-2 and cancer

Cyclooxygenase-2, also known as COX-2 or prostaglandin-endoperoxide synthase 2, is an enzyme with an important role in biosynthesis of prostanoid compounds such as prostaglandins. These compounds have a number of physiological functions, including vasodilation and mediation of inflammatory reactions. More recently they have been implicated in cancer development, including regulation of apoptosis, angiogenesis and invasion (Ghosh et al. 2010).

COX-2 exists alongside two other isozymes, COX-1 and COX-3. All of these enzymes perform very similar functions, but are differentially expressed according to the tissue type. COX-1 is constitutively expressed throughout most tissues, whereas COX-2 is an inducible enzyme expressed in only a few tissues. A number of cancer types, including colorectal cancer are known to have a tendency to over express COX-2 (Antonacopoulou et al. 2008), which is likely a key part in their development.

The implication of COX-2 in cancer development has led to a number of approaches to therapeutically target it. All NSAIDs are known to inhibit both COX-1 and COX-2, and this action is vital in their conventional use as analgesics, anti-inflammatories and antipyretics. Due to this non-specific inhibition, side effects such as GI bleeding can occur. This has led to

the development of more specific COX-2 inhibitors, which due to them not inhibiting the ubiquitously expressed COX-1, should have fewer side effects. However, these drugs still have issues, with rofecoxib (trade name Vioxx) having been withdrawn by drug company Merck due to an increase in risk of cardiovascular events (Bresalier et al. 2005).

5.1.2 NSAIDs and cancer incidence

Aspirin is known to reduce the incidence of colorectal cancer (Dube et al. 2007; Cuzick et al. 2009; Elwood et al. 2009; Half et al. 2009). In addition, NSAIDs in general have also been linked anti-cancer effects (Iwama et al. 2009; Zell et al. 2009). However, effects of these drugs have been disputed by certain studies (Bosetti et al. 2009), For aspirin, it is thought to be most effective in reducing incidence at high doses, although recent evidence has emerged to suggest that low dose aspirin may also be efficacious (Din et al. 2010; Rothwell et al. 2010).

5.1.3 NSAIDs and mortality?

The chemopreventative effect of NSAIDs naturally leads to the question of whether these drugs may be of benefit as an adjuvant treatment in colorectal cancer. A recent study established that there may be a reduction in mortality in patients treated with aspirin after diagnosis (Chan et al. 2009). In this study the greatest effects were observed in patients who began aspirin use post diagnosis, and also in patients with tumours expressing high levels of cyclooxygenase-2 (COX-2). Inhibition of COX-2 is the main mechanism through which aspirin's anti-cancer action is mediated. Another study using pooled randomized trial data determined there to be a reduction in mortality in a number of cancer types, including colorectal cancer (Rothwell et al. 2011). Both of these studies were relatively small in size in terms of colorectal cancer numbers, and it would therefore be highly beneficial to attempt to replicate these findings in a different, larger dataset.

5.1.4 Study rationale

Taking the previous evidence into account this study uses the GPRD as the source of a much larger colorectal cancer population, in order to further understand the effects of aspirin and NSAIDs on cancer mortality in a real world population. Other NSAID use (excluding aspirin) was also investigated. In addition to their anti-cancer action, aspirin and other NSAIDs have relatively few and minor side effects in contrast to the side effects of cytotoxic drugs discussed in section 4.1.3. Gastrointestinal toxicity such as discomfort, nausea, diarrhoea, and occasionally more serious bleeding and ulceration are the most important of these side effects. Elderly patients are at increased risk of these. However, careful management and drug selection (e.g. ibuprofen is associated with lower GI toxicity risk) can minimise the risk of such effects.

The putative anti-cancer efficacy and mild side effect profile therefore makes aspirin and other NSAIDs a good candidate for further investigation.

5.2 Methods

5.2.1 Study design

A prospective cohort study was carried out in the GPRD to determine the relationship between aspirin and NSAID usage and survival post cancer diagnosis.

5.2.2 Subjects

Any person with a recorded diagnosis of colorectal cancer within the GPRD (see Appendix II for medical codes) occurring at least one year after their entry to the database was selected. Patient data were collected from the beginning of the GPRD database (1987), up to the last available data in the database (2010). Patients with a previous diagnosis of colorectal cancer were excluded from the cohort, as were patients contributing less than 1 year of data to the GPRD.

5.2.3 Outcomes and exposures

The outcome to be observed was all cause mortality. Date of patient death was determined from the existence of one of two records; either a patient with a "Transfer out reason" specified as "death"; or by a "Statement of Death" (SoD) code (a "Clinical" or "Referral" event with a Read/OXMIS code indicating a death). Where both records existed, the date of death was determined preferentially from the SoD code. The follow-up time was determined to be the time between diagnosis and the death date determined by the above method. Follow up time for patients not dying in the study was determined either from the date that the patient transferred out from the GP or by the last data collection date for the GP.

The primary exposure was the use of aspirin, or another NSAID (section 10.01.01 of the British National Formulary (BNF)). To be exposed, a patient must have had a repeat prescription (≥2) within the period being examined for exposure. In order to minimise reverse causality (i.e. patients who die soon after diagnosis being less likely to receive a prescription) a fixed period of 1 year post-diagnosis was used to determine drug exposure and patients who died or were censored within this period were excluded from the analysis.

As in Chan et al's paper (Chan et al. 2009), pre-diagnosis exposure was considered to determine whether it influenced the effect of post-diagnosis exposure. This was done in two ways; 1) by adjusting for pre-diagnosis use in the Cox proportional hazards model and 2) by stratifying the analysis according to whether patients received aspirin/NSAIDs pre-diagnosis.

Potential associations were examined further by investigating the dose used. For aspirin low dose was decided to be 75 milligrams or under and high dose anything over 75 milligrams. Where multiple prescriptions with differing doses existed, the most frequently prescribed dose was used.

To allow comparison of the effect of high and low doses across all the NSAIDs, I standardized the definition of high dose and low dose between drugs relative to the maximum recommended doses for each drug (determined from the BNF). These standardized doses were then used to calculate the mean dose across all prescriptions for each patient individually. Patients with NSAID use were divided into 'high' dose or 'low' dose groups based on the median corrected dose of 0.32 times maximum recommended dose.

5.2.4 Other covariates

Data on gender, age, smoking status, alcohol use, body mass index (BMI) and comorbidity (coded as the Charlson Index (Charlson et al. 1987)) were extracted. Of these potential confounders, gender, age, comorbidity and smoking status were retained in multivariate models as *a priori* predictors of mortality risk. Other covariates were only retained in the multivariate model if they produced a 10% or greater change in the measured size of effect.

5.2.5 Statistical methods

I used Cox proportional hazards modelling to assess the effect of aspirin/NSAIDs on mortality risk, adjusting for multiple potential confounding variables as described above. Results are presented as hazard ratios (HR), with accompanying 95% confidence intervals (CIs).

Validity of the proportional hazards assumption was tested using a log-log plot. If the proportional hazards assumption was found to be violated, this non proportionality was then further characterised. The approximate time period that a change in effects occurred was determined by using observed/predicted survival curves, and observing where the observed curve deviated from the predicted. The data could then be stratified using this time period to investigate the effects before and after.

All data handling and analysis was done using Stata v11.1 SE (Statacorp, 4905 Lakeway Drive, College Station, Texas 77845 USA).

5.3 Results

5.3.1 Study population/covariates

13,944 patients with colorectal cancer were identified. 5,358 (38.4%) of these patients died during their period of registration and their median follow up time (survival time) for this group was 1.7 years. The remaining patients, alive up to the end of follow-up had a median time of post-diagnosis follow-up of 3.1 years (interquartile range 1.3-6.2). Aspirin use between diagnosis and 12 months post diagnosis was at 18.8% in patients surviving more than 12 months after diagnosis. 26.1% of patients received a prescription for aspirin prediagnosis. These results are summarised, along with other covariates used in the study in Table 5.1.

Table 5.1 Study population/covariates

		Aspirin nonuser (N=11,325)	%	Aspirin user (N=2,619)	%	NSAID nonuser (N=10,233)	%	NSAID user (N=3,711)	%
Women		6,067	53.6	1,632	62.3	5,493	53.7	2,206	59.4
Mean age (SD)		74.5	(11.7)	68.3	(8.5)	72.3	(11.5)	68.4	(11.4)
Mean BMI (SD)		27.0	(4.5)	26.2	(4.5)	27.1	(4.4)	26.1	(5.1)
Smoking status	No	6,074	53.6	1,306	49.9	5,503	53.8	1,877	50.6
	Ex	3,053	27.0	948	36.2	2,774	27.1	1,227	33.1
	Yes	1,645	14.5	322	12.3	1,462	14.3	505	13.6
	Missing	553	4.9	43	1.6	494	4.8	102	2.8
Alcohol use	No	7,790	68.8	1,890	72.2	1,541	15.1	620	16.7
	Ex	173	1.5	77	2.9	152	1.5	98	2.6
	Yes	1,714	15.1	447	17.1	7,079	69.2	2,601	70.1
	Missing	1,648	14.6	205	7.8	1,461	14.3	392	10.6
Mean Charlson Index (SD)		13.0	(9.5)	9.5	(13.0)	12.1	(7.3)	9.5	(7.2)

Numbers in table represent N (%) for categorical variables and Mean (SD) for continuous variables

5.3.2 Binary analysis

Post diagnosis aspirin use (Table 5.2) was associated with a decrease in mortality in colorectal cancer patients (multivariate HR=0.91 95% confidence interval (CI)=0.82-1.00). The effect did not occur in patients not prescribed aspirin before diagnosis (HR=0.99 CI=0.84-1.16), therefore the effect was entirely due to patients who did use aspirin pre diagnosis (HR= 0.86 CI= 0.76-0.98). If pre diagnosis aspirin use is considered exclusively, there was no association with survival (HR=1.04 CI=0.97-1.12). This analysis using pre diagnosis exposure included all patients excluded from the other parts of the study.

With NSAID use, there was a statistically significant increase in mortality when all patients were considered together (HR=1.29, CI=1.18-1.42). However, there was a greater increase in mortality observed in those beginning NSAID use after diagnosis, having not used them prior to diagnosis (HR=1.69, CI=1.45-1.97). As with aspirin, no statistically significant effects were observed when pre-diagnosis exposure only was considered (HR=1.05, CI=0.99-1.12).

Table 5.2 Binary analysis

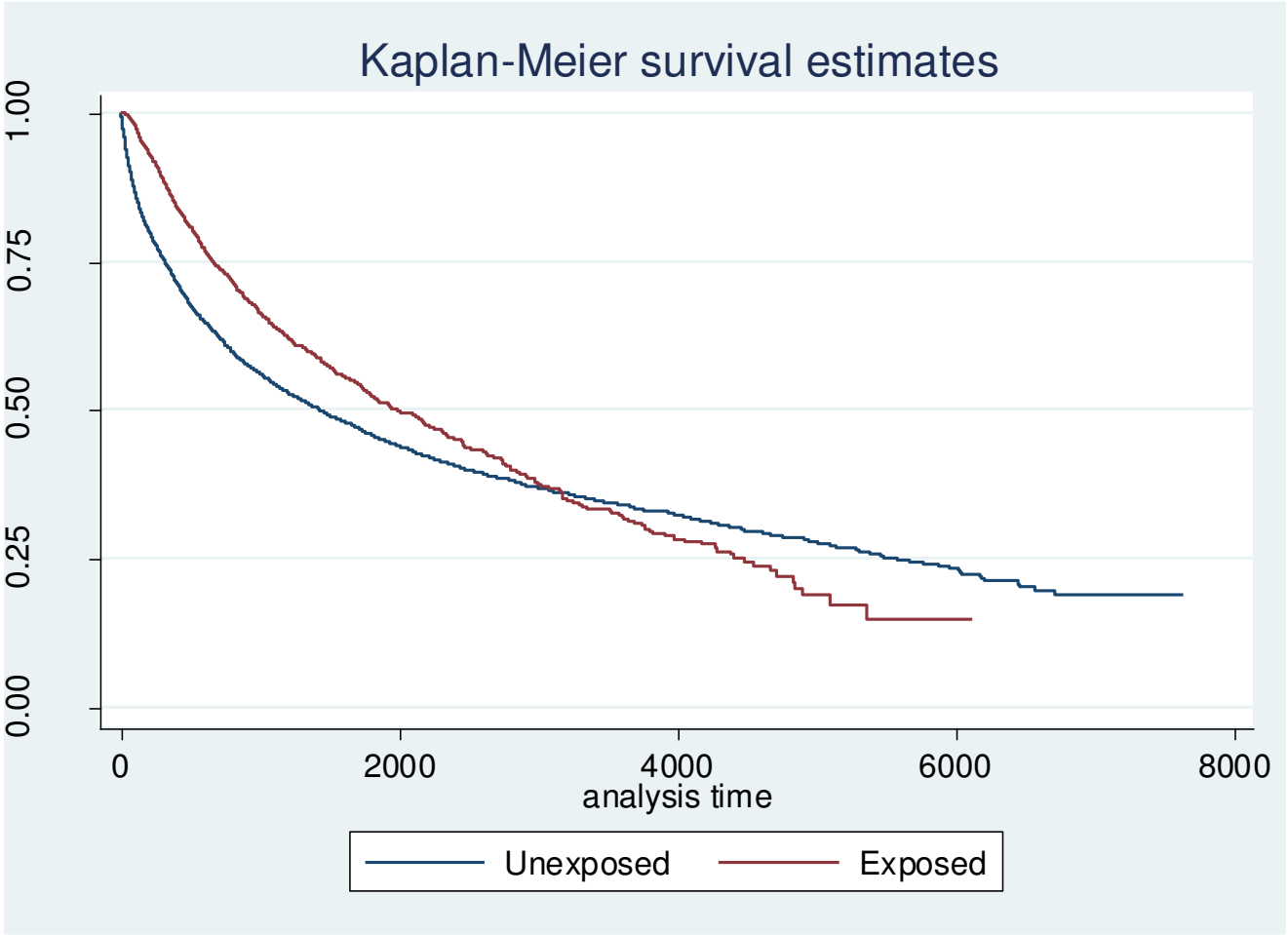
Pre diagnosis exposure strata	Drug type	Post diagnosis exposure	Patient status at end of follow-up		Age adjusted				Age and prediagnosis drug use adjusted				Multivariate*			
			Alive	Dead	HR	95%	CI		HR	95%	CI		HR	95%	CI	
All participants	Aspirin	Unexposed	6,925	4,400	1				1				1			
		Exposed	1,661	958	0.94	0.87	1.01		0.89	0.81	0.98		0.91	0.82	1.00	←
	NSAIDs	Unexposed	7,862	4,773	1				1				1			
		Exposed	724	585	1.32	1.21	1.43	←	1.32	1.21	1.43		1.29	1.18	1.42	
Aspirin/NSAID nonusers prediagnosis	Aspirin	Unexposed	6,231	3,910	1				-	-	-		1			
		Exposed	284	192	0.99	0.86	1.15		-	-	-		0.99	0.84	1.16	
	NSAIDs	Unexposed	4,835	3,162	1				-	-	-		1			
		Exposed	205	221	1.66	1.45	1.90	←	-	-	-		1.69	1.45	1.97	←
Aspirin/NSAID users prediagnosis	Aspirin	Unexposed	694	490	1				-	-	-		1			
		Exposed	1,377	766	0.84	0.75	0.94	←	-	-	-		0.86	0.76	0.98	←
	NSAIDs	Unexposed	3,027	1,611	1				-	-	-		1			
		Exposed	519	364	1.18	1.05	1.32	←	-	-	-		1.11	0.98	1.26	

*adjusted for: age, gender, smoking, BMI, alcohol use, comorbidity (Charlson index)

5.3.3 Kaplan-Meier curves- aspirin

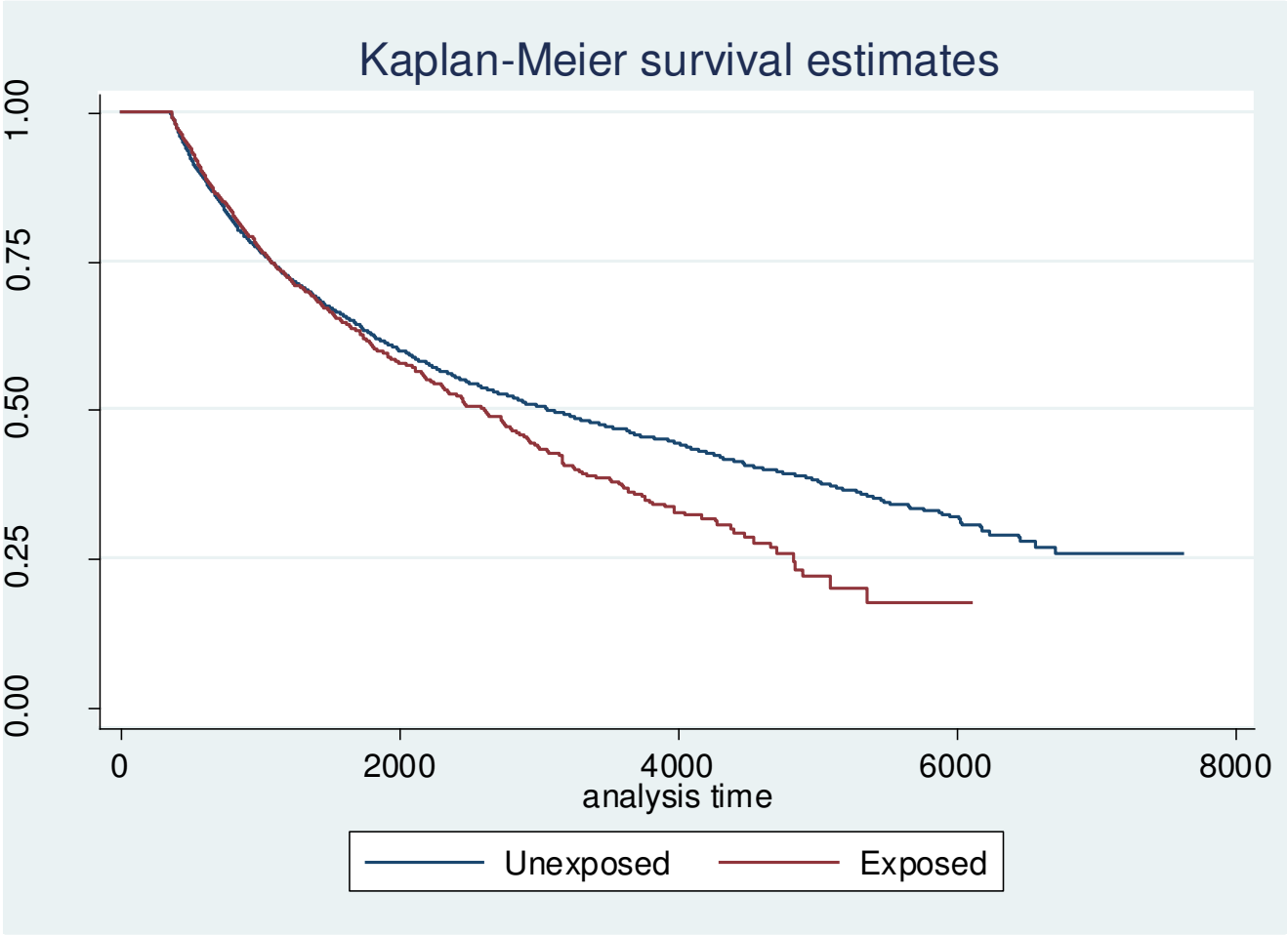
In a similar manner to the KM curves in chapter 4, these were first drawn without excluding patients who died or were censored during the first 12 months after diagnosis. This gives the impression of a large reduction in mortality for aspirin users early after diagnosis (Figure 5.1). With these patients excluded, the picture is very different (Figure 5.2), when the results are not adjusted for any confounders, there appears to be no benefit in mortality for either group early on, then after around 2000 days there is an increase in mortality for aspirin users. While these results do reflect the univariate Cox regression analysis, these results are confounded by a number of factors, particularly age. These factors are adjusted for in Figure 5.3, where there again appear to be differential effects between early and late follow up. Aspirin users appear to have reduced mortality initially, but it increases later on to become greater than non users. These differential effects are further explored in the proportional hazards assumption testing (Section 5.3.7).

Figure 5.1 Aspirin binary unadjusted analysis- without exposure time exclusion



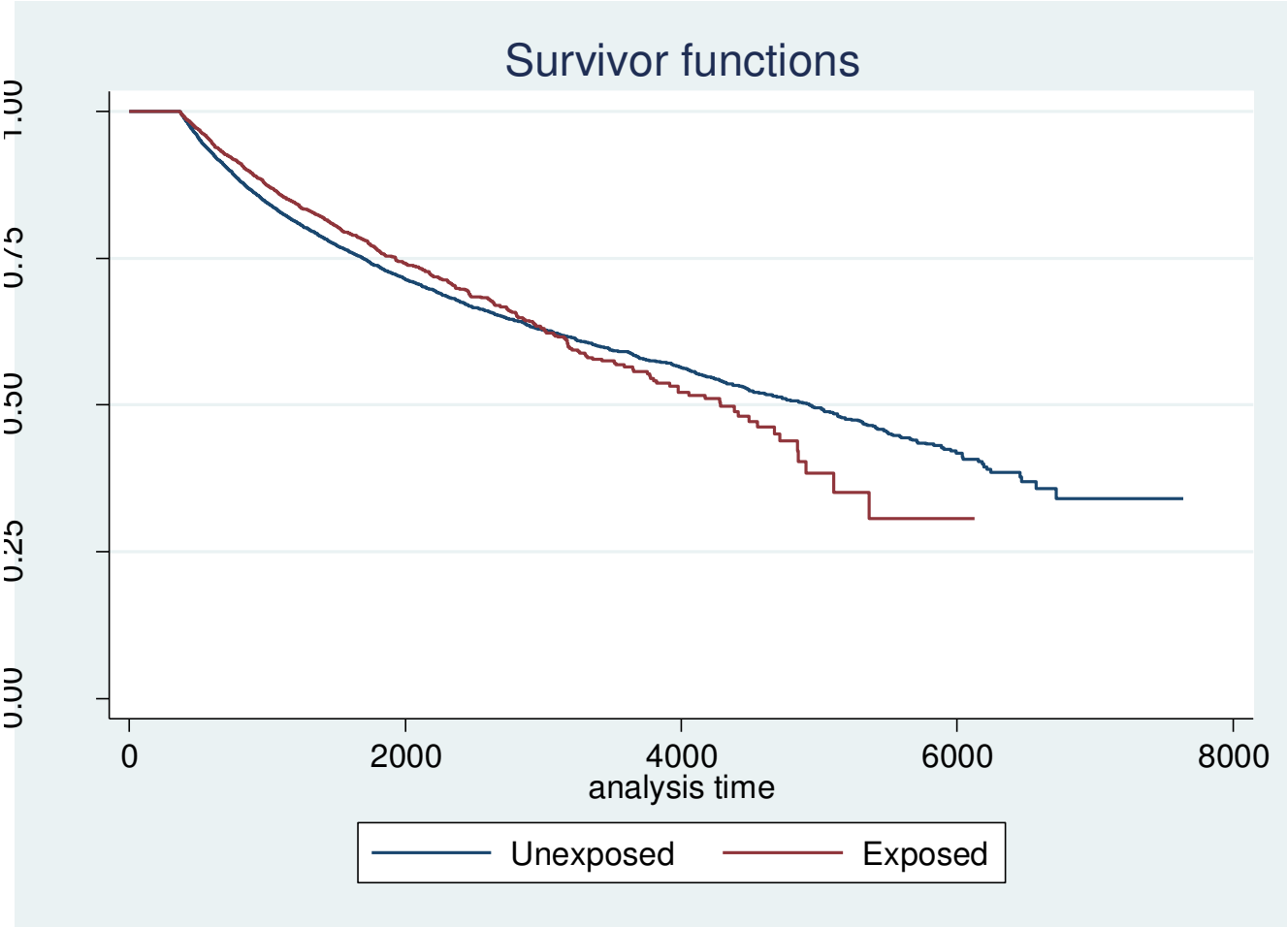
Colorectal cancer patient survival curves. Exposed patients received ≥2 prescriptions for aspirin in the 1 year following diagnosis. For this curve, patients dying or being lost to follow up during this "exposure" time were not excluded from the cohort.

Figure 5.2 Aspirin binary unadjusted analysis- with exposure time exclusion



Colorectal cancer patient survival curves. Exposed patients received ≥2 prescriptions for aspirin in the 1 year following diagnosis. Exclusion of patients dying or being lost to follow up during the "exposure" time leads to a change in shape of the initial part of the curve.

Figure 5.3 Aspirin binary multivariate analysis

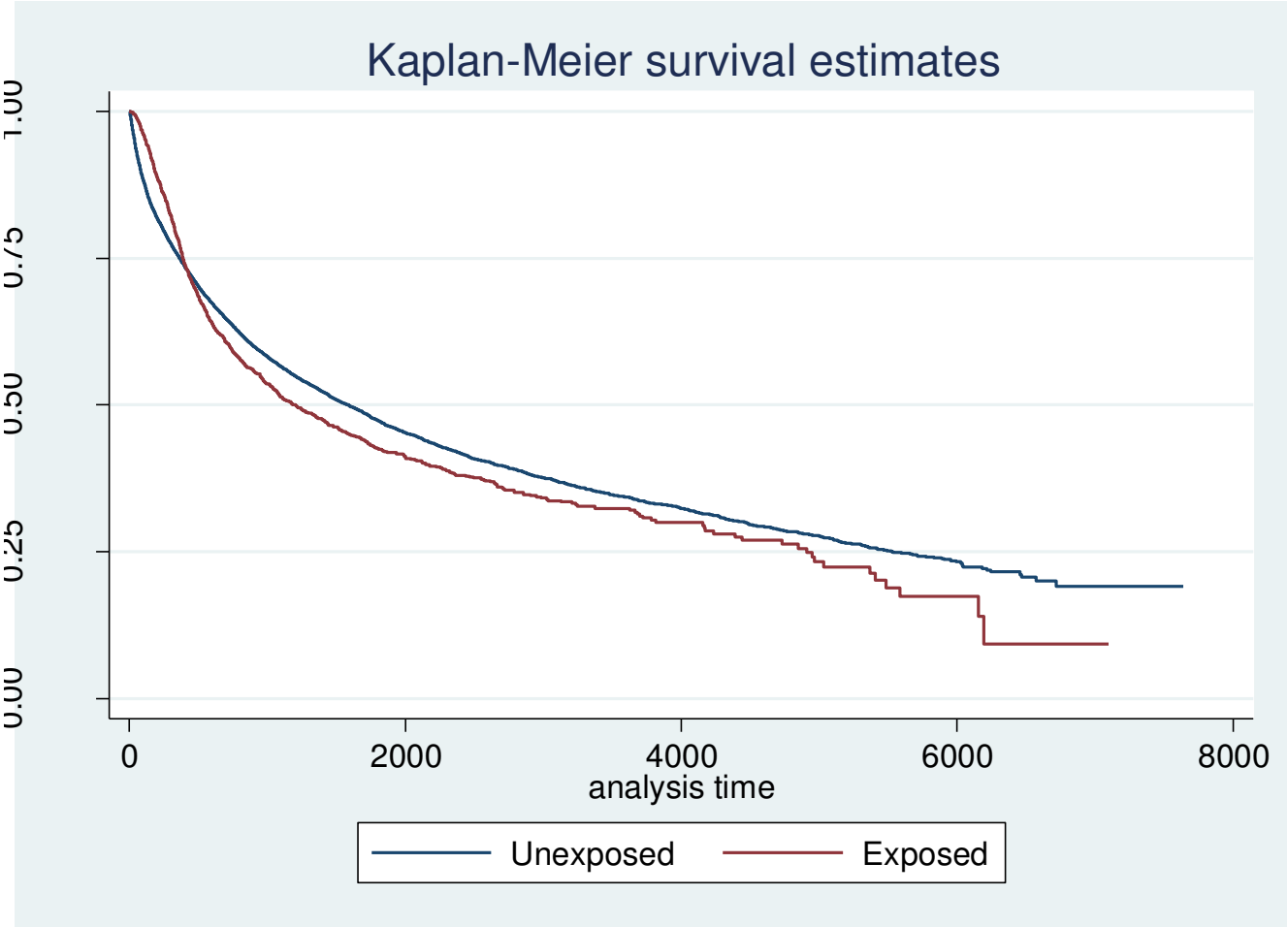


Multivariate colorectal cancer patient survival curves, adjusted for: age, gender, smoking, BMI, alcohol use, comorbidity (Charlson index).

5.3.4 Kaplan-Meier curves- NSAIDs

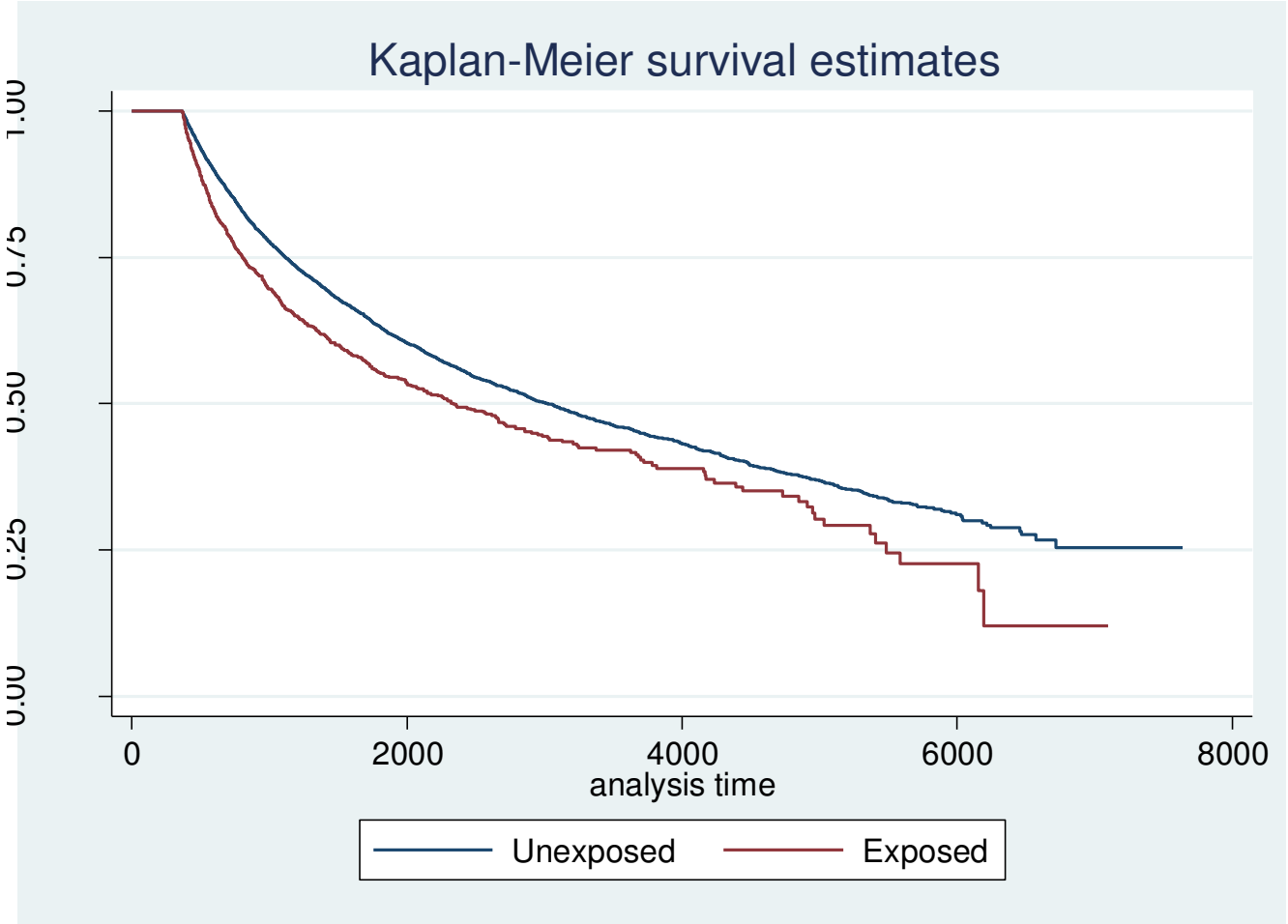
Once again the KM curve drawn without excluding patients lost before 12 months post diagnosis (Figure 5.4) gives the impression of slightly improved initial mortality for NSAID users, which is corrected by their exclusion (Figure 5.5). This unadjusted curve suggests an increase in mortality for NSAID users. When the curve is adjusted for the various confounders, this effect appears to be increased (Figure 5.6).

Figure 5.4 NSAIDs binary unadjusted analysis- without exposure time exclusion



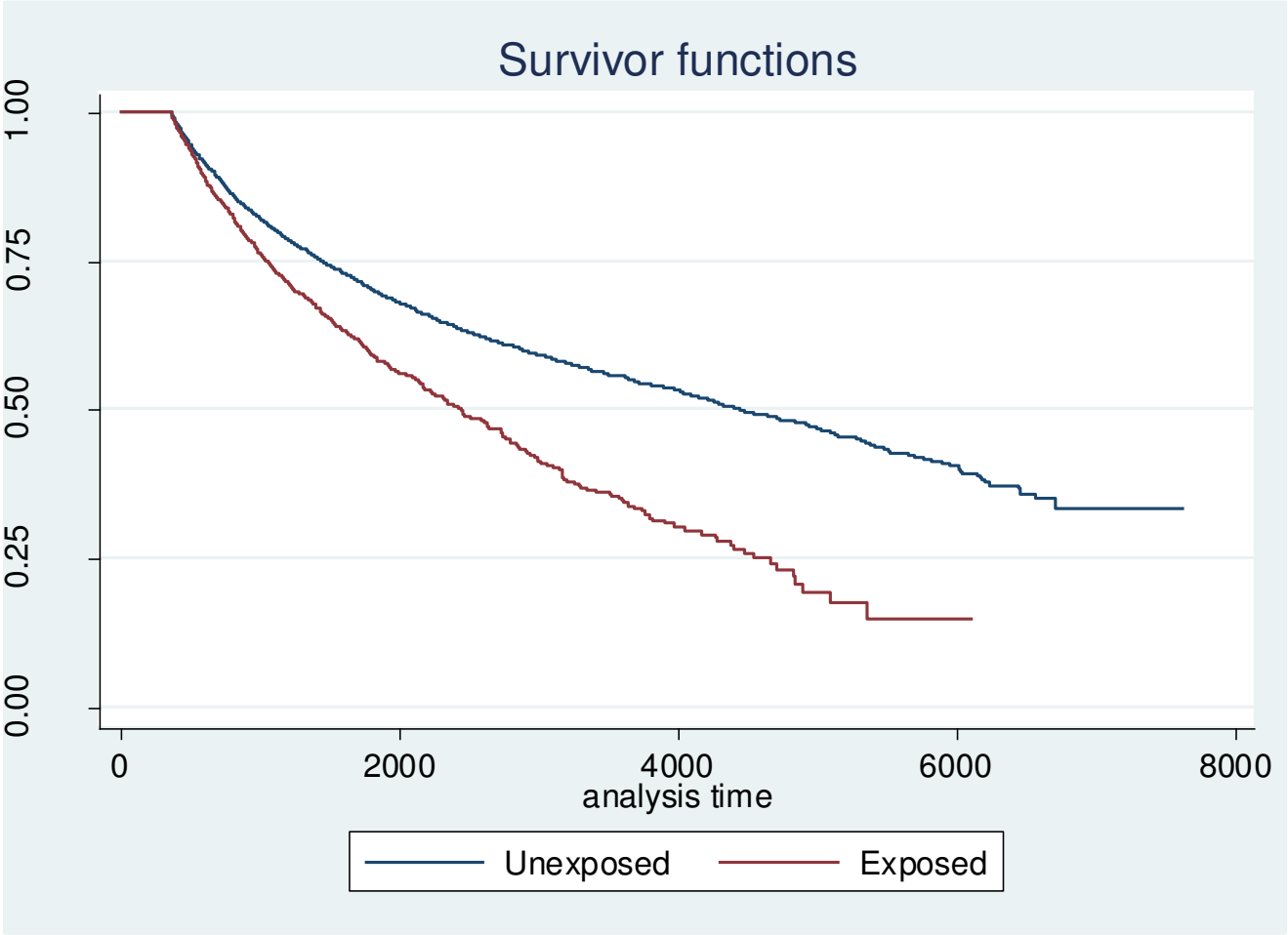
Univariate colorectal cancer patient survival curves. Exposed patients received ≥2 prescriptions for NSAIDs (excluding aspirin) in the 1 year following diagnosis. For this curve, patients dying or being lost to follow up during this "exposure" time were not excluded from the cohort.

Figure 5.5 NSAIDs binary unadjusted analysis- with exposure time exclusion



Univariate colorectal cancer patient survival curves. Exposed patients received ≥2 prescriptions for aspirin in the 1 year following diagnosis. Exclusion of patients dying or being lost to follow up during the "exposure" time leads to a change in shape of the initial part of the curve.

Figure 5.6 NSAIDs- binary multivariate analysis



Multivariate colorectal cancer patient survival curves, adjusted for: age, gender, smoking, BMI, alcohol use, comorbidity (Charlson index).

5.3.5 Dose response

Investigation of aspirin and NSAIDs' effects on mortality were continued by investigating the dose of drug used (Table 5.3). For aspirin, a dose response effect was not observed, as there was a small, non-significant decrease in mortality in those using low dose aspirin (HR=0.94 CI=0.86-1.02) and a small, non-significant increase in mortality in patients using high dose aspirin (HR=1.13 CI=0.97-1.32). For high dose NSAID use, a significant increase in mortality was observed for NSAID users (HR=1.29 CI=1.11-1.49).

Table 5.3 Dose response

Drug type	Post diagnosis Exposure	Patient status at end of follow up		Age adjusted			Multivariate*		
		Alive	Dead	HR	95%	CI	HR	95%	CI
Aspirin	Unexposed	6,925	4,400	1			1		
	Low dose	1,437	768	0.91	0.84	0.98←	0.94	0.86	1.02
	High dose	220	186	1.10	0.95	1.27	1.13	0.97	1.32
NSAIDs	Unexposed	7,862	4,773	1			1		
	Low dose	378	288	1.18	1.05	1.33←	1.18	1.04	1.35←
	High dose	290	233	1.35	1.19	1.54←	1.29	1.11	1.49←

*Adjusted for: age, gender, smoking, BMI, alcohol use, comorbidity (Charlson index)

5.3.6 Binary analysis- low dose only

If the original analysis (Table 5.2) is repeated with patients using high dose post diagnosis aspirin/NSAID excluded from the study, the results are broadly similar for aspirin, but with slightly greater size of effect (Table 5.4). For NSAIDs, the increase in mortality previously observed in patients who had not used them prior to diagnosis was increased somewhat, and still significant (HR=1.97 CI=1.64-2.36).

Table 5.4 Binary analysis- low dose only

Prior drug exposure group	Drug type	Post diagnosis exposure	Patient status at end of follow-up		Age adjusted				Age and prediagnosis drug use adjusted				Multivariate*			
			Alive	Dead	HR	95%	CI		HR	95%	CI		HR	95%	CI	
All participants	Aspirin	Unexposed	6,925	4,400	1				1				1			
		Low dose	1,441	772	0.91	0.84	0.98	←	0.86	0.79	0.94		0.89	0.80	0.98	←
	NSAIDs	Unexposed	7,862	4,773	1								1			
		Low dose	434	352	1.29	1.16	1.44	←	1.29	1.16	1.44		1.29	1.15	1.46	←
Aspirin/NSAID nonusers prediagnosis	Aspirin	Unexposed	6,231	3,910	1				-	-	-		1			
		Low dose	265	175	0.97	0.83	1.13		-	-	-		0.99	0.84	1.16	
	NSAIDs	Unexposed	4,835	3,162	1				-				1			
		Low dose	119	155	1.86	1.58	2.18	←	-				1.97	1.64	2.36	←
Aspirin/NSAID users prediagnosis	Aspirin	Unexposed	694	490	1				-	-	-		1			
		Low dose	1,176	597	0.81	0.72	0.92	←	-	-	-		0.83	0.73	0.95	←
	NSAIDs	Unexposed	3,027	1,611	1				-				1			
		Low dose	315	197	1.05	0.91	1.22		-				1.02	0.87	1.2	

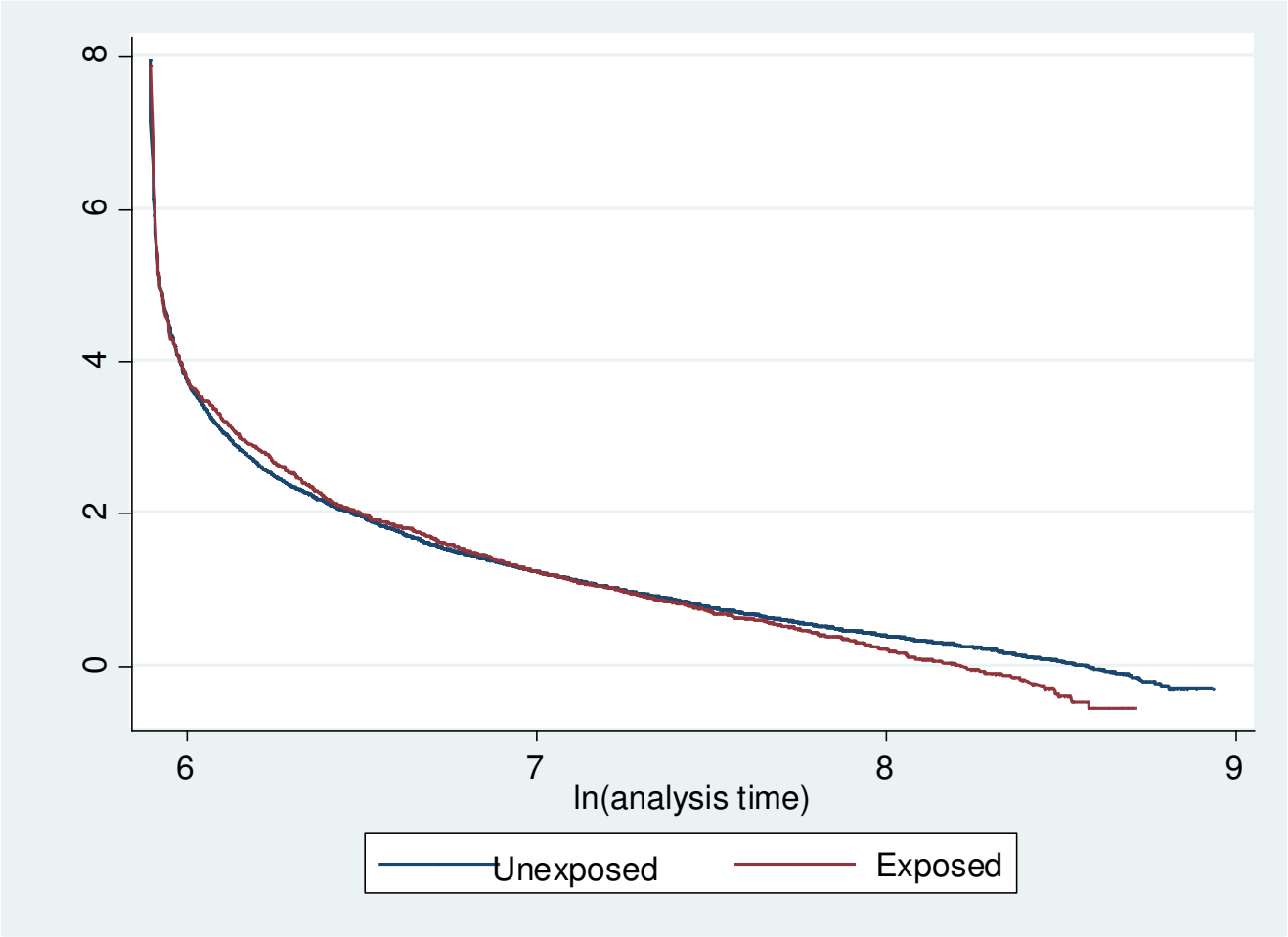
*Adjusted for: age, gender, smoking, BMI, alcohol use, comorbidity (Charlson index)

5.3.7 Proportional hazards assumption testing

The log-log plot for aspirin demonstrates that there are differing effects between the early and late analysis time (Figure 5.7). The curves for exposed and unexposed patients clearly cross and are not parallel. This is in contrast to the log-log plot for NSAIDs (Figure 5.9), where the curves are somewhat more parallel and do not cross.

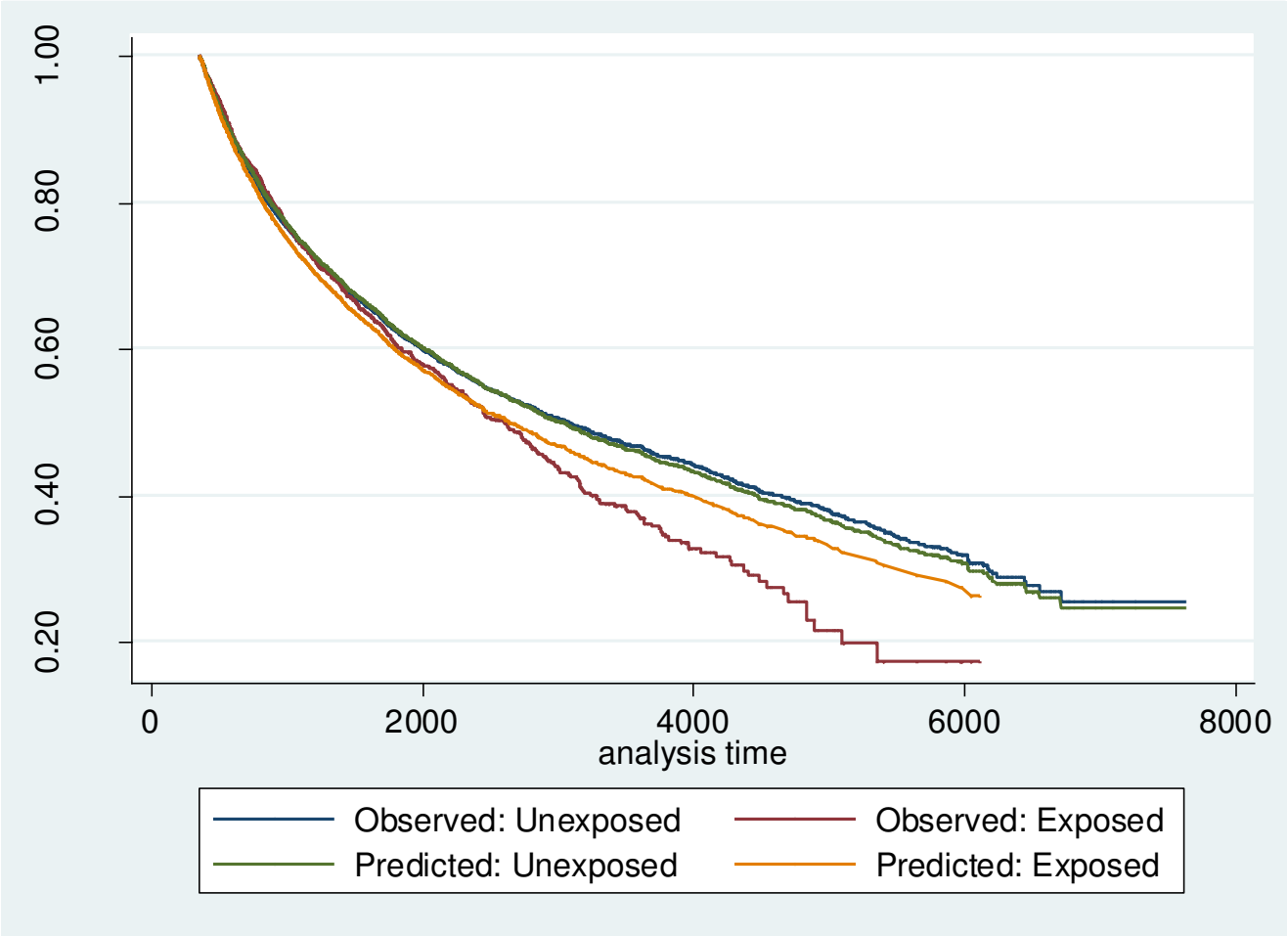
In order to estimate when the difference in effects occurred, observed vs predicted KM curves were drawn. For aspirin (Figure 5.8), there appears to be a change at around 2300 days (6.3 years). For other NSAIDs (Figure 5.10), there is some deviation from the predicted curve, but in a less striking manner than for aspirin.

Figure 5.7 Aspirin- log-log plot



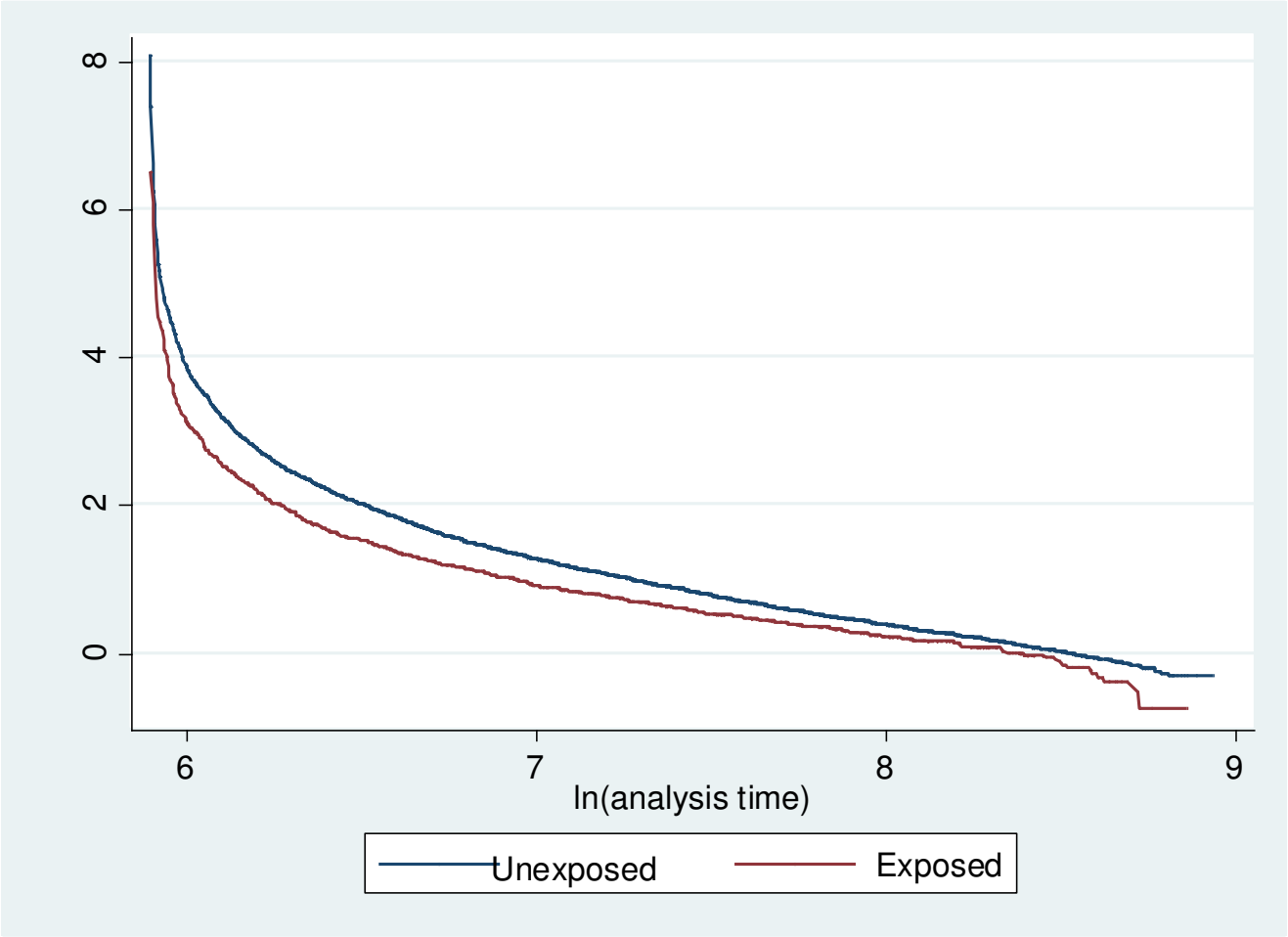
Assessment of the proportional hazards assumption for aspirin exposure.

Figure 5.8 Aspirin- observed vs predicted hazards



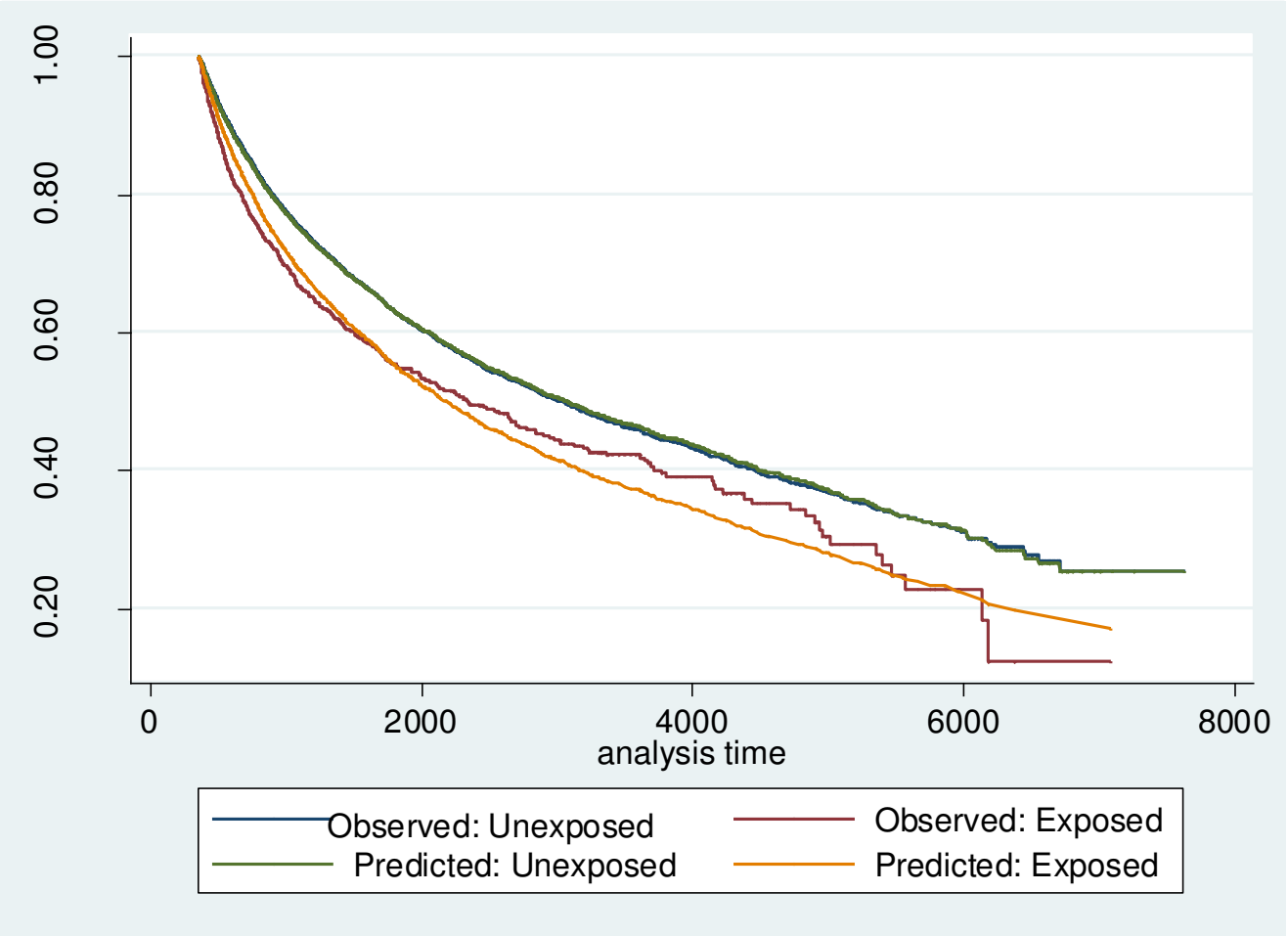
Determination of how observed effects on mortality deviate from effects predicted in the regression model.

Figure 5.9 NSAIDs- log-log plot



Assessment of the proportional hazards assumption for NSAID exposure.

Figure 5.10 NSAIDs- observed vs predicted hazards



Determination of how observed effects on mortality deviate from effects predicted in the regression model.

5.3.8 Non proportional hazards stratification

After it was determined that there were differential effects between early and late follow up after diagnosis, I tested for effects before and after this point (Table 5.5). For aspirin a reduction in mortality was observed up to 6.3 years (HR= 0.83 CI=0.75-0.91), whereas after this point the effect became an increase in mortality (HR=1.64 CI=1.32-2.03). Although NSAIDs did not display the same level of differential effects, the analysis was carried out in the same way, with a greater size of effect seen in the earlier follow up category (HR=1.32 CI=1.19-1.46).

Table 5.5 Non proportional hazards stratification

Drug type	Period examined	Post diagnosis exposure	Patient status at end of follow-up		Age adjusted				Age and prediagnosis drug use adjusted				Multivariate*			
			Alive	Dead	HR	95%	CI		HR	95%	CI		HR	95%	CI	
Aspirin	<6.3 years	Unexposed	7,262	4,520									1			
		Exposed	1,324	838	0.89	0.82	0.96	←	0.81	0.74	0.89	←	0.83	0.75	0.91	←
	>6.3 years	Unexposed	8,249	5,238									1			
		Exposed	337	120	1.70	1.40	2.08	←	1.72	1.40	2.11	←	1.64	1.32	2.03	←
NSAIDs	<6.3 years	Unexposed	6,853	4,089									1			
		Exposed	1,733	1,269	1.36	1.24	1.48	←	1.38	1.26	1.52	←	1.32	1.19	1.46	←
	>6.3 years	Unexposed	8,356	5,298									1			
		Exposed	230	60	1.07	0.82	1.40		1.08	0.83	1.41		1.16	0.88	1.52	

*Adjusted for: age, gender, smoking, BMI, alcohol use, comorbidity (Charlson index)

5.4 Discussion

5.4.1 Summary

It was found that there was a reduction in all-cause mortality for colorectal cancer patients using aspirin. This reduction was around 9% for all aspirin use, but increased to around 17% in the early follow up period. These were therefore modest in size but there were statistically significant reductions in mortality in various parts of the analysis. However no dose response relationship was found for aspirin, which may be due to confounding by indication. It is likely that patients with a poor prognosis would use high dose aspirin for pain management, whereas low dose (75mg) aspirin is not used in this way, which may have allowed its protective effects to be shown.

In contrast, NSAID use did not appear to give any reduction in mortality, and in fact showed an increase in mortality in some cases. The reason for this is likely to be confounding by indication, in a similar manner to aspirin use. I therefore do not believe that there are any causal associations between mortality in colorectal cancer patients and NSAIDs.

Though it is likely that NSAIDs are not associated with mortality in colorectal cancer patients, for aspirin, things are undoubtedly less clear cut. My findings are broadly similar to those of Chan et al's study (Chan et al. 2009) in that consistent reductions in mortality were observed. However, whereas Chan et al found their greatest size of effect in patients who started aspirin use for the first time post diagnosis, essentially no effect was found here. It is possible then that there are some tertiary factors affecting mortality, which also may be related to aspirin use.

5.4.2 Method refinements

During the analysis, it was found that different doses varied the effect on mortality. It was therefore decided to investigate mortality while excluding patients on high dose aspirin/NSAIDs. This gave a greater size of effect for aspirin use. This is likely a result of removing confounding that may have been present in high dose users, as high dose aspirin is most commonly used as an analgesic and may therefore be a proxy for poor prognostic markers such as bone metastases.

Although it was expected that the proportional hazards assumption would need to be tested, it was not known what the outcome of this testing would be. A substantial difference was found between the early and late effects in aspirin, which partially masked some of the mortality effects that occurred in aspirin users. Once the early and late follow up periods were analysed separately, it was clear that there was a more substantial reduction in mortality in the early part of follow up than was seen in the overall analysis. This new mortality estimate was approximately in line with the size of effect found in the Chan et al study, in a similar category. The effect of aspirin use in the late follow up period was to increase mortality. This may be due to aspirin use being a marker for previous cardiovascular disease, especially with the majority of use being low dose.

5.4.3 Strengths/weaknesses

This study used a dataset much larger than in all known previous studies on the same subject. As prescriptions are automatically recorded in the GPRD, the opportunity for recall bias to effect the ascertainment of exposures is limited. Since all relevant malignancies within the GPRD were selected, the possibility of selection bias was greatly reduced. However, the recording of all potential confounders may not be as reliable.

There is some missing data with respect to smoking, obesity and alcohol, and therefore a potential for residual confounding by these factors. However, these factors, even when combined had a relatively small confounding effect, and so it is likely that any residual confounding by these factors would be minor. The data used does however lack any data on factors such as cancer stage and histological grade. Though adjusting for factors such as comorbidity may have reduced the extent of confounding by these variables, it is still possible that increased morbidity would lead to both increased aspirin use (through pain management) and increased mortality. Indeed I believe this may be the reason for increases in mortality being observed in some cases. Adjusting for cancer stage and grade could therefore increase the size of the observed effects substantially. Related to this is COX-2 expression in colorectal tumours. It was determined that expression of COX-2 in a colorectal tumour led to a greater decrease in mortality (Chan et al. 2009). Lack of such histological data may therefore have contributed to the relatively small effect sizes found. It was not possible to look at cause-specific mortality in this dataset. Therefore another factor that would likely increase the observed size of effect is being able to look exclusively at cancer specific mortality. This would have the additional advantage of ascertaining to what extent the overall increase in mortality after 6 years was due to an increased risk of cardiovascular death.

5.4.4 Comparison with previous literature

While this study generally agrees with the results in previous literature on aspirin and colorectal cancer mortality, it does not entirely replicate them. One possible reason for this may be the type of data source.

Chan et al used the Nurses' Health Study (a questionnaire based study), and this may have led to substantial differences in determination of exposure. Rather than being based on prescribing, patients were simply asked if they were regular users of aspirin. This would

certainly lead to differences in terms of including patients using over the counter (and therefore frequently higher dose) aspirin. This may be important both in terms of the effects observed, and for any confounding by indication in this study. Another difference in the datasets is the length of follow up may have been greater in the Nurses' Health Study than for most patients in the GPRD.

Another study to look at aspirin use in relation to mortality used the California Teachers Study (Zell et al. 2009). This was a questionnaire based study, and therefore had relatively few participants. The study also used aspirin and ibuprofen use combined. However significant effects were noted for NSAID use, which were slightly higher, but comparable with my study in effect size. In contrast to my study, Zell et al looked only at NSAID use before cancer diagnosis, and therefore is somewhat at odds with my study, which found no effect here. However, with the dramatically different method of ascertainment of exposure between the different studies, this may in part explain any differences in timing of use etc.

The most recent findings in this area (Coghill et al. 2011) present a similar story. They found that regular NSAID use conferred a survival benefit in an observational study using the survey based Seattle Colorectal Cancer Family Registry. This included around 1,700 participants, but reports a significant survival benefit in NSAID users of comparable size to other studies (around 20%). This study again investigates only pre-diagnosis NSAID use, and it is therefore less generalisable in terms of using the drugs as cancer treatment.

Pooled randomised trial data were used by one group to determine the effect of long term aspirin use on mortality (Rothwell et al. 2011). While the outcome of this study (mortality) was effectively the same as in my study, the population being examined was not. As these were trials of primary or secondary prevention of cancer, the baseline population did not have cancer, or were in remission from cancer. This means that this study is as much a comment on the preventative power of aspirin as it is on the treatment efficacy. It is

therefore difficult to separate these results, though it is clearly valuable to show that the preventative properties of aspirin do translate to a decrease in cancer death. The study also adds value in that the majority of individual trials did not find significant effects in this outcome measure, but combining them gave sufficient power to do this.

In a similar manner to Rothwell et al, Din et al (2010) looked at aspirin and NSAID use in relation to survival from colorectal cancer. This study used the Study of Colorectal Cancer in Scotland, a large questionnaire based study. As it was a case control study it incorporated patient without cancer and therefore included incidence of colorectal cancer into the measure of survival. In a similar manner to my study, there was no association found for NSAIDs, but a survival benefit was found for low dose aspirin. Once again it is difficult to determine how much of this survival benefit is due to the change in cancer incidence. Additionally this study was mostly focused on cancer incidence rather than survival.

5.4.5 Interpretation

The body of evidence for the anti-cancer effect of aspirin is ever expanding, and it is therefore crucial that this is further investigated. While in observational studies consensus is beginning to form, due to the limitations of such studies, randomised intervention trials are the only way to fully determine whether these drugs can confer a benefit to cancer patients, and if so which patients are most likely to benefit. There is now strong evidence that aspirin may reduce colorectal cancer mortality, and now would seem a good time to carry out such a trial.

The minimum size of a clinical trial to investigate this, based on the overall effect on mortality found in this study (a hazard ratio of 0.91) would be 260 patients. The potential difference in the effect of aspirin in patients over expressing the COX-2 protein could also be investigated in such a study. Histological determination of COX-2 expression is entirely

practicable and may be an important indicator of response to aspirin. Given the potential gain for colorectal cancer patients, it would be imprudent to not investigate this further in patients. The sizes of effect observed in this and other similar studies are of comparable magnitude to clinical trials which examine alternative chemotherapy regimens (for example oxaliplatin plus 5-FU/leucavorin (André et al. 2009)) in comparison with the more standard 5-FU/leucavorin treatment (reviewed in (Jonker et al. 2011)). These types of study can be viewed as analogous to this study as here patients will either have been treated with standard therapy only, or standard therapy plus aspirin. It therefore seems plausible that a clinical trial of similar design to these others could find similar results. The additional benefit here is of course the greatly reduced toxicity of aspirin in comparison with drugs such as oxaliplatin.

6 ACE inhibitors and hepatocellular carcinoma

A case-control study using the GPRD

6.1 Introduction

Hepatocellular carcinoma (HCC) is amongst the commonest cancers globally and its incidence is thought to be rising in the UK, with incidence rates approximately trebling between 1971 and 2001 (West et al. 2006). Though survival rates have increased slightly in that time, prognosis is very poor. Known risk factors for HCC include cirrhosis, hepatitis B and C infection, sustained alcohol use, age and male gender (El-Serag et al. 2008; Kumagi et al. 2009).

6.1.1 Angiogenesis in cancer

On initial development, the structure of a tumour is much more homogeneous than that present in normal tissues, as it is derived from a single transformed cell. This means that support structures such as blood vessels are not present. A tumour can only grow to be a few millimetres across without a blood supply as growing bigger than this causes areas of hypoxia to develop within it, which leads to inhibition of growth and cell death.

In order to develop past this stage, the tumour must induce blood vessel growth (angiogenesis). This is often achieved by hijacking normal physiological mechanisms for angiogenesis, whereby the tumour cells release growth factors which encourage surrounding blood vessels to develop into the tumour mass. These growth factors include vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). These growth factors therefore offer a potential target for anticancer drugs and indeed various VEGF targeting monoclonal antibodies are now in clinical use, including bevacizumab.

Neoangiogenesis is thought to occur early in hepatocellular carcinogenesis (Yang et al. 2008b) due to the rapid nature of HCC growth. As a result of this dependence on angiogenesis, a focus on anti-angiogenic drugs in HCC treatment has emerged, including clinical trials of anti-angiogenic drugs, such as Sorafenib (Llovet et al. 2008).

6.1.2 Anti-cancer mechanism- ACE inhibitors

Angiotensin converting enzyme (ACE) inhibitors are used conventionally as an antihypertensive agent. However, laboratory findings demonstrate both anti-angiogenic activity and inhibition of liver cancer growth in rodent models (Volpert et al. 1996). It has been suggested that the anti-angiogenic activity is mediated by inhibition of vascular endothelial growth factor (VEGF) (Yoshiji et al. 2001). Research into the anti-cancer properties of ACE inhibitors has included examination of possible synergistic effects with other drugs, such as 5-fluorouracil (5-FU) (Yanase et al. 2007), interferon-β (Noguchi et al. 2003; Yoshiji et al. 2005b) and vitamin K2 (Yoshiji et al. 2005a; Yoshiji et al. 2006; Yoshiji et al. 2007; Yoshiji et al. 2009).

6.1.3 Previous epidemiology

Although laboratory evidence on ace inhibitors and HCC is compelling, studies in humans are limited in size and number. One study using 100 patients indicates possible protection against recurrence of HCC (Yoshiji et al. 2009), but only a single case study describes the potential use as a chemopreventative agent in humans (Yoshiji et al. 2007). Intriguingly, a different but related class of drugs, angiotensin receptor blockers, have recently been associated with an increase in cancer risk in randomized controlled trials (Sipahi et al. 2010).

6.1.4 HCC and high risk groups

HCC, having well defined high risk groups is an excellent candidate disease for the development of chemoprotective drugs. Risk factors for HCC mostly consist of chronic liver diseases, such as cirrhosis, haemochromatosis and hepatitis B/C infection. Other risk factors include alcohol use and diabetes.

This study assessed the potential therapeutic effects of ACE inhibitors in a large, population based study with robust measurement of ACE inhibitor exposure.

6.2 Materials and Methods

6.2.1 Design

A matched case-control study was conducted to determine the relationship between ACE inhibitor usage and cancer incidence using the General Practice Research Database.

6.2.2 Participants

Cases were defined as persons in the GPRD between 1987 and March 2002 with a recorded diagnosis of hepatocellular carcinoma (defined by Oxford Medical Information System or Read Codes). Two code sets were used, one including specific HCC codes only (referred to forthwith as definite), and the other adding codes for unspecified primary liver cancers (referred to as probable). The index date for cases was taken to be the first recorded diagnosis of HCC in cases and the same date for individually matched controls. Up to 10 controls were matched to each case by GP, sex and age at the index date (within 5 years). Patients with below 2 years of follow up at the index date were excluded.

6.2.3 Exposures

Exposures were defined as a record of prescription for an ACE inhibitor (BNF, section 2.5.5) prior to the diagnosis of HCC. Due to potential differences in the duration of data collection for each patient and to reduce the chance of reverse causality, the period used to determine a patient's exposure status was confined to between 24 months and 12 months before the index date. Exposure was defined as ≥2 prescriptions.

To allow assessment of dose response I determined the dose received for each prescription. This was standardised across drug types by dividing by the maximum recommended doses for each drug (determined from the BNF). These standardized doses

were then used to calculate the mean dose across all prescriptions for each patient individually. Patients with prior ACE inhibitor use were divided into 'high' or 'low' dose groups based on the median corrected dose of 0.225 times maximum recommended dose. Length/consistency of exposure was assessed by the number of days of exposure within the 1 year period that exposure was examined. Exposure was categorized as greater or less than 6 months in this period.

6.2.4 Other covariates

Risk factors for HCC for which data were available in this study were diagnosed chronic liver disease, diabetes, smoking and alcohol use. These were defined by a code for these conditions at any point during the patient's registration.

6.2.5 Statistical methods

I used conditional logistic regression, initially using univariate analysis, then using a multivariate model. The covariates assessed for possible confounding were alcohol use, smoking, diabetes, and chronic liver disease (cirrhosis, haemachromatosis and hepatitis B/C). These potential confounders were only retained in the model if they caused a 10% or greater change in the observed size of effect. Results are presented as odds ratios (ORs), with accompanying 95% confidence intervals (CIs). All data handling and analysis was done using Stata v11.1 SE (Statacorp, 4905 Lakeway Drive, College Station, Texas 77845 USA).

6.3 Results

6.3.1 Population

I identified a total of 335 HCC cases, matched to a total of 3,339 controls by age, gender and general practice. The median duration of data available prior to the index date was 5.7 years (range 1.0 to 12.3 years) for cases, and 5.0 years (range 1.0 to 12.3 years) for controls.

The full set of patients, based on the code list used in Table 6.2, gave the greatest level of study power. However, the patient cohort based on the code list in Table 6.4 was thought to be a more representative HCC population. It is this population which is analysed in Table 6.1, in terms of the various covariates investigated. The biggest difference between cases and controls here was for diagnosed chronic liver disease, where a 17 fold increase is seen in cases compared to controls.

Table 6.1 Covariates

		Controls	%	Cases	%
Number		2313		224	
Sex	Male	1,430	61.8	138	61.6
	Female	883	38.2	86	38.4
Age	<50	249	10.8	23	10.3
	50-60	385	16.7	36	16.1
	60-70	580	25.1	58	25.9
	70-80	661	28.6	64	28.6
	>80	438	18.9	43	19.2
Alcohol	Never user	255	11.0	35	15.6
	User	1,302	56.3	107	47.8
	Missing	756	32.7	82	36.6
Smoking	Never smoker	896	38.7	101	45.1
	Ex-smoker	169	7.3	15	6.7
	Current smoker	171	7.4	27	12.1
	Missing	1,077	46.6	81	36.2
Diagnosed chronic liver disease	No	2,289	99.0	185	82.6
	Yes	24	1.0	39	17.4
ACE inhibitor use	No	2,176	94.1	208	92.9
	Yes	137	5.9	16	7.1

6.3.2 Analysis using all possible cases

The study was initially carried out using a code list that included all the codes that could have been used to describe HCC. Some of these codes however were somewhat ambiguous and could have coded for other cancer types, such as cholangiocarcinoma. It is also conceivable that a few other cancer types that have metastasized to the liver may have been (incorrectly) coded as, for example "Carcinoma liver".

Table 6.2 Full code list

GPRD Medical Code	Read/OXMIS code	Read/OXMIS term
274664	1550A	MALIGNANT NEOPLASM LIVER
303102	1550AP	MALIGNANT NEOPLASM LIVER PRIMARY
219896	1550B	HEPATOMA
306049	1550C	CARCINOMA LIVER
201867	1550HC	MALIGNANT NEOPLASM HEPATOCELLULAR
206105	B15..00	Malignant neoplasm of liver and intra hepatic bileducts
251481	B150.00	Primary malignant neoplasm of liver
224132	B150000	Primary carcinoma of liver
233261	B150300	Hepatocellular carcinoma
206106	B150z00	Primary malignant neoplasm of liver NOS
288093	B152.00	Malignant neoplasm of liver unspecified
297513	B808000	Carcinoma in situ of liver
206291	B903000	Neoplasm of uncertain behaviour of liver
288302	BB5D.00	[M]Hepatobiliary tract adenomas and carcinomas
297580	BB5D400	[M]Liver cell adenoma
206320	BB5D411	[M]Hepatocellular adenoma
233466	BB5D500	[M]Hepatocellular carcinoma NOS
233467	BB5D511	[M]Hepatoma NOS
251676	BB5D512	[M]Hepatoma, malignant
297581	BB5D513	[M]Liver cell carcinoma
206321	BB5D700	[M]Combined hepatocellular carcinoma and cholangiocarcinoma
297582	BB5D800	[M]Hepatocellular carcinoma, fibrolamellar
242496	BB5Dz00	[M]Hepatobiliary adenoma or carcinoma NOS

Using the all patient population demonstrates that there are no significant associations between ACE inhibitor use and HCC, with the exception of the ≤6 months group in the duration analysis. Here a significant increase in ACE inhibitor use in HCC patients is observed.

Table 6.3 Analysis using full code list

Analysis type	ACE inhibitor use			Univariate				Multivariate*			
		Controls	Cases	OR	95% CI			OR	95% CI		
Ever use	Unexposed	3,003	294	1				1			
	Exposed	336	41	1.18	0.82	1.70		1.29	0.88	1.88	
Dose	Unexposed	2,974	287	1				1			
	Low (≤0.225)	202	26	1.32	0.85	2.04		1.49	0.95	2.33	
	High (>0.225)	163	22	1.29	0.79	2.09		1.38	0.83	2.29	
Duration	Unexposed	2,974	287	1				1			
	≤6 months	101	19	1.83	1.10	3.07	←	2.13	1.26	3.62	←
	>6 months	264	29	1.09	0.72	1.66		1.17	0.76	1.81	

*Adjusted for alcohol use, smoking, and chronic liver disease.

6.3.3 Analysis using probable and definite cases

This code list increases the likelihood of the selected cancer patients being HCC patients by excluding some of the more generic codes included in the previous list. This meant that essentially all the patients identified should be primary liver cancer patients, the large majority of which will be HCC patients.

Table 6.4 Probable/definite code list

GPRD Medical Code	Read/OXMIS code	Read/OXMIS term
274664	1550A	MALIGNANT NEOPLASM LIVER
303102	1550AP	MALIGNANT NEOPLASM LIVER PRIMARY
219896	1550B	HEPATOMA
201867	1550HC	MALIGNANT NEOPLASM HEPATOCELLULAR
206105	B15..00	Malignant neoplasm of liver and intrahepatic bile ducts
251481	B150.00	Primary malignant neoplasm of liver
224132	B150000	Primary carcinoma of liver
233261	B150300	Hepatocellular carcinoma
206106	B150z00	Primary malignant neoplasm of liver NOS
206320	BB5D411	[M]Hepatocellular adenoma
233466	BB5D500	[M]Hepatocellular carcinoma NOS
233467	BB5D511	[M]Hepatoma NOS
251676	BB5D512	[M]Hepatoma, malignant
297581	BB5D513	[M]Liver cell carcinoma
206321	BB5D700	[M]Combined hepatocellular carcinoma and cholangiocarcinoma
297582	BB5D800	[M]Hepatocellular carcinoma, fibrolamellar
242496	BB5Dz00	[M]Hepatobiliary adenoma or carcinoma NOS

As observed in Table 6.5, there was no significant difference in ACE inhibitor use between HCC cases and controls. 16 cases (7.7%) and 137 controls (6.3%) had prior ACE inhibitor use. Odds ratios were close to unity in the univariate analysis (OR=1.16, CI=0.67-2.00) and the multivariate model did not differ greatly (OR=1.18, CI=0.66-2.10).

The median adjusted dose was 0.225 as a proportion of the BNF maximum recommended dose. The adjusted dose is the daily dose for each prescription as a proportion of the maximum daily dose recommended in the BNF. Although there is some difference between high and low dose exposure (Table 6.5), these results are not significant. The greatest size of effect is in the low dose category, where there is a non-significant increase in low dose ACE inhibitors for cases (multivariate OR=1.51, CI=0.66-3.44). In addition to this there was no significant trend in terms of dose of prescription (multivariate p value for trend=0.47).

For the duration analysis, 28.1% of exposed patients were in the ≤6 months category and the remaining 71.9% in the >6 months category. There was a non-significant increase in ACE inhibitor use in HCC cases for both the ≤6 months category (multivariate OR=1.32, CI=0.45-3.82) and the >6 months category (OR=1.13, CI=0.58-2.21). No significant linear trend was observed (*P*=0.50).

Table 6.5 Analysis using probable/definite code list

Analysis type	ACE inhibitor use			Univariate			Multivariate*		
		Controls	Cases	OR	95% CI		OR	95% CI	
Ever use	Unexposed	2,176	208	1			1		
	Exposed	137	16	1.16	0.67	2.00	1.18	0.66	2.10
Dose	Unexposed	2,176	208	1			1		
	Low (≤0.225)	59	7	1.20	0.53	2.68	1.51	0.66	3.44
	High (>0.225)	78	9	1.13	0.55	2.31	0.98	0.45	2.12
Duration	Unexposed	2,176	208	1			1		
	≤6 months	34	4	1.20	0.42	3.44	1.32	0.45	3.82
	>6 months	103	12	1.14	0.61	2.14	1.13	0.58	2.21

*Adjusted for alcohol use, smoking, and chronic liver disease.

6.3.4 Analysis using highly specific code list

The codes used in this analysis all explicitly describe hepatocellular carcinoma. It is therefore highly unlikely that any other cancer types could be coded for by this code list.

Table 6.6 Definite code list

GPRD Medical Code	Read/OXMIS code	Read/OXMIS term
219896	1550B	HEPATOMA
201867	1550HC	MALIGNANT NEOPLASM HEPATOCELLULAR
251481	B150.00	Primary malignant neoplasm of liver
233261	B150300	Hepatocellular carcinoma
206320	BB5D411	[M]Hepatocellular adenoma
233466	BB5D500	[M]Hepatocellular carcinoma NOS
233467	BB5D511	[M]Hepatoma NOS
251676	BB5D512	[M]Hepatoma, malignant
297581	BB5D513	[M]Liver cell carcinoma
206321	BB5D700	[M]Combined hepatocellular carcinoma and cholangiocarcinoma
297582	BB5D800	[M]Hepatocellular carcinoma, fibrolamellar

The more specific HCC code set was used to eliminate the possibility that other cancer types might affect the results. As can be seen in Table 6.7, 2,149 patients were identified by this method. ACE inhibitor use between cases and controls while using this code list remains non-significantly different (OR=1.33 CI=0.68-2.59).

Table 6.7 Analysis using definite code list

Analysis type	ACE inhibitor use			Univariate			Multivariate*		
		Controls	Cases	OR	95% CI		OR	95% CI	
Ever use	Unexposed	1,424	136	1			1		
	Exposed	99	13	1.29	0.70	2.39	1.33	0.68	2.59
Dose	Unexposed	1,424	136	1			1		
	Low (≤0.225)	46	5	1.08	0.42	2.79	1.39	0.53	3.69
	High (>0.225)	53	8	1.47	0.68	3.19	1.28	0.53	3.06
Duration	Unexposed	1,424	136	1			1		
	≤6 months	26	3	1.15	0.34	3.87	1.39	0.41	4.74
	>6 months	73	10	1.34	0.67	2.69	1.30	0.61	2.81

*Adjusted for alcohol use, smoking, and chronic liver disease.

6.4 Discussion

6.4.1 Summary

Despite contrary *in vitro (Yoshiji et al. 2001; Noguchi et al. 2003; Yoshiji et al. 2005b; Yoshiji et al. 2005c; Yoshiji et al. 2006)* and human (Yoshiji et al. 2007; Yoshiji et al. 2009) evidence, this study found no significant association between ACE inhibitor use and risk of developing HCC. There was no suggestion of a protective effect in any of the analyses, with the binary analysis suggesting, if anything, a very small non-significant increase in risk. Dose appeared to have little effect on incidence also and did not show any consistent pattern in those patients exposed for longer.

6.4.2 Method refinements

The main modifications to the methods in this study involved the code lists used to select HCC cases. This involved starting by selecting as many HCC cases as possible and then systematically increasing the specificity of case selection by removing the more ambiguous codes. While this will have most likely excluded some HCC cases when using the more specific code lists, it did allow for comparison of the results between different cohorts.

It can be said with reasonable confidence that there were no substantial differences between the different cohorts in terms of associations between ACE inhibitor use and cancer incidence. This means that, despite some uncertainty in exactly what cancer types were coded for, particularly when using the less specific code lists, it is unlikely that any effect has been missed due to lack of precision in the case definitions used.

6.4.3 Strengths/weaknesses

As with any study that fails to discover an association, the statistical power of the study must be considered carefully. Though the power of this study is clearly not optimal, the

95% confidence intervals of the overall comparison exclude a protective odds ratio better than 0.66 (based on the probable/definite code list cohort). Hence these data suggest that if any protective association exists, the size of effect is likely to be small. Given the rarity of HCC, such a small change is unlikely to be clinically important. Yearly incidence of HCC in across the west is consistently under 10 per 100,000, while in some countries in East Asia and central Africa, incidence can approach closer to 100 per 100,000 (Bosch et al. 2004). Given this incidence, the number needed to treat based on an odds ratio of 0.66 is approximately 30,000 in western countries and 3,000 in the higher incidence countries. High risk groups might present a better target for these drugs, but as this is based on an entirely hypothetical odds ratio, which at best could not be ruled out, it is very unlikely.

An advantage of this study is that the use of routinely collected general practice records ensures that the recall bias affecting the ascertainment of drug exposures was minimised. Unfortunately I cannot be equally confident about the completeness of data on some confounders, such as smoking and alcohol use. Around 40% of patients did not have available data on these factors and this may have left residual confounding within the results. While some of the residual confounding is likely to be minimal (for example for smoking status), other confounders may continue to exhibit an effect. The most likely one of these is chronic liver disease. It is known that a large majority of HCC occurs in patients in high risk groups, such as those with chronic liver disease (for example cirrhosis, haemachromatosis and hepatitis B/C). However, these data suggest that only 17.4% of patients were diagnosed with chronic liver disease. This is difficult to explain, though may in part be due to cases not being diagnosed with chronic liver disease before diagnosis of HCC. This must therefore be acknowledged as a weakness of the study.

Although the codes used to define HCC in most of the analysis will have selected the majority of HCC cases, there is some potential for selection of cancer types such as

cholangiocarcinoma. I therefore carried out a sensitivity analysis, using only codes that were highly specific to HCC. Although the power was reduced somewhat, the results continued to suggest that there remains little association between ACE inhibitor use and HCC.

6.4.4 Previous literature

There is extensive laboratory research suggesting the anti-cancer effect of ACE inhibitors in HCC, particularly in murine models (Volpert et al. 1996; Yoshiji et al. 2001; Noguchi et al. 2003; Yoshiji et al. 2005a; Yoshiji et al. 2006). They use a variety of methods to assess the potential anti-cancer efficacy of ACE inhibitors, including a number of cell lines and animal models. They also go some way into investigating the mechanism of action of ACE inhibitors. While these studies undoubtedly have some merit, slight concern might be expressed given that the majority of these studies arise from just one research group in Japan. This does not discredit their individual findings, but it would certainly become a more compelling story if a greater variety of research groups were to investigate this.

The same group again produced two small clinical studies that suggest that ACE inhibitors do have an anti-cancer effect in HCC in patients in synergy with other agents (Yoshiji et al. 2007; Yoshiji et al. 2009). The first study is a case report and suggests that a precancerous condition (a dysplastic nodule) was regressed so as to be clinically undetectable after a year of ACE inhibitor treatment. While this case study does provide a rich source of information on the case itself, it can only ever make vague qualitative suggestions, which of course warrant further investigation in greater numbers. Their follow up to this was to look at secondary prevention (i.e. prevention of recurrence) of HCC. Using just 50 patients (100 including other studies in the paper) they suggest that around a 50% reduction in recurrence was observed after 2 years of treatment. While size of effect is impressive, the small number of patients in this study means that further large scale study is still required.

There are also epidemiological studies that suggest effects in other cancers, in terms of incidence (Lever et al. 1998; Sjoberg et al. 2007), where one study suggests that overall cancer incidence can be reduced by around 30% in ACE inhibitor users (Lever et al. 1998). This study, by the author's own admission may only be useful for hypothesis generation, perhaps because the comparison cohort was taken from a different data source than the cases, but also it also suggests that interventional trials are necessary to change treatment strategy. The other study, which looked specifically at oesophageal and gastric cancer in the GPRD (Sjoberg et al. 2007), found that there was only a benefit in oesophageal cancer incidence. The dose dependency that was also found suggests that the association found is more likely to be causal.

Survival may also be improved by ACE inhibitors (or angiotensin receptor blockers) (Wilop et al. 2009). In this case lung cancer patients were involved in an observational study to assess survival. An increase in median survival of 3.1 months was observed. While this small study is indeed valuable, more reliable data could be achieved in a randomised interventional study. In addition, this study does not distinguish between ACE inhibitors or angiotensin receptor blockers, meaning that it is difficult to tell what effect each class of drugs has.

Given these previous findings it is possible that my study, which found no such effects, does not represent the true effects. One epidemiological study does agree with my conclusions, but in general cancer incidence (Friis et al. 2001). Here a prescription database combined with the Danish Cancer Registry was used, allowing a large study size (17,897 cancer cases). However the comparison group used for this study was county specific cancer incidence rates. It is therefore perhaps questionable whether these groups are comparable. The study does suggest a (non-significant) decreased risk of upper digestive system cancers, which perhaps prompted the study by Sjoberg et al.

6.4.5 Implications

Given the relatively limited power of this study, it therefore can't be said with a high degree of certainty what the true effects are. It might be suggested however, that use of ACE inhibitors does not exert a large protective effect against HCC, and the prospect of further studies finding a substantial and clinically important protective effect seems unlikely. Smaller effects, or perhaps effects that are enhanced by synergy with other compounds, such as vitamin K2, cannot be ruled out.

7 Conclusions

Interpretation, implications and recommendations

7.1 Conclusions

The aims of this thesis were:

1. To determine the effects of tricyclic antidepressants on cancer incidence
2. To determine the effect of tricyclic antidepressants on post diagnosis cancer survival.
3. To investigate aspirin and colorectal cancer survival.
4. To determine if ACE inhibitors have an effect on hepatocellular carcinoma incidence.

7.2 Summary of findings

7.2.1 Tricyclic antidepressants and cancer incidence

31,953 cancers were identified, each matched with up to 2 controls. I found a statistically significant reduction in tricyclic prescriptions compared to controls in glioma (Odds Ratio (OR)= 0.59, 95% Confidence Interval (CI)= 0.42–0.81) and colorectal cancer patients (OR= 0.84, CI= 0.75–0.94). These effects were dose-dependent (p-values for trend, glioma= 0.0005, colorectal= 0.001) and time-dependant (p-values for trend glioma= 0.0008, colorectal= 0.008). The effects were cancer type specific, with lung, breast and prostate cancers largely unaffected by antidepressant use.

This biologically plausible, cancer specific, dose and time dependant inverse association suggests that tricyclics may have potential for prevention of both colorectal cancer and glioma. The other cancer types studied seem to be largely unaffected by tricyclic antidepressant use.

7.2.2 Tricyclic antidepressants and survival in glioma and colorectal cancer

A total of 1364 glioma and 16,519 colorectal cancer patients were used in the final analysis. There was a non-significant reduction in the hazard ratio for glioma patients treated with tricyclics (HR= 0.83, CI= 0.53-1.28). This was mainly found in patients who were not previously exposed to tricyclics (HR= 0.54, CI= 0.25-1.14). In contrast, a significant increase in the hazard ratio was found for colorectal cancer (HR= 1.40, CI= 1.22-1.60). This was mostly due to patients prescribed low dose tricyclics (HR= 1.55, CI= 1.31-1.83).

This study has shown no significant benefit in terms of mortality reduction in colorectal cancer or glioma patients treated with tricyclics. An apparent detrimental effect observed in colorectal cancer may be related to prescription of low dose tricyclics in the management of pain related to disseminated cancer. I cannot rule out small effects or an effect that occurs exclusively at higher doses.

7.2.3 Aspirin, NSAIDs and survival in colorectal cancer

In the final cohort used for analysis, there were a total of 13,994 colorectal cancer patients. Overall mortality, in the whole cohort, was slightly lower in patients treated with aspirin, (Hazard Ratio (HR)= 0.91 95% Confidence Interval (CI)= 0.82-1.00). This effect was observed only in patients treated with low dose aspirin (HR= 0.89 CI= 0.80-0.98). Differential effects on mortality were also observed depending on the length of time after diagnosis. Up to 6.3 years, a reduction in mortality was observed for aspirin users (HR= 0.83 CI= 0.75-0.91), whereas a after this period there was an increase in mortality (HR= 1.64 CI= 1.32-2.03). For NSAID use, there was no significant observed effect on overall mortality (HR= 1.07 CI= 0.98-1.15). Any effects that were observed for NSAID use displayed an increase in mortality, for example in high dose NSAID use (HR= 1.41 CI= 1.26-1.56).

While the findings of this study are not wholly consistent with previous findings, they do still provide further indication that aspirin may be beneficial in reducing mortality in colorectal cancer patients.

7.2.4 ACE inhibitors and incidence of hepatocellular carcinoma

335 HCC patients were identified, each matched to up to 10 controls by age, sex and general practice. The data show that HCC is associated with a small, non-significant increase in prior use of ACE inhibitors (OR= 1.16, CI= 0.67-2.00). ACE inhibitor use was 7.1% in cases and 5.9% in controls. No significant effects were found when investigating the effect of dose and exposure duration.

This study therefore found no clear protective effect of ever or long term use of ACE inhibitors against the development of HCC. My study suggests that it is unlikely that this class of drugs will be a clinically useful cancer chemoprevention therapy.

7.3 Implications

It is rare that descriptive epidemiology alone is sufficient to directly alter clinical practice. Clinical trials are required in order to fully establish the efficacy of a drug for a new indication. The value of this thesis lies in its ability to give an indication of potential effectiveness of a drug, and also in assisting in clinical trial design by estimating the size of effect that might be seen with a particular treatment. This can help in determining the study size required for an effective trial.

7.3.1 Tricyclic antidepressants and cancer incidence

The strong association between tricyclic usage and reduced glioma incidence is intriguing, but is of little immediate use in terms of cancer prevention. This is due to the rarity of glioma, which means that the cost/benefit ratio is poor in terms of the number of treated

people needed to prevent one glioma. For colorectal cancer the positive association found is a good indication of the potential anticancer action of tricyclics, but due to the relatively small size of effect found, using tricyclics for chemoprevention may be of limited value given the side effect profile of tricyclics.

7.3.2 Tricyclic antidepressants and survival in glioma and colorectal cancer

While little substantial evidence of improved mortality in tricyclic users was found, I cannot rule out that tricyclics could be useful in glioma treatment. The results obtained do still hint at a possible survival benefit and should certainly not deter any glioma patient currently using the drug to continue its use. It seems unlikely at this point that tricyclics could confer a survival benefit to colorectal cancer patients.

7.3.3 Aspirin, NSAIDs and survival in colorectal cancer

The evidence displayed here provides a small amount of support to previous findings that aspirin may benefit colorectal cancer patients. This is despite the GPRD not being an ideal setting to carry out such a study, with its lack or histological of stage data. Though aspirin may not yet be ready for full use as a colorectal cancer therapy, it seems appropriate at this point for clinical work to further establish its potential benefits.

7.3.4 ACE inhibitors and incidence of hepatocellular carcinoma

Based on my findings, it seems unlikely that ACE inhibitors have a substantial effect on HCC. Though a small effect may still be possible it is unlikely such an effect would be clinically significant, as HCC is not one of the most common cancers. Even using a hugely optimistic value of effect size, it is estimated that between 3,000 and 30,000 patients (depending on incidence rates) would need to be treated with ACE inhibitors to prevent one HCC case.

7.4 Recommendations for further studies

It may at some point be possible to identify high risk groups for glioma, through for example genetic profiling or molecular biomarkers. This would be of huge value as it could allow those identified as high risk to use tricyclics as a prophylactic against glioma development. Though a number of common cancer types were studied for their association with tricyclics here, there may be other cancer types that are particularly sensitive to mitochondrial inhibition. This is where molecular profiling of cancers may be important. Grouping cancers by site of origin does not necessarily give groups that are homogeneous in terms of drug response. Each tumour will have a specific set of malignant transformations. It is this that determines drug response, rather than the site of origin. Further clinical use of tricyclics in glioma patients is vital for to provide further evidence of its potential in treating glioma. As discussed at the end of chapter 4, the minimum trial size based on the effect size that seems most likely in this thesis (a hazard ratio of 0.83) would be 130. Given that there were around 4,500 incident primary brain tumours in the UK in 2007 (Cancer Research UK), with a large proportion of these being gliomas, it seems realistic that such a trial could be carried out.

Future linkage of the GPRD to additional cancer registries and hospital episode statistics (which is now starting to be implicated) may well provide the additional data required to fully replicate the previous findings on the association between aspirin and colorectal cancer. Clinical studies are however a vital next step in the development of this drug as an anti-cancer drug. Trials involving cancer prophylaxis and aspirin have been successful previously and it seems there is a good chance that the same may be true for its use in treatment. The likely minimum size of such a trial would be 260 patients based on data in this study. However, given the range of values for the potential size of effect in this area, using more patients may be advisable, particularly as colorectal cancer is very common.

For the ACE inhibitors and HCC, another epidemiological study may provide further insight into their association. Despite being powered sufficiently to provide some insight, the study in this thesis was still limited in power to detect small associations. As time passes the GPRD continues to accrue more data and the dataset used for this study was a extracted a number of years ago. This means that a larger cohort would now be available, which may allow detection of changes not found in this study. In addition, the GPRD continues to improve in quality in terms of recording of covariates, meaning that these factors could be better adjusted for.

Though a number of drugs and cancer types have been investigated in this thesis, there are others hypotheses that would be testable using this type of data. Peroxisome proliferator activated receptors (PPARs) are a class of proteins with a diverse range of physiological functions. Amongst these, it is thought that PPARγ may have a role in cancer (Grommes et al. 2004). There are two classes of drugs known to interact with PPARγ, glitazones and aminosalicilates. These are collectively known as PPAR antagonists and have been studied in relation to various types of cancer (Hatton et al. 2008), including prostate cancer (Jiang et al. 2009; Murtola et al. 2009) and melanoma (Freudlsperger et al. 2006).

Sodium valproate is conventionally used in the treatment of epilepsy and bipolar disorder. It has also been linked to an anti-cancer action in several cancer types, including neuroblastoma, glioma, breast cancer, prostate cancer and leukaemia (Singh et al. 2005). Its anti-cancer action is thought to be mediated trough inhibition of histone deacetylase (Gottlicher et al. 2001). Histone deacetylase is an important protein in both normal physiology and cancer development (Kortenhorst et al. 2006).

The value of this thesis and other similar epidemiological work is in exploring the effects of drugs in humans without the expense of an interventional trial. While this approach yields data that are far from the ideal of randomised, blinded, well dosed drug exposure inherent

in good clinical trials, it can still give results which give an indication of the efficacy of a drug in a new application. Given the continuing improvements to data quality and quantity and the ongoing linkage of databases to provide greater depth of information on patients, it seems that there is still plenty of potential for further studies to find new uses for old drugs.

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Appendices

GPRD

Excellence in Public Health Research

Date: 22nd February 2010

Epidemiological study to investigate the effect of tricyclic antidepressants and reduced incidence of various cancer types. Protocol No. 08_016

1. Data-set specification

Authors: Tarita Murray-Thomas, Dr. Tim Williams GPRD, MHRA, UK

Distribution: Alex Walker, University of Nottingham

2. Description of cohort as defined in the protocol

The baseline population will consist of all up-to-standard patients (UTS) in GPRD during the period 01/01/1987 – 31/12/2007 inclusive.

Cases will consist of all patients with an incident medical diagnosis of **brain tumour including glioma**, **colorectal**, **lung**, **breast** or **prostate** cancer in their clinical or referral record during 01/01/1987 – 31/12/2007 inclusive.
Cases will be ≥18 years of age on the index date of their diagnosis in the study window. The index date of diagnosis will be defined as the first date when the GP records a diagnosis of one or more of the relevant cancers of interest in the study window and during the patient UTS follow-up period.
Cases will have at least 5 years of UTS follow-up prior to the first recorded diagnosis of the relevant cancers in the study window.
A medical diagnosis of the relevant cancers of interest will be defined by the list of the Read OXMIS codes provided by the researcher and agreed with the GPRD research team (**Annex I to V**).

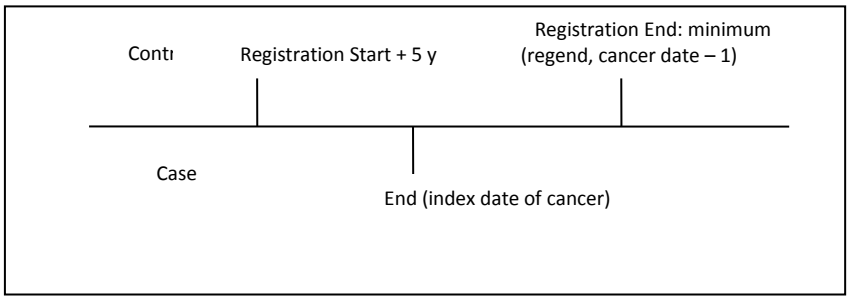
Cases with a diagnosis of any other cancer (including benign, malignant, secondary, cancers of uncertain behaviour or carcinomas in situ), **prior** to the diagnosis of the study cancers of interest will be excluded from the cohort. Cancer events recorded in the patient UTS and/or non-UTS follow-up period will be evaluated to identify cases for exclusion.

Controls will include all patients **without** a recorded medical diagnosis of the relevant cancers of interest **prior** to the index date of the case – in other words are cases allowed to become controls before the date they become a case. Controls will have at least 5 years of UTS follow-up prior to the index date of the case and will be matched on the same year of birth, gender and practice as that of the case. Controls will be matched to cancer cases on a ratio of up to 4:1

Controls with a diagnosis of **any** other cancer (including benign, malignant, secondary, cancers of uncertain behaviour or carcinomas in situ) prior to the index date of the case will be excluded from the cohort.

Index date matching will be undertaken as shown below.

Index date Matching



For the purposes of efficiency GPRD may not apply all cohort identification criteria. The criteria applied by GPRD however must result in a cohort that wholly contains the study cohort as defined in the protocol. The section below outlines the proposed GPRD data cut, and the researcher must be happy that they can generate the study cohort from this data cut, by further application of inclusion / exclusion criteria.

Cohort provided by GPRD data cut:

The following criteria will be applied by GPRD:

Cases

Inclusions

- All patients with a recorded incident diagnosis of brain tumour including glioma, colorectal, lung, breast or prostate cancer in their clinical or referral files during the period 01/01/1987 – 31/12/2007 inclusive.
- Age at index diagnosis: ≥ 18 years of age
- Gender - Male or Female
- Patient will have at least 5 years of UTS follow-up prior to the index date of diagnosis in the study window.

Exclusions

- Cases with a diagnosis of **any other** cancer ((including benign, malignant, secondary, cancers of uncertain behaviour or carcinomas in situ) **prior** to the diagnosis of the study cancers of interest
- Patients with less than 5 years of UTS follow-up prior to the index date of diagnosis in the study window.

The following criteria will **not** be applied by GPRD:

- None

Controls: Up to 4

Inclusions

- All patients **without** a recorded diagnosis of brain tumour including glioma, colorectal, lung, breast or prostate cancer in their clinical or referral record prior to the index date of the case.
- Patient will have at least 5 years of UTS follow-up prior to the index date of diagnosis of the case in the study window.

The controls will be will be matched to cases on:
Year of birth (±2)as the case
Same gender as the case
Same practice as the case

Exclusions

- Controls with a diagnosis of **any other** cancer (including benign, malignant, secondary, cancers of uncertain behaviour or carcinomas in situ) prior to the index date of the case.

Index date matching will be undertaken: The end date of the case will be set to the index date of the case.

Annex I: List of Read/OXMIS codes as evidence of Brain tumour & Glioma

GPRD Medical Code	Term Type	Read / OXMIS Code	Read / OXMIS Term
Brain			
219944	(OXMIS)	2259F	FIBROBLASTOMA MENINGEAL
265551	(OXMIS)	191 BC	CEREBRAL NEOPLASM
303132	(OXMIS)	191 A	MALIGNANT NEOPLASM CEREBRAL
210814	OXMIS	1929DG	OLIGODENDROGLIOMA
210816	OXMIS	1990NB	NEUROBLASTOMA DISSEMINATED
229004	OXMIS	191 MB	MEDULLOBLASTOMA BRAIN
229005	OXMIS	1929MN	MENINGIOMA BRAIN MALIGNANT
247109	OXMIS	190 B	RETINOBLASTOMA
247111	OXMIS	1929D	OLIGODENDROBLASTOMA
256329	OXMIS	1925GN	MALIGNANT GANGLIONEUROMA
265591	OXMIS	2259B	MENINGIOMA DIFFUSE
292899	OXMIS	1923	MENINGIOMA SPINAL CORD MALIGNANT
303133	OXMIS	1925NB	NEUROBLASTOMA
303134	OXMIS	1929AC	ASTROCYTOMA
303135	OXMIS	1929GL	GLIOMA
303218	OXMIS	2381	BRAIN TUMOUR
306093	OXMIS	2259A	MENINGIOMA
206161	READ	B510z00	Malignant neoplasm of cerebrum NOS
206162	READ	B515000	Malignant neoplasm of choroid plexus
215160	READ	B510000	Malignant neoplasm of basal ganglia
215161	READ	B511.00	Malignant neoplasm of frontal lobe
215162	READ	B512.00	Malignant neoplasm of temporal lobe
215163	READ	B512z00	Malignant neoplasm of temporal lobe NOS
215164	READ	B51y.00	Malignant neoplasm of other parts of brain
224192	READ	B515.00	Malignant neoplasm of cerebral ventricles
224193	READ	B515z00	Malignant neoplasm of cerebral ventricle NOS
224194	READ	B517300	Malignant neoplasm of pons
224306	READ	B7F4000	Spinal meningioma
233311	READ	B512100	Malignant neoplasm of uncus
242345	READ	B510.00	Malignant neoplasm cerebrum (excluding lobes and ventricles)
242346	READ	B515100	Malignant neoplasm of floor of cerebral ventricle
242347	READ	B516.00	Malignant neoplasm of cerebellum
251533	READ	B510400	Malignant neoplasm of hypothalamus
251534	READ	B510500	Malignant neoplasm of thalamus
251535	READ	B513.00	Malignant neoplasm of parietal lobe
251536	READ	B514.00	Malignant neoplasm of occipital lobe
251537	READ	B51y000	Malignant neoplasm of corpus callosum
260726	READ	B510300	Malignant neoplasm of globus pallidus
269967	READ	B51yz00	Malignant neoplasm of other part of brain NOS
270063	READ	B7F2000	Cerebral meningioma
270253	READ	ByuA.00	[X]Malignant neoplasm of eye, brain and other parts of cent
279035	READ	B510200	Malignant neoplasm of corpus striatum
279036	READ	B517.00	Malignant neoplasm of brain stem
279037	READ	B517000	Malignant neoplasm of cerebral peduncle
279038	READ	B51z.00	Malignant neoplasm of brain NOS
288139	READ	B517100	Malignant neoplasm of medulla oblongata

288140	READ	B51y100	Malignant neoplasm of tapetum
288141	READ	B51y200	Malignant neoplasm, overlapping lesion of brain
288433	READ	ByuA300	[X]Malig neopl, overlap lesion brain & other part of CNS
297389	READ	B51..00	Malignant neoplasm of brain
297390	READ	B51..11	Cerebral tumour - malignant
297391	READ	B510100	Malignant neoplasm of cerebral cortex
297392	READ	B512000	Malignant neoplasm of hippocampus
297393	READ	B517200	Malignant neoplasm of midbrain
297394	READ	B517z00	Malignant neoplasm of brain stem NOS
340924	READ	b51y.00	Malignant neoplasm of other parts of brain

Glioma

210814	(OXMIS)	1929DG	OLIGODENDROGLIOMA
229004	(OXMIS)	191 MB	MEDULLOBLASTOMA BRAIN
247111	(OXMIS)	1929D	OLIGODENDROBLASTOMA
265554	(OXMIS)	1929EP	EPENDYMOMA
265592	(OXMIS)	2259G	GLIOMA BENIGN
303134	(OXMIS)	1929AC	ASTROCYTOMA
303135	(OXMIS)	1929GL	GLIOMA
206401	READ	BBbA.00	[M]Myxopapillary ependymoma
206402	READ	BBbM.00	[M]Giant cell glioblastoma
206403	READ	BBbT.00	[M]Medulloblastoma NOS
215374	READ	BBb3.12	[M]Subependymal astrocytoma NOS
215375	READ	BBb3.13	[M]Subependymoma
215376	READ	BBb4.00	[M]Subependymal giant cell astrocytoma
215377	READ	BBbE.11	[M]Gemistocytoma
215378	READ	BBbJ.00	[M]Spongioblastoma polare
215379	READ	BBbL.12	[M]Spongioblastoma multiforme
215380	READ	BBbW.00	[M]Cerebellar sarcoma NOS
224439	READ	BBb0.12	[M]Gliosarcoma
224440	READ	BBb5.00	[M]Choroid plexus papilloma NOS
224441	READ	BBbE.00	[M]Gemistocytic astrocytoma
224442	READ	BBbP.00	[M]Primitive polar spongioblastoma
224443	READ	BBba.00	[M]Primitive neuroectodermal tumour
224444	READ	BBbz.00	[M]Glioma NOS
233553	READ	BBb..00	[M]Gliomas
233554	READ	BBb0.00	[M]Glioma, malignant
233555	READ	BBbC.00	[M]Astrocytoma, anaplastic type
233556	READ	BBbK.00	[M]Astroblastoma
242572	READ	BBb2.11	[M]Mixed glioma
242573	READ	BBb3.00	[M]Subependymal glioma
242574	READ	BBb6.00	[M]Choroid plexus papilloma, malignant
242575	READ	BBbF.00	[M]Fibrillary astrocytoma
242576	READ	BBbL.11	[M]Glioblastoma multiforme
251737	READ	BBb3.11	[M]Subependymal astrocytoma NOS
251738	READ	BBb7.00	[M]Ependymoma NOS
251739	READ	BBb8.11	[M]Ependymoblastoma
260947	READ	BBb2.00	[M]Mixed glioma
260948	READ	BBbU.00	[M]Desmoplastic medulloblastoma
270213	READ	BBbG.00	[M]Pilocytic astrocytoma
270214	READ	BBbL.00	[M]Glioblastoma NOS
270215	READ	BBbN.00	[M]Glioblastoma with sarcomatous component
270216	READ	BBbQ.00	[M]Oligodendroglioma NOS
270217	READ	BBbR.00	[M]Oligodendroglioma, anaplastic type
270218	READ	BBbS.00	[M]Oligodendroblastoma

279292	READ	BBb8.00	[M]Ependymoma, anaplastic type
279293	READ	BBbB.12	[M]Astroganglioma
279294	READ	BBbG.11	[M]Juvenile astrocytoma
279295	READ	BBbG.12	[M]Piloid astrocytoma
279296	READ	BBbX.00	[M]Monstrocellular sarcoma
288387	READ	BBb0.11	[M]Glioma NOS
288388	READ	BBb1.00	[M]Gliomatosis cerebri
288389	READ	BBb9.00	[M]Papillary ependymoma
288390	READ	BBbB.00	[M]Astrocytoma NOS
288391	READ	BBbH.00	[M]Spongioblastoma NOS
288392	READ	BBbZ.00	[M]Pleomorphic xanthoastrocytoma
297665	READ	BBbB.11	[M]Astrocytic glioma
297666	READ	BBbD.00	[M]Protoplasmic astrocytoma
297667	READ	BBbV.00	[M]Medullomyoblastoma

Annex II: List of Read/OXMIS codes as evidence of Colorectal cancer

GPRD Medical Code	Term Type	Read / OXMIS Code	Read / OXMIS Term
201875	(OXMIS)	1736CN	CARCINOMA ANUS
219895	(OXMIS)	1542A	MALIGNANT NEOPLASM ANAL CANAL
237937	(OXMIS)	1538B	SARCOMA COLON
247092	(OXMIS)	1542C	CARCINOMA ANAL CANAL
274663	(OXMIS)	1541A	MALIGNANT NEOPLASM RECTUM
292886	(OXMIS)	1736AN	MALIGNANT NEOPLASM ANUS
303101	(OXMIS)	1541C	RECTUM CARCINOMA
303203	(OXMIS)	2304	TUMOUR RECTAL
201865	OXMIS	1539AT	MALIGNANT NEOPLASM INTESTINE
210799	OXMIS	1530AD	ADENOCARCINOMA ASCENDING COLON
237936	OXMIS	1533A	MALIGNANT NEOPLASM SIGMOID
283743	OXMIS	1530AC	MALIGNANT NEOPLASM CAECUM
292877	OXMIS	1538AN	MALIGNANT NEOPLASM LARGE BOWEL NONRECTAL
292878	OXMIS	1538CN	LARGE BOWEL CARCINOMA NONRECTAL
303095	OXMIS	1530CC	CARCINOMA CAECUM
303096	OXMIS	1533AD	ADENOCARCINOMA SIGMOID COLON
303097	OXMIS	1538A	MALIGNANT NEOPLASM LARGE INTESTINE
303098	OXMIS	1538AD	ADENOCARCINOMA COLON
303099	OXMIS	1538C	COLON CARCINOMA
303100	OXMIS	1539A	MALIGNANT NEOPLASM BOWEL
206104	READ	B140.00	Malignant neoplasm of rectosigmoid junction
224129	READ	B133.00	Malignant neoplasm of sigmoid colon
224130	READ	B136.00	Malignant neoplasm of ascending colon
224131	READ	B14y.00	Malig neop other site rectum, rectosigmoid junction and anus
233260	READ	B141.00	Malignant neoplasm of rectum
242290	READ	B13z.11	Colonic cancer
242291	READ	B141.11	Carcinoma of rectum
242292	READ	B142.11	Anal carcinoma
242293	READ	B142000	Malignant neoplasm of cloacogenic zone
251477	READ	B137.00	Malignant neoplasm of splenic flexure of colon
251478	READ	B13z.00	Malignant neoplasm of colon NOS

251479	READ	B14..00	Malignant neoplasm of rectum, rectosigmoid junction and anus
251480	READ	B143.00	Malignant neoplasm of anus unspecified
260672	READ	B13y.00	Malignant neoplasm of other specified sites of colon
269916	READ	B130.00	Malignant neoplasm of hepatic flexure of colon
269917	READ	B14z.00	Malignant neoplasm rectum,rectosigmoid junction and anus NOS
270073	READ	B804000	Carcinoma in situ of rectosigmoid junction
278983	READ	B134.11	Carcinoma of caecum
278984	READ	B141.12	Rectal carcinoma
279149	READ	B803400	Carcinoma in situ of caecum
288088	READ	B13..00	Malignant neoplasm of colon
288089	READ	B131.00	Malignant neoplasm of transverse colon
288090	READ	B134.00	Malignant neoplasm of caecum
288091	READ	B138.00	Malignant neoplasm, overlapping lesion of colon
297335	READ	B132.00	Malignant neoplasm of descending colon
297336	READ	B135.00	Malignant neoplasm of appendix
297337	READ	B142.00	Malignant neoplasm of anal canal

Annex III: List of Read/OXMIS codes as evidence of Breast cancer

GPRD Medical Code	Term Type	Read / OXMIS Code	Read / OXMIS Term
201877	(OXMIS)	174 AN	MALIGNANT NEOPLASM NIPPLE
210809	(OXMIS)	174 CI	CARCINOMA BREAST INDURATED
256319	(OXMIS)	174 DL	ADENOCARCINOMA BREAST ULCERATION
303115	(OXMIS)	174 C	CARCINOMA BREAST
303116	(OXMIS)	174 DC	ADENOCARCINOMA BREAST
303185	(OXMIS)	217 AF	FIBROADENOMA BREAST
306054	(OXMIS)	174 A	NEOPLASM MALIGNANT BREAST
206147	READ	B342.00	Malignant neoplasm of upper-inner quadrant of female breast
206148	READ	B34yz00	Malignant neoplasm of other site of female breast NOS
215142	READ	B341.00	Malignant neoplasm of central part of female breast
215143	READ	B345.00	Malignant neoplasm of lower-outer quadrant of female breast
224176	READ	B340100	Malignant neoplasm of areola of female breast
224177	READ	B344.00	Malignant neoplasm of upper-outer quadrant of female breast
224178	READ	B347.00	Malignant neoplasm, overlapping lesion of breast
224179	READ	B34y000	Malignant neoplasm of ectopic site of female breast
233301	READ	B34z.00	Malignant neoplasm of female breast NOS
260706	READ	B340.00	Malignant neoplasm of nipple and areola of female breast
260707	READ	B340000	Malignant neoplasm of nipple of female breast
260708	READ	B340z00	Malignant neoplasm of nipple or areola of female breast NOS
260709	READ	B343.00	Malignant neoplasm of lower-inner quadrant of female breast
269951	READ	B346.00	Malignant neoplasm of axillary tail of female breast
297372	READ	B34..00	Malignant neoplasm of female breast
297373	READ	B34..11	Ca female breast
297374	READ	B34y.00	Malignant neoplasm of other site of female breast

Annex IV: List of Read/OXMIS codes as evidence of Lung cancer

GPRD	Term	Read /	Read / OXMIS Term

Medical Code	Type	OXMIS Code	
201873	OXMIS	1620C	TRACHEA CARCINOMA
219900	OXMIS	1620A	NEOPLASM MALIGNANT TRACHEA
256312	OXMIS	1621A	NEOPLASM MALIGNANT LUNG
283748	OXMIS	1621AB	NEOPLASM MALIGNANT BRONCHUS
303106	OXMIS	1621C	LUNG CARCINOMA
303107	OXMIS	1621CB	CARCINOMA BRONCHUS
303108	OXMIS	1621D	PANCOAST TUMOUR
303204	OXMIS	2313L	TUMOUR LUNG
215101	READ	B221100	Malignant neoplasm of hilus of lung
215102	READ	B223000	Malignant neoplasm of middle lobe bronchus
215103	READ	B224100	Malignant neoplasm of lower lobe of lung
215104	READ	B22z.00	Malignant neoplasm of bronchus or lung NOS
224142	READ	B220000	Malignant neoplasm of cartilage of trachea
224143	READ	B220z00	Malignant neoplasm of trachea NOS
224144	READ	B221z00	Malignant neoplasm of main bronchus NOS
224145	READ	B222.11	Pancoast's syndrome
224146	READ	B224.00	Malignant neoplasm of lower lobe, bronchus or lung
224147	READ	B22y.00	Malignant neoplasm of other sites of bronchus or lung
233275	READ	B225.00	Malignant neoplasm of overlapping lesion of bronchus & lung
242303	READ	B220100	Malignant neoplasm of mucosa of trachea
242304	READ	B223.00	Malignant neoplasm of middle lobe, bronchus or lung
251491	READ	B222100	Malignant neoplasm of upper lobe of lung
260679	READ	B222z00	Malignant neoplasm of upper lobe, bronchus or lung NOS
269932	READ	B22..00	Malignant neoplasm of trachea, bronchus and lung
269933	READ	B222.00	Malignant neoplasm of upper lobe, bronchus or lung
269934	READ	B22z.11	Lung cancer
278992	READ	B220.00	Malignant neoplasm of trachea
278993	READ	B221.00	Malignant neoplasm of main bronchus
288097	READ	B222000	Malignant neoplasm of upper lobe bronchus
288098	READ	B223100	Malignant neoplasm of middle lobe of lung
288099	READ	B223z00	Malignant neoplasm of middle lobe, bronchus or lung NOS
288100	READ	B226.00	Mesothelioma
297344	READ	B221000	Malignant neoplasm of carina of bronchus
297345	READ	B224000	Malignant neoplasm of lower lobe bronchus
297346	READ	B224z00	Malignant neoplasm of lower lobe, bronchus or lung NOS

Annex V: List of Read/OXMIS codes as evidence of Prostate cancer

GPRD Medical Code	Term Type	Read / OXMIS Code	Read / OXMIS Term
303125	OXMIS	185 C	PROSTATE CARCINOMA
303126	OXMIS	185 CA	ADENOCARCINOMA PROSTATE
306055	OXMIS	185 A	MALIGNANT NEOPLASM PROSTATE
233306	READ	B46..00	Malignant neoplasm of prostate
332056	READ	4M0..00	Gleason grading of prostate cancer
339526	READ	4M00.00	Gleason prostate grade 2-4 (low)
339842	READ	4M01.00	Gleason prostate grade 5-7 (medium)
339892	READ	4M02.00	Gleason prostate grade 8-10 (high)

GPRD

Excellence in Public Health Research

Date: 16th June 2008

Prospective study to further characterise the effects of tricyclic antidepressants on glioma and colorectal cancer.

Protocol 09_087R

1 Data-set specification

Authors: Tarita Murray-Thomas, MHRA, UK

Distribution: Alex Walker, University of Nottingham

Prospective Cohort Study

Study requirements as per protocol

Inclusions

Cases will consist of all patients with an incident medical diagnosis of glioma or colorectal cancer during the period 01/01/1987 -31/12/2009 inclusive. The index date of diagnosis of a glioma or colorectal cancer will be defined as the first date that the GP records such a diagnosis in the patient clinical or referral record. Patients will have at least 12 months of up-to-standard (UTS) follow-up prior to the index date of diagnosis of cancers of interest.

The list of Read codes for identifying glioma or colorectal cancer will be provided by the researcher and agreed with the GPRD Research Team (**Annex 1 & 2**).

Exclusions

Patients with <12 months of up-to-standard (UTS) follow-up prior to the index date of diagnosis of cancers of interest will be excluded from the cohort.

COHORT TO BE PROVIDED BY GPRD DATA CUT:

RETROSPECTIVE COHORT STUDY

Inclusion Criteria

- All patients with an incident medical diagnosis of **glioma or colorectal cancer** recorded during the period 01/01/1987 - 31/12/2009
- Age at index diagnosis**:** No restriction
- Gender: Male or Female
- Patients will have at least 12 months of up-to-standard (UTS) follow-up prior to the index date of glioma or colorectal cancer.

Exclusion Criteria

- Patients with <12 months of up-to-standard (UTS) follow-up prior to the index date of glioma or colorectal cancer.

Criteria that will not be applied by GPRD

- None

Annex I: Read codes for identifying glioma

Readoxmiscode	Readoxmisterm
191 MB	MEDULLOBLASTOMA BRAIN
1929AC	ASTROCYTOMA
1929D	OLIGODENDROBLASTOMA
1929DG	OLIGODENDROGLIOMA
1929EP	EPENDYMOMA
1929GL	GLIOMA
2259G	GLIOMA BENIGN
BBb..00	[M]Gliomas
BBb0.00	[M]Glioma, malignant
BBb0.11	[M]Glioma NOS
BBb0.12	[M]Gliosarcoma
BBb1.00	[M]Gliomatosis cerebri
BBb2.00	[M]Mixed glioma
BBb2.11	[M]Mixed glioma
BBb3.00	[M]Subependymal glioma
BBb3.11	[M]Subependymal astrocytoma NOS
BBb3.12	[M]Subependymal astrocytoma NOS
BBb3.13	[M]Subependymoma
BBb4.00	[M]Subependymal giant cell astrocytoma
BBb5.00	[M]Choroid plexus papilloma NOS
BBb6.00	[M]Choroid plexus papilloma, malignant
BBb7.00	[M]Ependymoma NOS
BBb8.00	[M]Ependymoma, anaplastic type
BBb8.11	[M]Ependymoblastoma
BBb9.00	[M]Papillary ependymoma
BBbA.00	[M]Myxopapillary ependymoma
BBbB.00	[M]Astrocytoma NOS
BBbB.11	[M]Astrocytic glioma
BBbB.12	[M]Astroganglioma
BBbC.00	[M]Astrocytoma, anaplastic type
BBbD.00	[M]Protoplasmic astrocytoma
BBbE.00	[M]Gemistocytic astrocytoma
BBbE.11	[M]Gemistocytoma
BBbF.00	[M]Fibrillary astrocytoma
BBbG.00	[M]Pilocytic astrocytoma
BBbG.11	[M]Juvenile astrocytoma
BBbG.12	[M]Piloid astrocytoma
BBbH.00	[M]Spongioblastoma NOS
BBbJ.00	[M]Spongioblastoma polare
BBbL.00	[M]Glioblastoma NOS
BBbL.11	[M]Glioblastoma multiforme
BBbL.12	[M]Spongioblastoma multiforme
BBbM.00	[M]Giant cell glioblastoma
BBbN.00	[M]Glioblastoma with sarcomatous component
BBbP.00	[M]Primitive polar spongioblastoma
BBbQ.00	[M]Oligodendroglioma NOS
BBbR.00	[M]Oligodendroglioma, anaplastic type
BBbS.00	[M]Oligodendroblastoma
BBbT.00	[M]Medulloblastoma NOS

BBbU.00	[M]Desmoplastic medulloblastoma
BBbV.00	[M]Medullomyoblastoma
BBbZ.00	[M]Pleomorphic xanthoastrocytoma
BBba.00	[M]Primitive neuroectodermal tumour
BBbz.00	[M]Glioma NOS
BBc6.00	[M]Ganglioglioma
BBc7.11	[M]Neuroastrocytoma
BBm0.00	[M]Microglioma
K055 G	EXCISION GLIOMA BRAIN

Annex 2: Read codes for identifying colorectal cancer

Readoxmiscode	Readoxmisterm
1530AC	MALIGNANT NEOPLASM CAECUM
1530AD	ADENOCARCINOMA ASCENDING COLON
1530CC	CARCINOMA CAECUM
1533A	MALIGNANT NEOPLASM SIGMOID
1533AD	ADENOCARCINOMA SIGMOID COLON
1538A	MALIGNANT NEOPLASM LARGE INTESTINE
1538AD	ADENOCARCINOMA COLON
1538AN	MALIGNANT NEOPLASM LARGE BOWEL NONRECTAL
1538C	COLON CARCINOMA
1538CN	LARGE BOWEL CARCINOMA NONRECTAL
1539A	MALIGNANT NEOPLASM BOWEL
1539AT	MALIGNANT NEOPLASM INTESTINE
1539C	CARCINOMA BOWEL
1541A	MALIGNANT NEOPLASM RECTUM
1541C	RECTUM CARCINOMA
159	GASTROINTESTINAL MALIGNANCY
159 A	NEOPLASM MALIGNANT GASTROINTESTINAL TRAC
159 C	GASTROINTESTINAL CARCINOMA
2304	TUMOUR RECTAL
B13..00	Malignant neoplasm of colon
B130.00	Malignant neoplasm of hepatic flexure of colon
B131.00	Malignant neoplasm of transverse colon
B132.00	Malignant neoplasm of descending colon
B133.00	Malignant neoplasm of sigmoid colon
B134.00	Malignant neoplasm of caecum
B134.11	Carcinoma of caecum
B136.00	Malignant neoplasm of ascending colon
B137.00	Malignant neoplasm of splenic flexure of colon
B138.00	Malignant neoplasm, overlapping lesion of colon
B13y.00	Malignant neoplasm of other specified sites of colon
B13z.00	Malignant neoplasm of colon NOS
B13z.11	Colonic cancer
B14..00	Malignant neoplasm of rectum, rectosigmoid junction and anus
B140.00	Malignant neoplasm of rectosigmoid junction

B141.00	Malignant neoplasm of rectum
B141.11	Carcinoma of rectum
B141.12	Rectal carcinoma
B14y.00	Malig neop other site rectum, rectosigmoid junction and anus
B14z.00	Malignant neoplasm rectum,rectosigmoid junction and anus NOS
B1z0.00	Malignant neoplasm of intestinal tract, part unspecified
B1z0.11	Cancer of bowel
Byu1200	[X]Malignant neoplasm of intestinal tract, part unspecified

A full set of data files will be provided with this analysis. In addition the cohort identifying list of codes will be included. To maximise the quality of data, the cohort will be limited to acceptable patients only, as defined by a standard set of conditions relating to registration details.

The files are provided as raw data, in tab-delimited ASCII files. These files can be uploaded into statistical software such as Stata or SAS, or into data management packages such as Microsoft Access for further data processing and analysis.

Appendix III: Glossary of survival analysis methods

Below is a list of technical terms used within chapters 4 and 5 of this thesis. The aim of this is to explain the use of the various types of analysis and technical plots used, in terms of how they help to interpret the results.

Cox proportional hazards modelling is a method used to model survival times. It creates an estimate of the ratio of risks (called the **hazard ratio**) between two groups. This allows a quantitive measurement of the size and direction of the effects observed. The model assumes that the hazard rate remains stable over time. This is known as the proportional hazards assumption and is assessed in various ways in this thesis.

Kaplan-Meier curves are a method of producing a graphical representation of mortality in populations. The curve depicts the proportion of the start population still alive at each time point. Each death in the population causes the curve to drop by the proportion of the population that the dying patient represents. As patients are lost to follow up during the study, the population gets smaller and each subsequent death causes a greater drop in the curve. When curves representing two populations are drawn on the same plot, a comparison can be made of how (and when) morality in the two groups differs. This can also give an initial indication of whether the proportional hazards assumption is met, though this is better determined by using the log-log plot.

Log-log plots are a method of determining whether the hazard rate remains proportional over time between two groups in a survival study. They are created by plotting the logarithm of the survival curve verses the logarithm of study time. If the curves produced by in this graph are approximately parallel and do not cross, the proportional hazards assumption can be said to be met. However any deviation from parallel may be an

indication that the proportional hazards assumption is not met. This lack of proportionality can then be further assessed.

An **observed/predicted** plot can be used to assess how much and when the proportional hazards assumption is not met. It is created by drawing the same curve as in the Kaplan-Meier plot and then on top of that, drawing a curve created using Cox predicted values. If the two lines are separated at any point, then it is likely the proportional hazards assumption has not been met. Additionally, it can be seen where the deviation occurs in the time scale. This is useful when further analysing the data.

A number of approaches can be taken if non-proportionality is found to exist. Firstly the model can be reassessed to determine if there are any missing covariates which may, when adjusted for, cause proportionality to be restored. If this is not possible however, often the best strategy to use is **stratification** of the data according to the time after study initiation. This allows the effects for each time period to be assessed independently and this may reveal effects for different time periods that were masked by the non-proportionality in the overall model.

The **Charlson Index** is a measure of comorbidity in a patient. It encompasses 22 different conditions, such as cardiovascular disease, cancer and AIDS. Each patient is assigned a score for each condition that they are diagnosed with, and these are then totalled to produce an overall score. Scores associated with each condition are 1, 2, 3 or 6 depending on the impact of the disease on mortality.

Appendix IV: Other achievements

I have completed 2 modules in statistics within the School of Community Health Sciences. The results of exams and assignments in these modules are as follows:

Research Methods in Epidemiology and Basic Statistics	Statistics assignment: 80.5% Statistics exam: 77.8% Epidemiology exam: 56.3% **Overall Mark: 67.4%**
Advanced Research Methods in Epidemiology and Statistics	Exam: 86.5% Assignment: 61% **Overall Mark: 74%**

I attended the National Cancer Research Institute (NCRI) conference in Birmingham in October 2008. I also attended the same conference in 2009, where I was invited to speak in a proffered paper presentation, and also to present in a poster session:

http://www.ncri.org.uk/ncriconference/2009abstracts/abstracts/Para6.htm

I have also completed a programme of Graduate School research training courses. These can be seen on the next page.

Certificate of Attendance

Mr Alex John Walker
Community Health Sciences

has completed the following short research training course(s)

Advanced Statistics 2 - Multiple logistic regression and further model building strategies (M&HS)
1 training point

Nature of the PhD and the supervision process
1 training point

Getting going on your thesis and getting your work published
2 training points

Faculty postgraduate Research Forum (Medicine and Health Sciences Faculty)
4 training points

Advanced Statistics 1 - Introduction to multivariate methods and multiple linear regression (M&HS)
1 training point

Ethics Committee - animal and patient (Medicine and Health Sciences Faculty)
1 training point

Critical analysis of scientific literature (Medicine and Health Sciences Faculty)
1 training point

Advanced Statistics 3 - Survival data and Cox regression (M&HS)
1 training point

Preparing your first year report and writing scientific abstracts (M&HS Faculty)
1 training point

Planning research and time management
1 training point

Building a database with MS Access
2 training points

Clinical Trials - the basics (Medicine and Health Sciences Faculty)
2 training points

Basic Statistics (Medicine and Health Sciences Faculty)
1 training point

Building a bibliography (an online learning course)
1 training point

Preparing and presenting an effective CV for PhD and MPhil students
1 training point

Professor Claire O'Malley
Dean of the Graduate School

This Cerficate of Attendance is issued by the Graduate School, University of Nottingham, for attendance at short courses offered at the University for research students. 1 training point is equivalent to half a day of tutor contact time or independent study.

Appendix V: Publications arising from this work

The following papers have been published in relation to this thesis. A copy of the first paper follows this page. The other article was in press at the time of writing.

Walker, A. J., T. Card, T. E. Bates and K. Muir (2011). "Tricyclic antidepressants and the incidence of certain cancers: a study using the GPRD." *Br J Cancer* **104**(1): 193-7.

and

Walker, A.J., J. West, M.J. Grainge, T.R. Card (2011). "Angiotensin converting enzyme inhibitors and hepatocellular carcinoma in the General Practice Research Database" *Cancer Causes and Control* (in press)