From Department of molecular medicine and surgery
Karolinska Institutet, Stockholm, Sweden

ASPECTS OF BILIARY TRACT CANCER - INCIDENCE AND REPRODUCTIVE RISK FACTORS

Carl Kilander, M.D.

Karolinska Institutet

Stockholm 2017

Published by Karolinska Institutet.
Printed by E-print AB 2016
Front page illustration: Christina Gyllenhammar Kilander

ISBN 978-91-7676-382-7

“Allt stort som skedde i världen,
skedde först i någon människas fantasi.”

“Ibland är det som om livet plockade ut en
av sina dagar och sa: ‘Dig ska jag ge allt!
Du ska bli en av de där rosenröda dagarna som
skimrar i minnet när alla andra är glömda.’”

- A. Lindgren

1 ABSTRACT

Biliary tract cancer, including cancer of the extra-hepatic bile ducts, cancer of the Ampulla of Vater and gallbladder cancer, is a devastating disease with poor prognosis. The incidence of biliary tract cancer is decreasing worldwide, for unknown reasons. One of two aims of this thesis was to evaluate the Swedish Cancer Register regarding biliary tract cancer and to assess the incidence of biliary tract cancer in Sweden. Secondly, an association between sex hormone exposure, particularly estrogen, and biliary tract cancer has been suggested. Thus, the second aim of this thesis was to evaluate the effect of sex hormone exposure on biliary tract cancer risk.

Study I and II were based on data from the Swedish Cancer Register, the Swedish Patient Register and the Swedish Causes of Death Register. **Study I** was a validation study of biliary tract cancer diagnoses in the Cancer Register, using data from the Patient Register and the Cause of Death Register as comparison. Overall, 44% of patients diagnosed with biliary tract cancer between 1990 and 2009 were not found in the Cancer Register. The underreporting increased with increasing patient age and later time period. **Study II** indicated a decreasing incidence trend of biliary tract cancer. However, the mortality rates were higher than the incidence rates after the mid 1980's and onwards. Moreover, the incidence trends based on data from the Patient Register suggested a more stable or only slightly decreasing trend. Thus, even though the incidence of biliary tract cancer may be decreasing, the extent of the decline is likely over-estimated.

Study III investigated the association between reproductive factors and biliary tract cancer. Women and men were included in the study, but analyzed separately, to enable differentiation between hormone exposure and other factors. The risk of cancers of the extra-hepatic bile ducts (including the Ampulla of Vater) is likely not influenced by reproductive factors because similar associations were seen in women and men. However, an association between reproductive factors and gallbladder cancer was observed in women, but not as clearly in men. **Study IV** investigated the risk of biliary tract cancer in a cohort of men with prostate cancer, a proxy for estrogen exposure (prostate cancer treatment). There was no clear association, although a slightly decreased risk was indicated. However, men with the highest presumed estrogen exposure had an increased risk of biliary tract cancer, though the association was not statistically significant. **Study V** investigated the association between menopausal hormone therapy and biliary tract cancer. A slightly reduced risk of gallbladder cancer was suggested for users of menopausal hormone therapy compared to non-users, but the association was not statistically significant. However, an increased risk of gallstone disease was noted however.

In conclusion, there is substantial under-reporting of biliary tract cancer to the Swedish Cancer Register, especially in the elderly and in the later time period. The decreasing incidence trends of biliary tract cancer in Sweden are likely over-estimated, as a consequence of under-reporting. Furthermore, the role of sex hormones in the etiology of biliary tract cancer is uncertain. Sex hormone exposure may influence the risk of gallbladder cancer specifically but not biliary tract cancer as a whole. Future etiological research should separate gallbladder cancer from other extra-hepatic cancers.

2 LIST OF PUBLICATIONS

I. Kilander C, Mattsson F, Ljung R, Lagergren J, Sadr-Azodi O.
Systematic underreporting of the population-based incidence of pancreatic and biliary tract cancers.
Acta Oncologica 2014;53:822-9

II. Kilander C, Lagergren J, Sadr-Azodi O.
The population-based incidence and mortality of biliary tract cancer in Sweden.
Manuscript submitted

III. Kilander C, Mattsson F, Lu Y, Ljung R, Lagergren J, Sadr-Azodi O.
Reproductive risk factors and risk of biliary tract cancer in a population-based study.
Acta Oncologica 2015;54:1152-8

IV. Kilander C, Mattsson F, Lu Y, Ljung R, Lagergren J, Sadr-Azodi O.
Exogenous oestrogen and the risk of biliary tract cancer – a population-based study in a cohort of men treated for prostate cancer.
Acta Oncologica 2016;55(7):846-50

V. Kilander C, Lagergren J, Sadr-Azodi O, Brusselaers N.
Menopausal hormone therapy and risk of biliary tract cancer.
Manuscript

3 TABLE OF CONTENTS

4 LIST OF ABBREVIATIONS

AJCC	American Joint Committee on Cancer
APC	Annual Percent Change
ATC	Anatomical Therapeutic Chemical
CA19-9	Carbohydrate Antigen 19-9
CI	Confidence Interval
CR	The Cancer Register
DAG	Directed Acyclic Graph
DR	The Causes of Death Register
ERCP	Endoscopic Retrograde Cholangiopancreatography
EUS	Endoscopic Ultrasound
HR	Hazard Ratio
ICD	International Classification of Disease
IQR	Inter-quartile Range
OR	Odds Ratio
PR	The Patient Register
SIR	Standardized Incidence Ratio
TNM	Classification of Malignant Tumors
US	The United States of America
WHO	World Health Organization

5 INTRODUCTION

Biliary tract cancer, referring to cancers of the extra-hepatic bile ducts, the Ampulla of Vater and the gallbladder in this thesis, is a rather rare group of diseases in the Western world.(1) There are approximately 350 new cases of biliary tract cancer annually in Sweden and the incidence has been decreasing world-wide over the past 40 years.(2-4) Less than 30 % of patients are eligible for curative surgery at presentation, however, the 5-year survival without surgery is less than 10 % in Sweden.(5)

The Swedish Cancer Register is utilized for cancer incidence estimations in Sweden. The register has been shown to be of excellent over-all quality, but the completeness for some specific cancer sites has been questioned.(6, 7) Incidence estimations based on register data will be only as valid as the data themselves. Thus, the first aim of this thesis was to evaluate the Cancer Register regarding completeness of biliary tract cancer diagnoses and to estimate the incidence of biliary tract cancer in Sweden.

There is a female predominance of biliary tract cancer, specifically for gallbladder cancer where the female-to-male ratio ranges between 2 and as high as 5-6 in some countries.(8, 9) Often, the association between gallstone disease and biliary tract cancer is cited as an explanation.(10) However, estrogen exposure in itself has been suggested to influence biliary tract cancer development, but available literature on this matter leaves the research question unresolved.(11) Therefore, the second aim of this thesis was to evaluate if sex hormone exposure affects the risk of developing biliary tract cancer.

6 BACKGROUND

6.1 BILIARY TRACT CANCER

6.1.1 History of biliary tract cancer

One of the first accounts of biliary tract cancer in medical research presented in a systematic manner can be found in a paper by the American surgeon Dr. Musser published in the late 19th century, describing masses of the gallbladder and extra-hepatic bile ducts discovered accidentally during laparotomy.(12) Later, during the 20th century, Sako and colleagues reviewed the available literature and published a report including 560 cases of biliary tract cancer identified between 1935 and 1954 describing the localization and clinical features of the disease.(13) Etiological investigations have not been undertaken until more recently but the knowledge regarding biliary tract cancer development remains insufficient.

The treatment of hepatobiliary masses, including distal extra-hepatic cancers, was first investigated in the late eighteen hundreds, but was made more systematic by Whipple and colleagues when the pancreatico-duodenectomy (later named Whipple's procedure) was first described.(14, 15) Since then, numerous treatment modalities have emerged for managing biliary tract cancer, but surgical resection remains the only treatment that may offer a chance of cure.(16) In the 19th century, an era when advanced treatment options simply were not available, cancers of the biliary tract were effectively a sentence to death. Unfortunately, the prognosis for patients who are diagnosed with biliary tract cancer has not improved satisfactorily into modern times.(5)

6.1.2 Clinical presentation, diagnosis and staging

Clinical presentation

The clinical characteristics of patients reported by forerunners in biliary tract cancer research are recognized in more modern descriptions of the disease. Symptoms typically include intermittent pain in the epigastric area or right upper quadrant, anorexia (loss of appetite) and nausea.(17) Symptoms of advanced tumor stages of the disease include cholestasis and jaundice, due to biliary obstruction, subsequent bacterial translocation with cholangitis and sepsis.(10) Secondary symptoms, such as weight loss, fatigue and symptoms from distant metastases occur frequently. Because of the slow initial tumor progression, the majority of patients present in an advanced stage, when the disease is already locally advanced and/or

systemic.(18) Most patients are diagnosed at older ages and may suffer from other diseases and co-morbidities, making a clinical diagnosis even more challenging.(19)

A subset of patients with gallbladder cancer present with acute cholecystitis (gallbladder inflammation) and reports estimate that gallbladder cancer is diagnosed in around 1-2 % of patients undergoing cholecystectomy (gallbladder removal) for supposedly benign gallbladder disease.(20) However, higher rates have been seen in some case series and in the elderly.(21, 22) Conversely, around 8 % of patients with gallbladder cancers are diagnosed after routine cholecystectomy for benign gallbladder disease in Sweden.(5)

Diagnosis

Acquisition of biopsies for histopathological evaluation of suspected cancerous tissue remains an important part of the initial work-up for establishing a correct biliary tract cancer diagnosis.(23) The majority of cancers in the biliary tract are carcinomas, mainly adenocarcinoma.(5, 24) Access to the biliary tract using minimally invasive procedures is limited however, and concerns for tumor dissemination following trans-abdominal biopsies have been raised.(25, 26) Available guidelines recommend abdominal ultrasound and routine blood work as initial diagnostic tests followed by computed tomography of the abdomen and thorax to evaluate tumor stage and the presence of distal metastases.(23) Non-invasive imaging techniques, such as trans-abdominal ultrasound, computed tomography and magnetic resonance tomography have improved enormously over the past 3 decades, and accurate diagnoses can readily be made with these non-invasive methods.(27, 28) Trans-abdominal ultrasound is commonly used to evaluate patients with abdominal pain located in the right hypochondria and may readily visualize a dilated biliary tree when biliary obstruction is present, but the sensitivity for detecting a gallbladder mass may be limited.(27, 29) Computed tomography has higher sensitivity for diagnosing malignant obstructions of the biliary tract, especially gallbladder cancer when combined with positron emission tomography.(30, 31) Furthermore, magnetic resonance imaging is used increasingly with excellent results to diagnose biliary tract cancer in a non-invasive manner, but may be inferior to the invasive procedure endoscopic retrograde cholangiopancreatography (ERCP).(32-34)

ERCP is an important modality in biliary tract cancer diagnostics and allows for simultaneous tissue sampling; however high false negative rates have been described.(1, 35) More recent ERCP-based techniques, including cholangioscopy, have enabled direct visualization of cancerous tissue, increasing the diagnostic accuracy.(36) Furthermore, endoscopic ultra-sound (EUS) has emerged as an important diagnostic modality and is being used increasingly

in biliary tract cancer work-up and diagnosis.(37) Additionally, EUS in conjunction with fine-needle aspiration, for tissue sampling, has improved the diagnostic accuracy even further.(38)

There are, presently, no reliable biomarkers that offer clear guidance in the differential diagnosis of biliary tract cancer. The carbohydrate antigen 19-9 (CA19-9) has been used to some extent but studies report lacking performance in the ability to accurately discriminate biliary tract cancer from benign lesions.(39) Some reports have suggested that CA19-9 can be used as a prognostic marker in advanced biliary tract cancer and to evaluate treatment response.(40)

Staging

When biliary tract cancer has been confirmed, tumor staging is imperative before patients can be assigned a specific treatment, which largely depends on the extent of local tumor growth and potential dissemination. In staging of biliary tract cancer, the commonly used TNM system for classification developed by the American Joint Committee on Cancer (AJCC), is advocated.(41) The T-stage denotes the extent of tumor growth locally, the N-stage assesses any lymph node involvement and the M-stage describes if any distant metastases are present.(42) Patients are then categorized into TNM group I to IV, depending on tumor characteristics. Histo-pathological examination of biopsies in combination with information acquired from invasive or non-invasive procedures/imaging techniques form the basis of the staging procedure. In cases where curative surgery may come in question, but the extent of tumor dissemination is unclear after initial evaluation, staging may involve a diagnostic laparoscopy to minimize unnecessary resections.(43)

6.1.3 Risk factors

Only a handful of risk factors for biliary tract cancer have been established with variations across geographic regions, probably reflecting genetic variations and differences in lifestyle risk factors.

Primary sclerosing cholangitis

Primary sclerosing cholangitis is an auto-immune, inflammatory disease resulting in strictures and stenosis (narrowing) of the bile ducts.(44) Patients diagnosed with primary sclerosing cholangitis have an increased risk of developing biliary tract cancer and the incidence of primary sclerosing cholangitis is increasing in Europe.(45, 46) The condition itself is relatively uncommon, but the etiological contribution to biliary tract cancer may be of

importance.(47) There are even reports suggesting that screening of biliary tract cancer in patients with primary sclerosing cholangitis may be indicated.(48)

Diabetes and obesity

Numerous studies have shown an increased risk of biliary tract cancer, specifically gallbladder cancer and extra-hepatic bile duct cancers, in diabetic individuals.(49) In contrast, a study based on the European Prospective Investigation into Cancer and Nutrition cohort (EPIC cohort) reported an increased risk of gallbladder cancer in diabetic patients, but failed to show any clear associations between diabetes and cancers of the extra-hepatic bile ducts/Ampullary cancers.(50) A study from Denmark showed an increased risk of biliary tract cancer in hospitalized patients with diabetes.(51) Diabetes and obesity often co-exist in patients with the metabolic syndrome, which has become an epidemic in the modern Western world.(52) However, it has been shown that diabetes increases the risk of biliary tract cancer independent of obesity.(53) Conversely, obesity in itself has been reported to increase the risk of gallbladder cancer, but not cancers of the extra-hepatic bile ducts or Ampullary cancers.(54, 55) Furthermore, the association between obesity and gallbladder cancer may be independent of diabetes.(56) An American study revealed an increased risk of cancers of the extra-hepatic bile ducts in obese subjects.(57) Additionally, one study showed that obese adults appear to have a higher risk of biliary tract cancer-specific mortality; however the results were not adjusted for diabetes and there was no differentiation was made between tumor locations.(58)

Gallstone disease

Gallstone disease (cholelithiasis) normally refers to the presence of gallstones in the gallbladder. Gallstones are, most often, formed by precipitation of cholesterol in fat-saturated bile but may also consist of other elements such as bilirubin and calcium. However, the precise mechanisms behind gallstone formation remains incompletely understood.(59) Cholelithiasis is a common condition in Western populations, with an estimated 10-20 % prevalence and is one of the most well-documented risk factors for biliary tract cancer.(60-63) Additionally, cholecystectomy has been shown to decrease the risk of cancers of the extra-hepatic bile ducts.(64) However, one Swedish study showed that the risk of cancers of the extra-hepatic bile ducts is higher 1-4 years following cholecystectomy, compared to the background population, but decreased to the level of the background population over time.(61) This finding is likely explained by the relatively large proportion of biliary tract

cancers diagnosed *en passant* after surgery. Cholelithiasis is more frequent in populations from South America, where also biliary tract cancer incidence is high.(65)

Gallstone disease is more common in women, especially in the obese.(66) Furthermore, it has been shown that estrogen exposure, i.e. during pregnancy, modulates bile composition, gallbladder kinetics and increases the risk of gallstone disease.(67-69) Considering the associations between gallstone disease and obesity, diabetes and sex hormone exposure, it has been argued that gallstone disease may be seen as a mediator of metabolic, genetic and hormonal factors influencing risk for biliary tract cancer development.(53, 70, 71) However, about a fifth of all gallbladder cancers removed for histopathological examination have no concomitant gallstones.(24)

Other risk factors

A number of additional factors have been suggested to influence the risk of biliary tract cancer, but may be of limited importance or lack proper support in the literature. More specifically, exposure to thorium dioxide (an agent used in radiology in the early 20th century), tobacco smoking, Caroli's disease, certain liver fluke infections (parasitic infections mostly limited to Asian populations) and biliary cysts have been described as potential risk factors for biliary tract cancer.(10, 57, 72, 73)

6.1.4 Treatment

Curative treatment

Any treatment of biliary tract cancer with a curative intent requires radical surgery.(1) Advancements in oncological treatment and minimally invasive procedures have to some extent improved the prognosis, but have yet to replace surgical intervention as the corner stone of curative treatment.(74) The over-all 5-year survival in patients with biliary tract cancer is 5-15% in Sweden.(5) Similar over-all mortality rates have been described internationally depending on cancer classification and geographical area.(75-78) At the time of biliary tract cancer diagnosis, roughly 25% of patients are considered for surgery in the Western world, but some centers have reported a higher rate of curatively intended surgery.(1, 5, 79) However, long-term survival is heavily dependent on surgical intervention at an early tumor stage.(16) Additionally, tumor stage is a strong predictor of survival. For T1 gallbladder cancer, where simple cholecystectomy may be enough, the long-term survival in operated patients ranges from 65 to 99%.(5, 80, 81) For T2 gallbladder cancer, radical cholecystectomy including lymphadenectomy (removal of lymph nodes), bile duct resection,

partial hepatectomy (partial removal of the liver) and possibly resection of adjacent organs, has been shown to be the correct surgical strategy because the sub-serosal plane, in which the dissection for a simple cholecystectomy is commonly performed, is often violated by T2 tumors.(82) Consequently, the survival of patients with T2 gallbladder cancer ranges from 35 to 75%.(83, 84) Surgery for gallbladder cancer of stages T3-4 typically involves additionally extensive resections and normally a poor long-term survival, ranging from 0 to around 25 %.(84, 85)

Curative treatment and prognosis for cancers of the extra-hepatic bile ducts are also strongly related with tumor stage. Because of the more heterogeneous anatomical location, surgical procedures may involve pancreatico-duodenectomy for more distal tumors or bile duct resections with or without partial hepatectomy for more proximal tumors.(18) The 5-year survival has been reported to range from 5 to 50% following curatively intended surgery and, similarly to gallbladder cancer, less advanced tumor stage is a strong predictor of survival.(5, 86, 87)

For advanced biliary tract cancer (stage III and IV) with advanced regional lymph node involvement, or when distant metastasis is present, the over-all 5-year survival in Sweden is under 10%.(5) Patients with stage IV tumors are seldom selected for surgery because of an exceedingly poor prognosis regardless of treatment. There is currently no consensus regarding locally advanced disease and resectability, but tumor dissemination to lymph nodes around the aorta or further may be a contraindication for curative surgery.(88) However, some centers have reported more favorable outcomes even in advanced disease.(89)

Neoadjuvant treatment typically require biliary drainage via placement of stents in the bile ducts in the presence of obstruction in order to decrease the risk of cholangitis and optimize patients for surgery.(23, 90) Portal vein embolization may be performed in patients scheduled for curative resection including extended hepatectomy when the remnant liver size may be insufficient.(91, 92) There is currently no strong evidence to support the use of neoadjuvant chemo-radiotherapy in the general management of biliary tract cancer, however some benefits have been observed for certain patient categories.(93-96)

Adjuvant chemo-radiotherapy is sometimes implemented in the treatment of biliary tract cancer, however randomized clinical trials evaluating the subject are lacking.(95) One meta-analysis showed a borderline significant improvement in prognosis when adjuvant therapy was employed, and some studies have indicated potential survival benefits in certain patient groups.(96, 97)

Non-curative treatment

In the palliative setting, the median survival in patients without specific treatment ranges from 3 to 9 months.(98, 99) When obstructive jaundice is present, biliary stenting for bile duct patency can increase quality of life and decrease the risk of cholangitis.(100, 101) Furthermore, relieve of obstructive jaundice is often a requirement before any specific palliative treatment.(88) Recently, a large randomized controlled trial reported a survival benefit using a combination of chemotherapeutic drugs compared to a single-drug regimen, and provided support for the use of chemotherapy in palliative treatment of biliary tract cancer.(102) Photodynamic therapy has emerged as a new treatment modality for unresectable biliary tract cancer, however complications are frequent and high-grade evidence is lacking.(103) External radiotherapy has been used rather sparsely but may prolong survival in some groups.(104) A best supportive care approach, focusing on pain management and symptomatic treatment of e.g. nausea and itching may be indicated in some patients when specific palliative treatment is inappropriate.(96)

6.2 CANCER INCIDENCE AND REGISTERS

6.2.1 Cancer epidemiology

Epidemiological studies often involve calculating the occurrence of a disease in distinct groups. Cancer incidence represents an important measurement of cancer occurrence in epidemiological research, and may be used in cancer control programs as well as in academic research. In general terms, the incidence of a disease can be defined as the number of new cases in a specific population during a specific time period. However, the probability of a disease occurring in a specific population during a specific time is sometimes used synonymously.(105) Repeated incidence estimations are often calculated at different time points in order to monitor incidence changes over time.

Disease occurrence can be expressed in different ways, yet incidence and prevalence are perhaps the most common modalities. Correspondingly, disease incidence may be described in different forms, but a distinction into rates and proportions can be made. The concept of an incidence rate implies an element of time in the calculations. Mathematically, the numerator is the number of new cases in the studied population during the specific time period and the denominator is the accumulated observation time of the population at risk. In cancer incidence measurements, the observation time is often expressed in person-years. The same concept applies to other occurrence measurements, such as mortality rates. Instead of rates, various proportions can be calculated to describe the incidence. The incidence proportion can

be calculated by dividing the number of incident cases by the size of the population. This proportion is equivalent to the risk of the disease. Instead of risk, the odds of a disease occurring is the number of new cases in the population divided by the number of disease-free individuals at the end of the time period. Time is not directly considered when expressing proportions, however the time period where the risk or odds are valid must be specified.

The second dimension of disease occurrence, prevalence, reflects the number of individuals with the disease at a specific time point; mathematically, a proportion. The prevalence is a function of incidence and survival/cure rate in combination and is often used in cross-sectional studies where time is not considered in the same sense as in an incidence estimate. However, as with any proportion, it is important to specify the time point at which the measurement is taken.

6.2.3 Cancer registers

Pioneers in cancer registration first started to catalogue cancer in the mid-20th century and currently, more than 200 cancer registers exist worldwide. The International Association of Cancer Registries was founded in 1966 and continuously works for a standardized data collection procedure to improve comparability between registers of different regions. The purpose of cancer registers has traditionally been to collect data on new cancer cases in the population and to produce basic statistics about cancer occurrence. However, modern authorities have highlighted the synergistic effects of cancer control programs in parallel with cancer registers and made arguments that the role of cancer registers could be expanded.

In Sweden, the National Board of Health and Welfare maintains the national cancer register. Incidence and survival statistics are presented annually and reports of changes in cancer occurrence over time are presented continuously. Additionally, the cancer register in Sweden has been used increasingly in epidemiological research over the past two decades and the quality of the register is considered high.(6) However, the number of academic studies dedicated to evaluating the quality of data in the cancer registers remains surprisingly low.

6.2.4 Incidence of biliary tract cancer

The relative rarity of biliary tract cancer was recognized originally in the 19th century and has been confirmed by more recent studies.(4, 9) According to the Swedish Cancer Register, approximately 350 patients are diagnosed with biliary tract cancer annually in Sweden, and the incidence rate was 3.5 cases/ 100,000 person-years in 2014.(3) The majority of diagnosed biliary tract cancers are gallbladder cancers, which are known to be more frequent in women

than men.(8) The incidence rates of biliary tract cancer have been decreasing in Sweden over the past 25 years.(3)

Internationally, the incidence of biliary tract cancer is a matter of debate. There is a growing body of literature emphasizing the importance of distinguishing the sub-sites of biliary tract cancers in analyzing cancer incidence.(4, 106, 107) One recent American study based on data from the "Surveillance, Epidemiology and End Results" program showed decreasing incidence trends of gallbladder cancer and increasing incidence trends of extra-hepatic tumors.(108) Another American study based on different data showed a similar result. (109) The incidence of Ampullary cancers specifically, is also decreasing in Europe and in the US. (76, 110) Furthermore, a female predominance in gallbladder cancer has been shown in most parts of the world.(107, 111) European studies have often reported a decreasing incidence of gallbladder cancer, whereas the trends for extra-hepatic tumors are somewhat less clear. A number of European studies have shown stable or slightly decreasing incidence of cancers of the extra-hepatic bile ducts, excluding gallbladder cancer, whereas some have shown increasing trends (112-116) Interestingly, one report has shown an increasing incidence of biliary tract cancer in parts of Asia.(117) However, that study was based on data from Shanghai in China, an area which may have become more like Western countries over the past 30 years due to political/financial changes and may not accurately represent the incidence trends for the whole of China.

The reasons for the seemingly decreasing incidence trends of gallbladder cancer are not well understood but have been attributed to the increasing use of cholecystectomy.(4, 24) However, evidence of a causal relationship between a more extensive use of cholecystectomy and decreased incidence of gallbladder cancer remains scarce, and the decline in gallbladder cancer incidence started before the introduction of the laparoscopic cholecystectomy.(8)

Some reports have highlighted misclassification of biliary tract cancers as a possible explanation for differences in incidence trends between registers. (62, 106) However, the National Board of Health and Welfare in Sweden have guidelines directing the diagnosis in cases where anatomical origin and location is difficult to establish.(118) Yet, the potential differences between tumor locations are important to acknowledge in etiological research to account for potential biological differences between tumors of the biliary tract.

6.3 REPRODUCTIVE FACTORS AND HORMONES

6.3.1 Sex hormones

Sex hormones are steroid derivatives involved in numerous physiological functions in the human body. Typically, estrogen and progesterone are considered female sex hormones and have a primary regulatory role in the menstrual cycle, but are also important in energy homeostasis and bone metabolism.(119) Sex hormones are synthesized from cholesterol precursors and are regulated by complex feed-back and feed-forward mechanisms involving the hypothalamus and the pituitary gland. The levels of estrogen and progesterone vary considerably over time, making measurements of hormone levels difficult to interpret in the context of accumulated exposure. Therefore, proxies have been used extensively in academic research as indicators of hormone exposure.

6.3.2 Endogenous sex hormones

Endogenous sex hormones originate from within the body itself in contrast to exogenous hormones which are added to the body from external sources. “Reproductive factors” is a term often used to describe a number of states or events associated with reproduction, such as age at menarche, number of children or pregnancies (parity), age at the birth of the first child, age at menopause, and the occurrence of breast feeding. Reproductive factors have been used as proxies for endogenous hormone exposure in a variety of studies previously. The rationale behind using reproductive factors as proxies for hormone exposure is based on the changes in estrogen and progesterone levels women experience during these events. (120) Estrogen levels naturally increase during pregnancy to extreme levels.(121) In theory, multiparous women (women with more than one child) have been exposed to a higher accumulated dose of estrogen over time, compared to a woman with no children. In parallel, estrogen levels naturally fall after menopause, and the accumulated estrogen exposure in women experiencing early menopause may be lower compared to women with a more normal menopausal age.(122) In Sweden, the age at natural menopause has been estimated at around 50 years, and similar findings have been reported in other European countries.(123-125)

6.3.3 Exogenous sex hormones

Hormone therapy is used to treat numerous conditions in modern medicine, ranging from contraceptive therapy and menopausal hormone supplementation, to treatment of certain types of cancers. The regimens and compounds vary with indication but a disruption of the normal hormone homeostasis is the end effect regardless of treatment regimen. An inhibitory effect is sometimes desired, which is the case of breast cancer treatment, where estrogen

receptor blockers or estrogen synthesis inhibitors are applied to inhibit cancer growth in estrogen-dependent tumors.(126) In parallel with the treatment of breast cancer, the treatment of prostate cancer with estrogens to achieve androgen deprivation was among the pioneers in hormonal cancer treatments. High doses of estrogen were the mainstay treatment for prostate cancer until the late 1970's, when luteinizing hormone releasing hormone agonists were introduced.(127) The therapeutic arsenal available in the modern treatment of prostate cancer has improved significantly since then to include operative, medical and radiation-based techniques making high dose estrogen regimens infrequent.(128)

One group of patients still exposed to exogenous sex hormones are women with symptoms related to hormone deficiency. Menopausal hormone therapy is indicated to relieve vaginal atrophy and vaso-motor symptoms in menopausal women, but also as prophylaxis against osteoporosis in certain subsets of women.(129) The use of menopausal hormone therapy has decreased internationally over the past decades, likely because of the many studies indicating an increased risk of breast cancer and cardio-vascular disease in women exposed to hormone therapy.(130) Furthermore, the risk of ovarian and endometrial cancer may be increased.(131, 132) On the contrary, some studies have shown a protective effect of menopausal hormone therapy on certain gastrointestinal cancers, such as colon and gastric cancer.(133, 134) Also, menopausal hormone therapy increases the risk of developing gallstone disease and cholecystitis at standard therapeutic doses.(135)

6.3.4 Sex hormones in biliary tract carcinogenesis

Biliary tract cancer, specifically gallbladder cancer, is more common in women than men and a role of estrogen has been suggested as an explanation for this sex difference.(136, 137) Previous reports have often investigated the effect of endogenous estrogen exposure in women using reproductive factors as proxies. The reproductive factors parity and age at the birth of the first child have been used previously in medical research as surrogate measures of endogenous estrogen exposure.(138, 139) Previous investigations of the association between reproductive factors and biliary tract cancer as a whole entity, have reported conflicting results. A Norwegian study found no statistically significant association between parity and biliary tract cancer and an Italian study showed a non-significant association, but also failed to show any association between age at the birth of the first child and biliary tract cancer. (140, 141) Studies investigating the association between reproductive factors and gallbladder cancer specifically have been more consistent, showing an increased risk with increasing parity.(70, 142-147) Similarly, older age at the birth of the first child has also been shown to be inversely associated with gallbladder cancer.(70, 146, 147) One study investigated the

effect of age at the last birth and found a positive association with gallbladder cancer.(143) Investigations in cancers of the extra-hepatic bile ducts excluding gallbladder cancer have failed to show any statistically significant associations.(57, 70, 147)

Early menarche, late menopause and menopausal status have been shown to increase the risk of biliary tract cancer in some studies, supporting the "hormonal hypothesis".(57, 137, 143, 147, 148) However, some studies have failed to confirm the results.(141, 149) Some studies even reported later menarche to be a risk factor for gallbladder cancer. (57, 145, 147) It is possible that differences in study design, study population, or classification of exposure or outcome may have contributed to the inconsistencies.

The risk of developing biliary tract cancer following exogenous hormone exposure has not been studied to a great extent and the available literature does not provide a coherent picture. Some studies have demonstrated an increased risk of biliary tract cancer after hormone replacement therapy for menopausal symptoms or oral contraceptives.(150, 151) However, opposite results have been observed in some studies and some studies have failed to show any clear associations whatsoever.(57, 146, 152-154) Failure to discriminate between gallbladder cancer and other extra-hepatic tumors may in part account for the differences between studies. Furthermore, it has been shown that exogenous hormone exposure modulates bile composition and increases the risk of cholelithiasis, benign gallbladder disease and biliary tract surgery, suggesting a pathway for biliary tract carcinogenesis by exogenous estrogen exposure via gallstone disease.(155-157)

Investigations in the molecular changes in biliary tract cancer have provided further proof of a role of sex hormones in the etiology of biliary tract cancer. It is known that estrogen-mediated processes regulate cholangiocyte (cells of the bile duct) proliferation under physiological conditions and more recently, that estrogen is important in the pathophysiology of the bile ducts in biliary tract cancer.(158) In vitro studies have shown that estrogen exposure increases cancer cell growth in bile duct cancer cells and that Tamoxifen (an estrogen receptor antagonist) inhibits cancer cell growth.(159, 160) Furthermore, histological evaluations of gallbladder cancer tissues have revealed increased expression of estrogen receptors, and anti-hormonal therapies have been suggested as a potential treatment target for biliary tract cancer.(161) Moreover, genetic alterations in genes involved in estrogen regulation have been associated with the risk of biliary tract cancer.(162, 163)

7 AIMS OF THIS THESIS

The overall aims of this thesis were to clarify the incidence of biliary tract cancer in Sweden and to investigate the role of sex hormone exposure in biliary tract cancer etiology.

The specific aims were:

- To investigate the completeness of the Swedish Cancer Register regarding biliary tract cancer.
- To evaluate the incidence trends of biliary tract cancer in Sweden.
- To clarify if parity or age at the birth of the first child (proxies for endogenous sex hormone exposure) influence the risk of biliary tract cancer.
- To examine the risk of biliary tract cancer in men exposed to exogenous estrogen during prostate cancer treatment.
- To assess the association between menopausal hormone therapy and the risk of biliary tract cancer.

8 DATA SOURCES AND METHODS

8.1 OVERVIEW

Table 1. Overview of materials and methods used in Studies I-V.

	Study I	**Study II**	**Study III**	**Study IV**	**Study V**
Study design	Population-based validation study	Population-based incidence study	Population-based case-control study	Population-based cohort study	Population-based cohort study
Data sources	The Swedish Cancer Register, the Swedish Patient Register and the Swedish Causes of Death Register	The Swedish Cancer Register, the Swedish Patient Register and the Swedish Causes of Death Register	The Swedish Cancer Register, the Swedish Patient Register, the Swedish Causes of Death Register, the Multi-Generation Register and the Register of the Total Population	The Swedish Cancer Register, the Swedish Patient Register, the Swedish Causes of Death Register and the Register of the Total Population	The Swedish Cancer Register, the Swedish Patient Register, the Swedish Causes of Death Register and the Prescribed Drug Register
Source population	All Swedish residents	All Swedish residents	Individuals in the Multi-Generation Register	Men with prostate cancer	Swedish women in the Prescribed Drug Register
Time period	1990-2010	1970-2010	1960-2008	1961-2008	2005-2012
Exposure	N/a	N/a	A) Parity B) Age at the birth of the first child	Prostate cancer treatment	Menopausal Hormone Therapy
Outcome	Biliary tract cancer	A) Biliary tract cancer B) Gallbladder cancer	A) Biliary tract cancer B) Gallbladder cancer C) Cancers of the extra-hepatic bile ducts/Ampulla	A) Gallbladder cancer B) Cancers of the extra-hepatic bile ducts/Ampulla	A) Gallbladder cancer B) Cancers of the extra-hepatic bile ducts/Ampulla
Statistical methods	Frequency distributions and relative proportions	Standardized incidence rates and join-point regression	Conditional logistic regression	Standardized Incidence Ratios	Conditional logistic regression and Cox Proportional Hazards models

8.2 DATA SOURCES

The five studies that are included in this thesis are based on data from Swedish national health-care registers. Record linkage between the registers was made possible by the use of the Swedish personal identity number uniquely identifying all Swedish residents.(164) In all the included studies, the patient data have been made anonymous to adhere to patient integrity regulations.

8.2.1 The Swedish Cancer Register

The Cancer Register was established in 1958 and collects data on newly diagnosed cancers in Sweden. The register is kept by the National Board of Health and Welfare. Since the 1980's however, newly diagnosed cancers are typically reported to one of six regional cancer registers, spread out across Sweden, where each case undergoes routine controls and subsequent data registration. Thereafter, in October each year, the regional cancer registers submit the registered data from the preceding year to the national register at the National Board of Health and Welfare.(165) The regional centers use structured procedures for inputting new data and perform basic operations to ensure the correctness of the data. There is no direct communication between the Cancer Register and other health-care registers such as the Patient Register or the Causes of Death Register. One important implication of this lack of communication is that a cancer diagnosis reported in one of these other register will not automatically be registered in the Cancer Register. This characteristic has important implications for academic research comparing the registers to one another. However, health-care practitioners in Sweden are required by law to report newly diagnosed malignant tumors and some benign tumors to the register regardless of the foundation of the diagnosis. Additionally, pathology departments are also required to report tumors diagnosed during histopathological and cytological examinations of specimens collected from tissue sampling. Thus, in the case of histologically verified cancers, newly reported cases should have two independent registrations.

The Cancer Register contains data about the cancer diagnosis according to the 7th version of the International Classification of Diseases (ICD), date of diagnosis, tumor stage, histopathology (when collected), foundation of the diagnosis, patient characteristics and information about the health-care facility where the diagnosis was made.

Recently, concerns that clinicians less often report new cancer cases unless verified by histopathology have been raised.(166) However, histopathological confirmation is not a requirement for registration in the Cancer Register. Under-reporting of cancer could seriously

influence the completeness of the data in the Cancer Register and thereby its usability in epidemiological research. The Cancer Register has been evaluated in only a handful of studies. Mattson et al. reported a non-reporting of less than 2% in the latter part of the 1970's in a comparison with death certificates.(167) A more recent study showed that the overall completeness is high (96%), but also that existing under-reporting seems to be site-dependent and also increases with age.(6)

8.2.2 The Swedish Patient Register

The Patient Register was first established in the 1960's to collect data on in-patient care, psychiatric care in particular. The scope of the register was extended and became nationwide, capturing all in-patient care, from 1987 onwards. Furthermore, data from specialized out-patient visits from public and private health care providers are also available from 2001 onwards. The data collected by the register includes discharge diagnoses according to the current version of the ICD, surgical procedures, patient characteristics (age, sex etc.) and administrative information, such as data about the health care provider and date of admission.(169) The quality and reliability of the Patient Register has been evaluated by the National Board of Health and Welfare and others, and the positive predictive value of a diagnosis in the Patient Register is approximately 90%.(170)

8.2.3 The Swedish Causes of Death Register

The Causes of Death Register collects information about all deaths among Swedish residents and even though the collection of mortality statistics in Sweden started much earlier, the register in its current shape was established in the 1960's. The register is maintained by the National Board of Health and Welfare and is believed to cover more than 99 % of all Swedish residents.(171) Every person registered as a Swedish resident at their time of death should, at their time of death, be reported to the Causes of Death Register, regardless of the place of death. The data in the register are based on death certificates, containing not only patient data but also primary and underlying causes of death. Typically, it is the responsibility of the physician who acknowledges the death to sign and submit the death certificate after making an ample evaluation of the cause of death. The physician specifies the immediate cause of death and potential underlying causes of death in the certificate. The quality of the register is, to a large extent, determined by two key factors intimately linked to the production of the death certificate, the rate of non-reporting and the correctness and completeness of the death certificates. Firstly, the rate of non-reporting is low, but has increased from less than 1% in the 1980's to almost 3% in 2008. Moreover, less than 1% of all death certificates are

incomplete or inadequately specified. Secondly, there is substantial variation in the quality of the issued death certificates. Malignant tumors, specifically, have been found to be correctly specified as the cause of death in around 90% of all cases.(171)

The reliability of cause of death statistics is sometimes suggested to correlate with the autopsy rate. In Sweden, the autopsy frequency has decreased from around 50% in the 1970's to only 12% in 2009. However, improvements in diagnostic instruments, such as laboratory tests and imaging, may allow for a reliable diagnosis to be made without the need for a clinical autopsy. Therefore, it is not likely that the quality of statistics concerning causes of death have decreased to the same extent as the autopsy rates.(171)

8.2.5 The Register of the Total Population and the Multi-Generation Register

The Register of the Total Population is one of the major population registers available in Sweden and is maintained by Statistics Sweden. All Swedish residents are included in the register and are identified by the personal identity number. Data such as birth date, sex, marital status, birth country, educational level, and migration can be extracted from the register. The register is of high overall quality and completeness should not be a major concern for the use of the register in most epidemiological research.(172) The Multi-Generation Register is a subset of the Total Population Register and contains data on the family connection between parents and offspring. The number of children and age at the birth of each child can be extracted from the register. The register contains data on more than 9 million individuals and the coverage of parenthood is more than 98 % from 1961 onwards.(173) Due to lack of data for some time periods, individuals born in 1932 who died before 1961 may be missing from the register.

8.2.6 The Swedish Prescribed Drug Register

The Prescribed Drug Register was established in 2005 and has since then collected information about all dispensed drugs in Sweden. The register is maintained by the National Board of Health and Welfare. According to Swedish law, pharmacies are required to submit data regarding dispensed drugs to the Electronic Health Authorities, where a basic control of the data is completed. The data are submitted electronically and the information is then submitted to the National Board of Health and Welfare for entry into the register. Data submission occurs on a monthly basis and includes the dispensed item and dosage, dispense date, the personal identity number, the Anatomical Therapeutic Chemical (ATC) code of the drug etc. Over-the-counter drugs and drugs used in in-patient care are not included in the register.(174)

8.3 STUDY DESIGN AND METHODS

8.3.1 Study I

To evaluate the completeness of the Cancer Register, Study I compared biliary tract cancer records in the Cancer Register with biliary tract cancer records in the Patient Register between 1987 and 2010. Three sub-populations were thus created:
1) patients with records in both registers (CR-and-PR);
2) patients with records in the Patient Register only (PR-not-CR) and;
3) patients with records in the Cancer Register only (CR-not-PR).

Furthermore, the cause of death, assessed from the Causes of Death Register, were compared with the cancer records in the three sub-populations to evaluate the accuracy of biliary tract cancer diagnoses. Biliary tract cancer was identified by the ICD-7 codes 1551-1559. Biliary tract cancer cases in the Cancer Register with a diagnosis based on an unexpected finding on autopsy records only were excluded because of the unlikeliness that these patients would have a concordant entry in the Patient Register. Further exclusions were made for biliary tract cancer diagnosed before the study period and a registered death before cancer diagnosis. Case registration in each register is independent of registration in the other two.

8.3.2 Study II

Building on the results of the first study, Study II assessed the incidence and mortality trends of biliary tract cancer in Sweden between 1970 and 2010 using the Cancer Register, the Patient Register and the Causes of Death Register. For the Patient Register specifically, the data acquisition was limited to the years 1987-2010 because of the incomplete national coverage of the register before 1987. Cancer of the extra-hepatic bile ducts was identified by the diagnosis codes 155.2, 156.11, 156B and C24.0 in the ICD-7, ICD-8, ICD-9 and ICD-10, respectively. Corresponding codes identifying ampullary cancer were 155.3, 156.21, 156C, C24.1, and for gallbladder cancer the codes 155.1, 156.01, 156A and C23.9 were used. Furthermore, the codes 155.8-155.9 156.99, 156W-X and C24.8-9 denoted biliary tract cancer without clear origin or with extensive growth.

8.3.3 Study III

Study III was a case-control study, nested with the Multi-Generation Register, investigating the association between reproductive factors and biliary tract cancer. The Cancer Register, Patient Register, Causes of Death Register and the Register of the Total Population provided data of cancer diagnoses, comorbidities, date of death, educational level and migration status,

respectively. The study period was between 1960 and 2008. Previously cancer-free women and men, above 15 years of age, with a first diagnosis of biliary tract cancer identified in the Cancer Register were considered cases. Biliary tract cancer was denoted by the ICD-7 codes 1551-1553 (Table 2). Furthermore, only adenocarcinomas, denoted by the histology code 096 (WHO C24.1 coding system) were included to ensure uniform tumor biology. Ten age- and sex-matched controls were randomly selected for every incident case using density-based sampling.(175) Subjects with a history of cholecystectomy were not eligible as controls for the gallbladder cancer specific analysis. To be eligible, controls had to be resident in Sweden, alive at the time of the cancer diagnosis of the corresponding case and have no previous history of gastro-intestinal malignancy. Parity (number of children) and age at the birth of the first child were the study exposures and were used as proxies for endogenous sex hormone exposure. The study end-points were biliary tract cancer, death, migration, or end of study period, whichever occurred first. Women and men were included, but analyzed separately.

8.3.4 Study IV

This was a population-based cohort study that investigated the risk of biliary tract cancer in a cohort of men exposed to exogenous estrogen. The cohort consisted of men with a histologically verified diagnosis of prostate cancer between 1961 and 2008 identified in the Cancer Register. The ICD-7 code 177 identified prostate cancer. Prostate cancer diagnosis was used as proxy for exogenous estrogen exposure. The cohort was *a priori* divided into two groups, based on time period, to account for the changes in prostate cancer treatment occurring during the study period. Before 1980, prostate cancer was typically treated with high-dose estrogen regimens to achieve androgen deprivation, whereas less estrogen-heavy treatments gained ground after 1980.(127, 128) The cohort was thus split into an early, and more estrogen exposed group (1961-1980), and a later, and less estrogen exposed group (1981-2008). The outcome was histologically verified adenocarcinoma of the biliary tract cancer, identified in the Cancer Register by the ICD-7 codes 1551-1553 and the histology code 096 (WHO C24.1). The risk of biliary tract cancer in the two periods was compared to the risk in the general male population. Potential confounding factors, death and migration were assessed from the Patient Register, the Causes of Death Register and the Register of the Total population, respectively.

8.3.5 Study V

Study V was a cohort study investigating the association between menopausal hormone therapy and biliary tract cancer in Swedish women between 1st of July 2005 and 31st of

December 2012. The cohort was based on data from the Prescribed Drug Register and included all women exposed to menopausal hormone therapy between 1st of July 2005 and 31st of December 2011, and women identified in the Prescribed Drug Register without such therapy. The register contains information about more than 90% of all Swedish women between 40 and 64 years of age, and more than 95% of women aged 65 years or more. Women with a history of cancer prior to the start of the study, assessed form the Cancer Register, were excluded.

The cohort was initially constructed using a comprehensive matching procedure, first on group level at a 1:3 ratio. Thus, 3 unexposed women were selected for each exposed woman. Study participants were matched exactly for three variables: history of delivery (yes/no), hysterectomy (yes/no) and history of thrombotic events (yes/no), producing eight strata. Thereafter, additional matching for age (year of birth), diabetes (yes/no), alcohol-related disease (yes/no), obesity (yes/no), and smoking-related disease (yes/no), using the nearest neighbor strategy were performed within each stratum. The extensive matching, even for factors not strongly related to hormone therapy and biliary tract cancer was undertaken to capture effects of potential confounding from unmeasured factors. Matching variable status were assessed from the Patient Register. ICD-codes for the matching variables are presented in Table 2.

Women with at least one prescription of a drug used for systemic menopausal hormone therapy were considered exposed. Women exposed to hormone therapy before 40 years of age before the start of the study were excluded. The ATC codes "G03C" and "G03F" were used to identify estrogen containing drugs used for treatment of menopausal symptoms. Only systemic (oral or transdermal) preparations were considered. The cohort was followed-up for adenocarcinoma of the biliary tract, assessed from the Cancer Register. Gallbladder cancer and cancers of the extra-hepatic bile ducts and the Ampulla were identified by the corresponding ICD-10 codes (Table 2). Adenocarcinoma was denoted by the code "096" (WHO C24.1 definition). For gallbladder cancer specifically, women with cholecystectomy prior to the start of the study were excluded because they would not be at risk.

Secondly, an unmatched cohort was constructed using the same data sources as described above, but the matching procedure was omitted. All women identified in the Prescribed Drug Register were included in the unmatched cohort. The outcome was classified according to the ICD-7 (Table 2). Exclusions were the same as for the matched cohort.

Table 2. Diagnoses codes of variables used in Studies I-V, by ICD version.

Variable	ICD-7	ICD-8	ICD-9	ICD-10
Outcomes				
Gallbladder cancer	1551	156,01	156A	23.9
Cancers of the extra-hepatic bile ducts	1552	156,11	156B	24.0
Ampullary cancer	1553	156,21	156C	24.1
Biliary tract cancer, other	1558-9	156,99	156W-X	24.8-9
Matching/confounding variables				
Delivery	660'-678'	.	.	.
Diabetes mellitus	260'	250'	250'	E10.0-E11.9
Obesity	287'	277'	278'	E65.9-E66.9
Primary sclerosing cholangitis	Not defined	Not defined	Not defined	K83.0A
Alcohol-related disease	307', 322.0-322.2, 581.1	291.1-3, 291.9, 303.0-303.2, 303.9, 571.0	303', 305A, 357F, 425F, 535D, 980', 291', 571A-571D	T51.9, F10', G31.2, K29.2, K70', K86.0, R78.0, Z71.4, K85.2, E24.4, G62.1, G72.1, I42.6
Smoking-related disease	450', 451', 501.99-505.00, 527.10-527.11	440', 441', 443.9-445, 490.9-491, 492	440', 441', 557B, 490', 491'	I70', I71', I73, K55.1, J41', J42', J43', J44
Gallstone disease	584'	574'	574'	K80', K85.1, K56.3, K81
Thrombotic events	.	451', 452', 453', 450'	451', 452', 453', 415'	I80', I81', I82', I26'
Surgical procedures				
Cholecystectomy	.	.	5350-5359 [a]	JKA20, JKA21 [a]
Hysterectomy	.	.	7210-7228 [b]	LCD00-97 [b]

[a] 6th classification of surgical procedures (1963-1996), [b] KVÅ97 (1997-)

8.4 STATISTICAL ANALYSES

8.4.1 Study I

Firstly, relative proportions in the three sub-populations were calculated as a measure of concordance between the Cancer Register and the Patient Register. The cancer date in the Cancer Register was used as the index date. In cases reported to the Patient Register only, the date of first admission with cancer as the discharge diagnosis was used as the index date.

Secondly, the results were stratified by sex, age group (<60, 60-69, 70-79, or ≥80 years of age), and 5-year calendar periods (1990-1994, 1995-1999, 2000-2004, or 2005-2009). The analyses were restricted to 1990 and onwards to ensure that each date of cancer occurrence was the first reported cancer diagnosis in the Patient Register.

Thirdly, the concordance between the cause of death and biliary tract cancer diagnosis in the three sub-populations was calculated and the analysis was stratified for number of hospital discharges with a biliary tract cancer diagnosis (1,2,3,4, or ≥5). Cause of death was categorized into seven groups: pancreatic cancer, biliary tract cancer, esophageal or gastric cancer, liver cancer, metastatic or advanced cancer, non-gastrointestinal, and non-malignant disease.

Lastly, the survival probability in relation to the time since biliary tract cancer diagnosis was calculated for the three sub-populations.

8.4.1 Study II

Annual incidence and mortality rates of biliary tract cancer per 100,000 individuals between 1970 and 2010 were calculated. Rates were age-specific and age-adjusted. For the Patient Register, incidence rates were calculated from 1990 onwards, to prevent inclusion of prevalent cases before the register became nationwide. Each year's age distribution was used in the denominator and incidence/mortality rates were standardized using the Swedish background population in 2010 as a reference. Analyses were made for biliary tract cancer as a whole, but also for gallbladder cancer separately.

The results from the Cancer Register were further stratified on the basis of the diagnosis and which were categorized into five groups:

1. Radiology-based;
2. histopathology after biopsy;
3. cytology-based;
4. autopsy findings;
5. surgery/autopsy without histopathology, clinical or laboratory findings.

To analyze trends of incidence/mortality over time, log-linear joinpoint regression was used to calculate Annual Percent Change (APC) with 95% Confidence Intervals (CI) to detect statistically significant trends changes.

8.4.1 Study III

Conditional logistic regression was used to calculate Odds Ratios (OR) with 95% CI to estimate the association between reproductive factors and biliary tract cancer. Cancer of the extra-hepatic bile ducts, ampullary cancer and gallbladder cancer were analyzed separately.

Parity was categorized into four groups: 0, 1, 2, or ≥3 and subjects with one child were used as reference category. The age at the birth of the first child was categorized into three groups: ≤22, 23-29, or ≥30 years of age and subjects aged 22 years or less at the birth of the first child were used as the reference category.

The statistical model included the matching variable age and further adjusted for comorbidities (binary), country of birth (Sweden or outside Sweden), and level of education (four categories: elementary school, secondary school, university or missing). Comorbidities included diabetes, alcohol abuse, and smoking-related diseases. Only a very small number of cases (eight in total) had a history of primary sclerosing cholangitis and therefore no adjustment was therefore performed for this condition.

To address any potential effect of menopausal status, the analyses were stratified for age at cancer diagnosis/index date. Thus, study subjects aged less than 50 years were compared with subjects aged 50 years or more. However, because of the limited sample size, the effect of menopausal status was analyzed for gallbladder cancer only.

8.4.1 Study IV

Standardized incidence ratios (SIR) with 95% CI were calculated as the ratio of the observed to expected number of newly diagnosed biliary tract cancers. The expected number of cancers was the product of the age- and calendar year-specific incidence rate and the observed number of person-years in the Swedish male population, using five-year intervals. The two groups of the cohort were followed until biliary tract cancer diagnosis, emigration, death, or the end of the study period; whichever occurred first. To reduce potential surveillance bias, the first year of follow-up was discarded.

Cancers of the extra-hepatic bile ducts and ampullary cancer were analyzed as one entity and gallbladder cancer was analyzed separately. For the gallbladder cancer analysis specifically, cases with a history of cholecystectomy prior to the start of the study were excluded. Furthermore, cholecystectomy represented an additional outcome.

To evaluate a potential effect of confounding factors, sensitivity analyses excluding individuals with obesity, diabetes, primary sclerosing cholangitis, alcohol related disease, and gallstone disease without record of cholecystectomy were performed.

Finally, to address any effect of a prolonged exposure, the analyses were stratified by latency time between diagnosis of prostate cancer and biliary tract cancer. Latency time was categorized into two groups: >1-5 years and >5 years.

8.4.1 Study V

The matched cohort

The association between menopausal hormone therapy and biliary tract cancer in the matched cohort was evaluated using conditional logistic regression models, calculating an OR with 95% CI. The cohort was followed-up for adenocarcinoma of the biliary tract. Gallbladder cancer was analyzed separately and cancers of the extra-hepatic bile ducts were analyzed together with ampullary cancers. End-points were biliary tract cancer, end of follow-up, death or diagnosis of a cancer other than biliary tract cancer, whichever occurred first.

The exposure, menopausal hormone therapy, was categorized as a binary variable (ever/never), i.e. prevalent users at the start of follow-up in 1 July 2005 and women ever exposed to menopausal hormone therapy during the follow-up were included. The analyses were further stratified for the type of regimen (estrogen only or combination regimen).

The multivariable logistic regression model accounted for the variables used in the matching procedure, which is appropriate if the models are adjusted for factors other than the matching variables.(176) The model was further adjusted for osteoporosis.

Additionally, to evaluate the effect of gallstone disease on the association between hormone therapy and gallbladder cancer, individuals with a history of gallstone disease prior to the start of the study were excluded. Additionally, gallstone disease was used as an outcome in a second regression model to assess the association between hormone therapy and gallstone disease.

The unmatched cohort

In the unmatched cohort, the association between biliary tract cancer and menopausal hormone therapy was assessed using Cox proportional hazard models, providing hazard ratios (HR) with 95 % CI. Person-years were calculated from the start of the study until registered biliary tract cancer, end of follow-up (December 31st 2012), or death, whichever occurred

first. Moreover, cohort members were censored if diagnosed with a malignancy other than biliary tract cancer. For gallbladder cancer specifically, date of cholecystectomy represented an additional censor point. The proportional hazards assumption was tested on the basis of the Schoenfeld residuals. There was no deviation from proportional hazards noted.

The exposure was categorized as ever/never and was included as a time-dependent variable to account for changes in exposure over time. The analyses were stratified according to the type of MHT regimen, either estrogen only or estrogen/progestogen combinations. The multivariable analyses were adjusted for age (year of birth), diabetes (yes/no), primary sclerosing cholangitis (yes/no), hysterectomy (yes/no) and gallstone disease (yes/no) (Table 2). Gallstone disease was included as a covariate to address the effect of hormone therapy on biliary tract cancer, accounting for the expected increased frequency of gallstone disease in the exposed. To make detection of all outcomes possible, biliary tract cancers diagnosed within a 90-day window after cholecystectomy were considered in the analyses.

8.5 SOFTWARE FOR STATISTICAL ANALYSES

Statistical analyses of Studies I, III and IV were performed using SAS Statistical Package, version 9.2 (SAS Institute Inc., Cary NC, USA.)

Statistical analyses for Studies II and V were performed using STATA 13 statistical software (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP.).

Joinpoint analysis in Study II was performed using SEER*Stat (Surveillance Research Program, National Cancer Institute SEER*Stat Software [seer.cancer.gov/seerstat] version 4.3.1.0.

8.6 ETHICAL CONSIDERATIONS

The Regional Ethical Review Board in Stockholm has approved all studies in this thesis. Studies I to V are strictly register-based and there has been no contact with individual patients or control subjects. All data about the study subjects have been made anonymous before presented to the researcher's making reverse identification of study participants impossible. All results are presented at group level to prevent any identification of the study participants. Data storage, management and analyses have been performed on firewall- and password protected servers at Karolinska Institutet, and located in secure offices accessed by key card and password.

9 RESULTS

9.1 STUDY I

9.1.1 Concordance between the Cancer Register and the Patient Register

In total, 14,273 biliary tract cancer cases were identified in the Cancer Register and/or the Patient Register (Figure 1 and Table 3). Overall, 44% (6,303) of the study subjects were identified in the Patient Register only. Correspondingly, 48% (6,799) of the study subjects were identified in both registers. A total of 8% (1,171) were identified in the Cancer Register only. The vast majority of the biliary tract cancer cases (92%) were identified in the Patient Register.

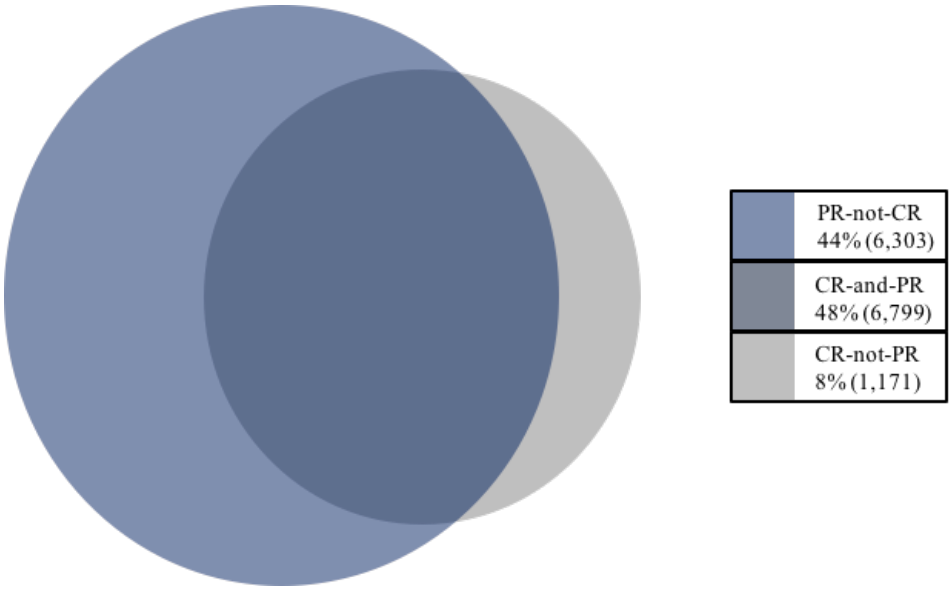

Figure 1. Distribution of biliary tract cancer cases in the Cancer Register (CR) and the Patient Register (CR) between 1990 and 2010. PR-not-CR: Cases in the Patient Register only. CR-and-PR: Cases in both registers. CR-not-PR: Cases in the Cancer Register only.

Biliary tract cancer patients were more often women, and women were somewhat more frequently reported to the Cancer Register (58%) compared to men (52%) (Table 3). Additionally, most of the study subjects were 70 years or older at the time of cancer diagnosis (63%). In parallel, subjects aged 80 years or more were less often reported to the Cancer Register (46%) compared to subjects aged less than 60 years (59%). The absolute number of new cases was relatively stable over the study period but only 43% of study subjects diagnosed between 2005 and 2009 were registered in the Cancer Register, compared to 73% diagnosed between 1990 and 1994.

Table 3. Number of patients with biliary tract cancer diagnosis in the Swedish Cancer Register (CR) and the Swedish Patient Register (PR) between 1990 and 2009.

	Total	PR	CR	CR-and-PR	PR-not-CR	CR-not-PR
Total, (%)	14,273 (100)	13,102 (92)	7,970 (56)	6,799 (48)	6,303 (44)	1,171 (8)
Sex, (%)						
Male	5,348 (100)	4,899 (92)	2,786 (52)	2,337 (44)	2,562 (48)	449 (8)
Female	8,925 (100)	8,203 (92)	5,184 (58)	4,462 (50)	3,741 (42)	722 (8)
Age, (%)						
<60	2,051 (100)	1,886 (92)	1,218 (59)	1,066 (52)	833 (41)	152 (7)
60-69	3,207 (100)	2,944 (92)	1,995 (62)	1,732 (54)	1,212 (38)	263 (8)
70-79	4,863 (100)	4,425 (91)	2,849 (59)	2,419 (50)	2,014 (41)	430 (9)
≥80	4,152 (100)	3,847 (93)	1,908 (46)	1,582 (38)	2,244 (54)	326 (8)
Time period, (%)						
1990-1994	3,454 (100)	2,989 (87)	2,515 (73)	2,072 (60)	939 (27)	443 (13)
1995-1999	3,428 (100)	3,161 (92)	2,030 (59)	1,758 (51)	1,398 (41)	272 (8)
2000-2004	3,650 (100)	3,413 (94)	1,804 (49)	1,572 (43)	1,846 (51)	232 (6)
2005-2009	3,741 (100)	3,539 (95)	1,621 (43)	1,397 (37)	2,120 (57)	224 (6)

PR-not-CR: Cases in the Patient Register only. CR-and-PR: Cases in both registers. CR-not-PR: Cases in the Cancer Register only.

9.1.2 Concordance between the Cancer Register, the Patient Register and the Causes of Death Register

Altogether, 93% of all study subjects died during the study period and the distribution was similar in all three sub-populations (Table 4). The overall concordance between biliary tract cancer diagnosis and biliary tract cancer as cause of death in the sub-population identified in both registers was 84%. Corresponding agreement for the sub-populations reported to the Patient Register or the Cancer Register only was 59% and 51%, respectively. The concordance between biliary tract cancer diagnosis and biliary tract cancer as the cause of death, increased with an increasing number of hospital discharges with a diagnosis of biliary tract cancer in both sub-populations, where such analysis was possible (Table 4). For the sub-population identified in both registers, the concordance increased from 81% in subjects with only one hospital discharge, to 87% in subjects with five or more discharges. Corresponding concordance for the sub-population in the Patient Register only increased from 50% only in subjects with one hospital discharge to 68% in subjects with five or more such discharges.

Approximately 80% of all study subjects died within the first year of cancer diagnosis (Figure 2). The probability of survival in the sub-population reported to both the Cancer Register and

the Patient Register was higher compared to the sub-populations reported to only one of the two registers, but decreased over time.

Table 4. The concordance between biliary tract cancer diagnosis in the Swedish Cancer Register, the Swedish Patient Register, and the Causes of Death Register, stratified by number of hospital discharges with a biliary tract cancer diagnosis between 1990 and 2010.

Sub-population	Total	Dead	Death in biiary tract cancer
PR-not-CR (%)	6,303	5,833 (93)	3,438 (59)
Number of hospital discharges with biliary tract cancer diagnosis (%)			
1	3,020	2,755	1,391 (50)
2	1,252	1,181	751 (64)
3	652	622	419 (67)
4	355	335	238 (71)
≥5	1,024	940	639 (68)
CR-not-PR (%)	1,171	1,082 (93)	552 (51)
CR-and-PR (%)	6,799	6,359 (93)	5,358 (84)
Number of hospital discharges with biliary tract cancer diagnosis (%)			
1	2,106	2,009	1,620 (81)
2	1,274	1,200	1,019 (85)
3	875	834	714 (86)
4	555	513	442 (86)
≥5	1,989	1,803	1,563 (87)

PR-not-CR: Cases in the Patient Register only. CR-and-PR: Cases in both registers. CR-not-PR: Cases in the Cancer Register only.

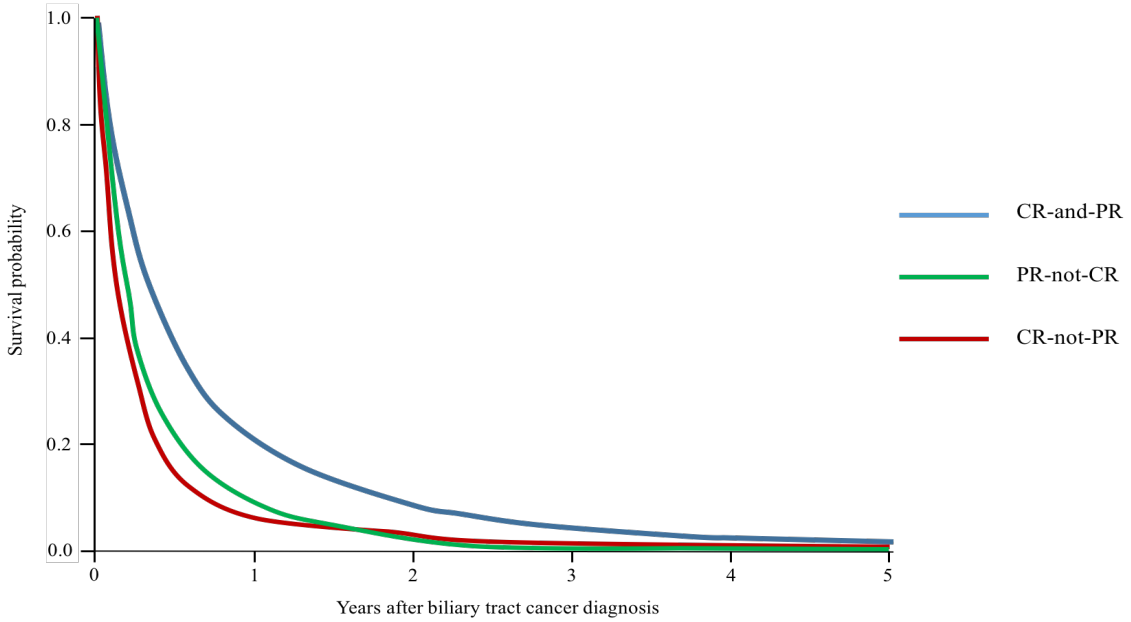

Figure 2. Survival probability of biliary tract cancer cases registered in the Cancer Register (CR) and the Patient Register (PR). CR-and-PR: Cases in both registers. PR-not-CR: Cases in the Patient Register only. CR-not-PR: Cases in the Cancer Register only.

9.2 STUDY II

9.2.1 Study subjects

A total of 21,350 cases of biliary tract cancer were identified in the Cancer Register between 1970 and 2010 (Table 5). Correspondingly, 24,769 cases were identified in the Causes of Death Register. In the Patient Register, 16,505 biliary tract cancer cases were identified between 1987 and 2010, of which 2,593 were diagnosed before 1990. Gallbladder cancer was the most common sub-site in all three registers (66%, 62% and 56% in the Cancer Register, the Patient Register and the Causes of Death Register respectively). The total number of biliary tract cancers in the Patient Register differed from that in Study I, because of the inclusion of other time periods. Almost 7% of study subjects in the Patient Register were diagnosed with biliary tract cancer of multiple sub-sites.

Table 5. Distribution of patients with biliary tract cancer diagnosis in the Cancer Register, the Patient Register, and the Causes of Death Register between 1970 and 2010.

	Cancer Register (%)	Patient Register (%)[α]	Causes of Death Register (%)
Total	21,350 (100)	16,505 (100)	24,769 (100)
Gallbladder cancer	14,181 (66)	10,233 (62)	13,963 (56)
Sex			
Male	6,950 (33)	6,107 (37)	7,983 (32)
Female	14,400 (67)	10,398 (63)	16,786 (68)
Age			
0-49	745 (3)	641 (4)	666 (3)
50-54	733 (3)	592 (4)	684 (3)
55-59	1,291 (6)	1,021 (6)	1,263 (5)
60-64	2,083 (10)	1,554 (9)	2,094 (8)
65-69	2,869 (13)	2,128 (13)	3,108 (13)
70-74	3,692 (17)	2,604 (16)	4,125 (17)
75-79	4,010 (19)	3,018 (18)	4,698 (19)
80+	5,927 (28)	4,947 (30)	8,131 (33)
Time period			
1970-1974	2,913 (13)	.	2,399 (10)
1975-1979	3,268 (15)	.	2,886 (12)
1980-1984	3,577 (17)	.	3,476 (14)
1985-1989	3,311 (16)	2,593 (16)[β]	3,597 (15)
1990-1994	2,515 (13)	2,989 (18)	3,288 (13)
1995-1999	2,030 (9)	3,161 (19)	3,054 (12)
2000-2004	1,804 (8)	3,413 (21)	2,870 (12)
2005-2010	1,932 (9)	4,349 (26)	3,199 (13)

α 1987-2010, β 1987-1989

9.2.2 The Cancer Register

Overall, the incidence of biliary tract cancers decreased over time in both men and women (Figures 3 and 4). The annual incidence rate among men peaked at almost 8 new cases/100,000 persons in the mid 1980's, where after the incidence rate decreased by 4.2% annually (95% CI -4.8 to -3.6) until the end of the study period. In women, the incidence rate was stable (APC 0.2, 95% CI -1.1 to 1.5) between 1970 and 1983. After 1983 however, the incidence patterns were similar to those seen in men (APC -4.7, 95% CI -5.2 to -4.2).

In the gallbladder cancer specific analyses, the results were similar to the analyses of biliary tract cancer in total, but the decreasing incidence trends starting in the mid 1980's were perhaps more pronounced (APC -6.1, 95% CI -6.8 to -5.3 for men and APC -5.3, 95% CI -5.9 to -4.8 for women) (Figures 5 and 6).

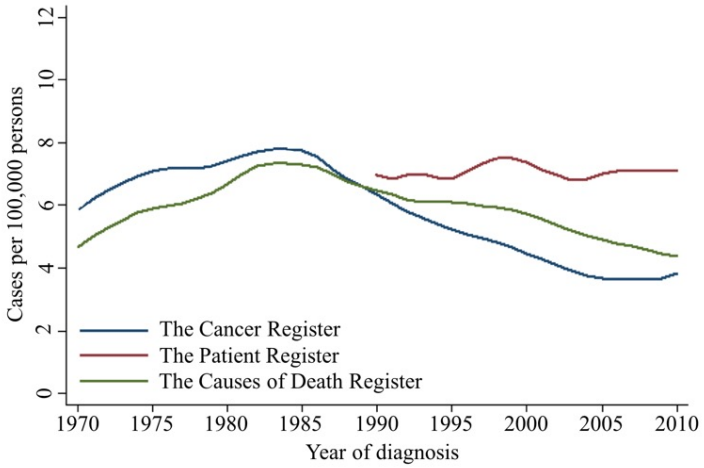

Figure 3. Standardized incidence/mortality rates of biliary tract cancer in men between 1970 and 2010.

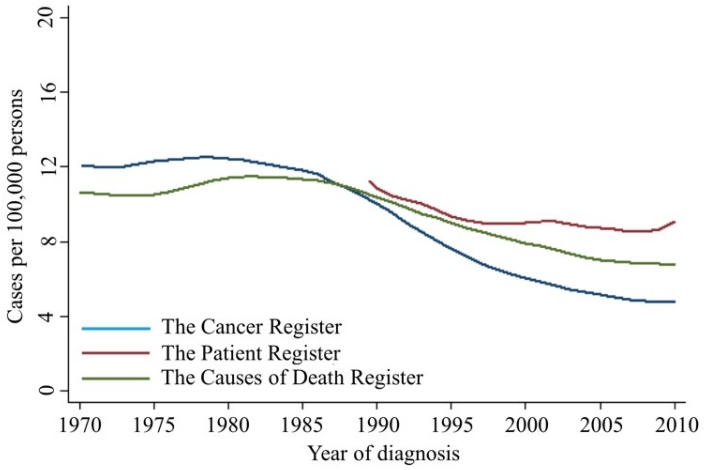

Figure 4. Standardized incidence/mortality rates of biliary tract cancer in women between 1970 and 2010.

At the start of the study period, most biliary tract cancer diagnoses were based on autopsy findings in both sexes (Figures 7 and 8). However, the number of male cases based on autopsy reports decreased by 10.0% (95% CI -11.1 to -8.9) annually from 1986 and onwards. A similar trend was seen in women (APC -11.1, 95% CI -12.0 to -10.3). In men, the number of cases based on histopathology increased by 4.0% (95% CI 1.4 to 6.7) annually between 1970 and 1983. Correspondingly, a similar finding was seen in women (APC 1.5, 95% CI 0.3 to 2.7). By 2010, the majority of biliary tract cancer diagnoses were based on histopathology in men and women even though a decreasing trend in the use of histopathology was seen from 1990 onwards.

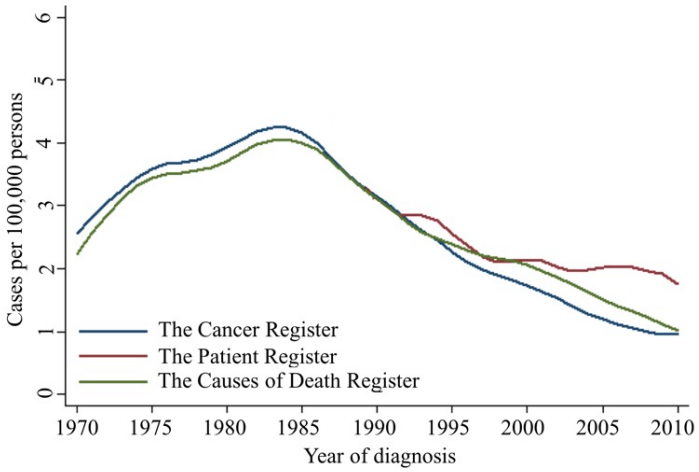

Figure 5. Standardized incidence/mortality rates of gallbladder cancer in men between 1970 and 2010.

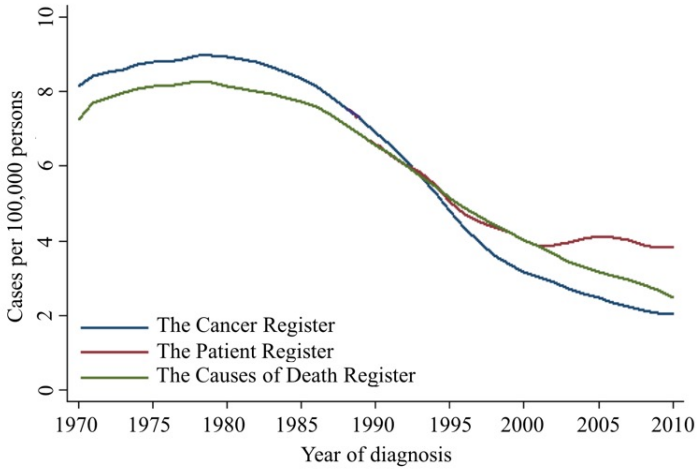

Figure 6. Standardized incidence/mortality rates of gallbladder cancer in women between 1970 and 2010.

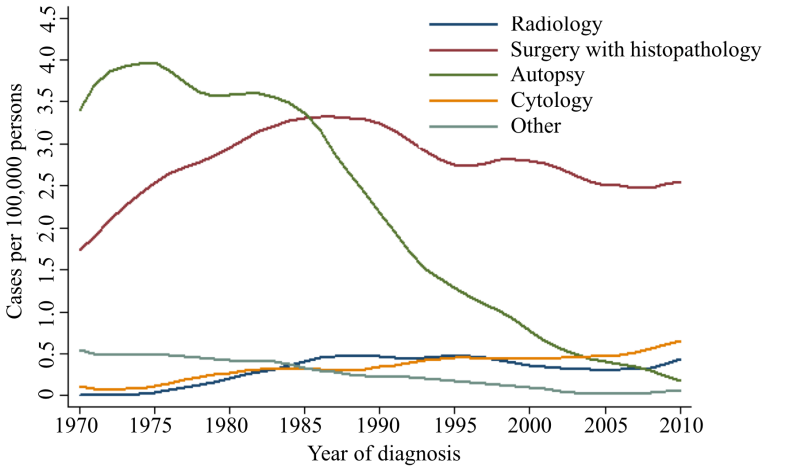

Figure 7. Foundation of biliary tract cancer diagnosis reported in the Cancer Register in men between 1970 and 2010.

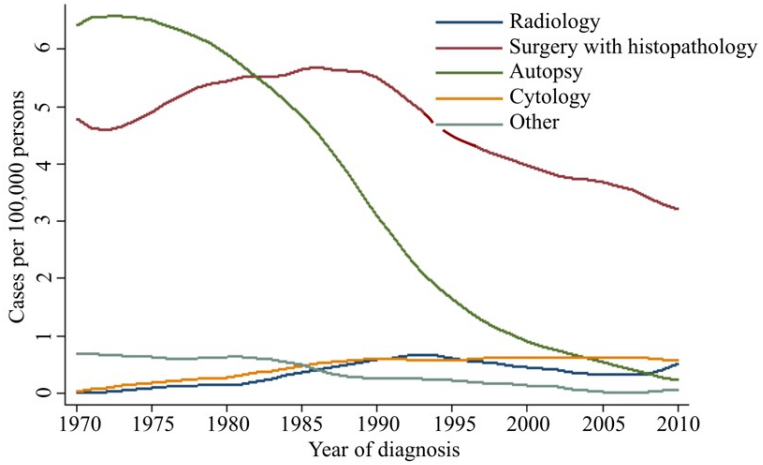

Figure 8. Foundation of biliary tract cancer diagnosis reported in the Cancer Register in women between 1970 and 2010.

9.2.3 The Patient Register

The incidence rate of biliary tract cancer assessed from the Patient Register was stable over the study period in men (APC 0.1, 95% CI -0.8 to 0.9). In women, a slightly decreasing trend of borderline significance was noted (APC -0.9, 95% CI -1.6 to -0.1).

For gallbladder cancer specifically, the rate in men decreased by 2.4% (95% CI -3.3 to -1.4) annually between 1990 and 2010, but the curve flattened out towards the end of the study period. In women, a decreasing trend between 1990 and 1997 was seen (APC -6.3, 95% CI -8.4 to -4.6). After 1997 however, the incidence trend was stable (APC -0.9, 95% CI -2.3 to 0.6).

9.2.4 The Causes of Death Register

The mortality rates of biliary tract cancer in men peaked in 1983, where after a decreasing trend was observed (APC -1.8, 95% CI -2.5 to -1.2). In women, the mortality rate was stable until 1986 (APC 0.7, 95% CI -0.3 to 1.7), but decreased by 2.4% (95% CI -3.0 to -1.9) annually thereafter until 2010. From 1987 onwards, the mortality rate was higher than the incidence rate (in the Cancer Register) in both men and women (Figures 3 and 4).

The mortality rates of gallbladder cancer, specifically, also decreased during the study period (Figures 5 and 6). In men, the mortality rate increased up until 1985 (APC 3.1, 95% CI 1.2 to 5.0) but decreased by 4.8% (95% CI -5.6 to -4.1) annually thereafter. In women, the mortality trend was stable before 1985 but decreased by 4.4% (95% CI -5.0 to -3.7) annually thereafter.

9.3 STUDY III

9.3.1 Study subjects

A total of 1,896 biliary tract cancer cases were identified, of which; 1169 cases had gallbladder cancer; 432 cases had cancer of the extra-hepatic bile ducts; and 295 cases had ampullary cancer. The mean age at diagnosis was approximately 57 years, and diabetes was more common in cases than controls. Female cases were more often multiparous (more than one child) and younger at the birth of the first child, compared to controls.

9.3.2 Reproductive factors and gallbladder cancer

Women with three children or more had increased odds of gallbladder cancer (OR 2.06, 95% CI 1.68 to 2.51) compared to women with one child (Table 6). Furthermore, the odds of gallbladder cancer decreased with increasing age at the birth of the first child in women (OR 0.54, 95% CI 0.41 to 0.70).

The association between parity and gallbladder cancer was unchanged in women aged 50 years or more at diagnosis (OR 2.34, 95% CI 1.87 to 2.93 for women with ≥3 children), whereas no association was seen in women aged less than 50 years (Table 7). The association between a woman's age at the first birth and gallbladder cancer was unchanged by stratification for age at diagnosis.

There was a positive association between increasing number of children and gallbladder cancer in men (OR 1.70, 95% CI 1.20 to 2.40 for men with ≥3 children). However, no association between age at the birth of the first child and gallbladder cancer was observed. The association between number of children and gallbladder cancer remained in men aged 50

years or more at diagnosis and no association was seen in men aged less than 50 at diagnosis (data not shown).

Table 6. The association between reproductive factors and biliary tract cancer in men and women, expressed as adjusted odds ratios (OR) with 95% confidence intervals (CI) by sub-site.

	Gallbladder cancer	**Cancers of the extra-hepatic bile ducts**	**Ampullary cancer**
	OR (95 % CI)[α]	OR (95 % CI)[α]	OR (95 % CI)[α]
Women			
Number of children			
0	1.00 (0.76 - 1.32)	1.40 (0.84 - 2.33)	0.47 (0.20 - 1.13)
1	Reference	Reference	Reference
2	1.59 (1.30 - 1.94)	1.64 (1.09 - 2.46)	1.51 (0.88 - 2.60)
≥3	2.06 (1.68 - 2.51)	1.65 (1.07 - 2.55)	1.56 (0.89 - 2.73)
Age at the birth of the first child (years)			
≤22	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
23-29	0.71 (0.60 - 0.83)	0.60 (0.43 - 0.84)	0.97 (0.61 - 1.53)
≥30	0.54 (0.41 - 0.70)	0.61 (0.36 - 1.04)	0.86 (0.45 - 1.64)
Men			
Number of children			
0	1.26 (0.85 - 1.87)	1.87 (1.15 - 3.01)	2.42 (1.39 - 4.23)
1	Reference	Reference	Reference
2	1.43 (1.01 - 2.01)	1.78 (1.16 - 2.73)	3.32 (1.98 - 5.54)
≥3	1.70 (1.20 - 2.40)	2.57 (1.65 - 3.98)	2.77 (1.61 - 4.77)
Age at the birth of the first child (years)			
≤22	Reference	Reference	Reference
23-29	1.03 (0.72 - 1.48)	0.75 (0.50 - 1.11)	0.71 (0.45 - 1.13)
≥30	1.05 (0.69 - 1.59)	0.67 (0.42 - 1.06)	0.73 (0.44 - 1.23)

[α] Model is adjusted for diabetes, alcohol use, smoking, birth country and educational level.

9.3.3 Reproductive factors and cancers of the extra-hepatic bile ducts and the Ampulla

Women and men with three or more children had increased odds of cancer of the extra-hepatic bile ducts (OR 1.65, 95% CI 1.07 to 2.55 for women and OR 2.57, 95% CI 1.65 to 3.98 for men). Similar associations were observed for Ampullary cancers (OR 1.56, 95% CI 0.89 to 2.73 for women and OR 2.77, 95% CI 1.61 to 4.77 for men). Furthermore, there was an inverse association between age at the birth of the first child and cancer of the extra-hepatic bile duct, but the results were generally similar in men and women (Table 6). Women

and men aged 30 years or more had slightly decreased odds of Ampullary cancer, though the associations were not statistically significant.

Table 7. Association between reproductive factors and gallbladder cancer in women stratified for age at diagnosis, expressed as odds ratios (OR) with 95% confidence intervals (CI) .

	<50 years at diagnosis	**≥50 years at diagnosis**
	OR (95 % CI) $^{\alpha}$	OR (95 % CI) $^{\alpha}$
Number of children		
0	1.01 (0.58 - 1.76)	0.99 (0.72 - 1.37)
1	Reference	Reference
2	1.11 (0.72 - 1.71)	1.72 (1.37 - 2.16)
≥3	1.16 (0.73 - 1.84)	2.34 (1.87 - 2.93)
Age at the birth of the first child (years)		
≤22	Reference	Reference
23-29	0.65 (0.46 - 0.94)	0.72 (0.60 - 0.86)
≥30	0.46 (0.23- 0.90)	0.55 (0.41 - 0.75)

$^{\alpha}$ Model is adjusted for diabetes, alcohol use, smoking, birth country and educational level.

9.4 STUDY IV

9.4.1 Study subjects

The study included 203,131 men with prostate cancer, providing 849,307 person-years of follow-up time after exclusion of the first year of follow-up. In total, 22.5% (45,744 individuals) of all study subjects were considered more exposed to estrogen therapy, and the remaining 77.5% (157,387 individuals) were considered less exposed. A total of 7,068 individuals were excluded in the gallbladder cancer-specific analyses because of a history of cholecystectomy before the start of the study. The median follow-up time was 3.8 years (Inter-quartile range (IQR): 1.6 to 7.2 years).

9.4.2 Risk of gallbladder cancer

The median follow-up time was 3.9 years in the more exposed group and 3.2 years in the less exposed group. Altogether, 17 gallbladder cancers were observed in the more exposed group, compared to the expected 19.5 (SIR 0.87, 95% CI 0.51 to 1.39). Correspondingly, 24 gallbladder cancers were identified in the less exposed group compared to the expected 29.7 (SIR 0.81, 95% CI 0.52 to 1.21). In the sensitivity analysis, the point estimates decreased, but there were no clear differences between the more exposed and the less exposed groups (Table 8). A decreased risk of gallbladder cancer was also indicated when the two groups were

combined (OR 0.83, 95% 0.60 to 1.13) and was statistically significant in the sensitivity analysis excluding cohort members with known risk factors for biliary tract cancer (OR 0.67, 95% CI 0.44 to 0.97).

Men in the more exposed group with a latency time of more than five years (prolonged exposure) had an increased point estimate of gallbladder cancer, though the association was not statistically significant (SIR 1.34, 95% CI 0.71 to 2.29). The stratified analysis did not change the results for the less exposed group (Table 8).

Table 8. Standardized incidence ratios (SIR) with 95% confidence intervals (CI) for biliary tract cancer between 1961 and 2008, stratified for latency time.

	Number of observed cases	Number of expected cases	Main analysis SIR (95% CI)	Sensitivity analysis SIR (95% CI) [b]
Gallbladder cancer				
1961-2008	41	49.2	0.83 (0.60-1.13)	0.67 (0.44-0.97)
1961-1980[c]	17	19.5	0.87 (0.51-1.39)	0.63 (0.31-1.13)
>1-5 years	4	9.8	0.41 (0.11-1.04)	.
>5 years	13	9.7	1.34 (0.71-2.29)	.
1981-2008	24	29.7	0.81 (0.52-1.21)	0.69 (0.40-1.11)
>1-5 years	15	18.4	0.81 (0.46-1.34)	.
>5 years	9	11.3	0.80 (0.36-1.52)	.
Cancers of the extra-hepatic bile ducts [a]				
1961-2008	36	45.5	0.79 (0.55-1.10)	0.73 (0.48-1.06)
1961-1980[c]	13	14.4	0.91 (0.48-1.55)	0.80 (0.39-1.48)
>1-5 years	4	5.6	0.71 (0.19-1.83)	.
>5 years	9	7.5	1.20 (0.55-2.28)	.
1981-2008	23	31.1	0.74 (0.47-1.11)	0.69 (0.40-1.10)
>1-5 years	13	15.1	0.86 (0.46-1.47)	.
>5 years	10	12.7	0.78 (0.38-1.44)	.

[a] Extra-hepatic bile ducts also included Ampullary cancer

[b] The anlaysis excluded subjects with: gallstone disease who did not undergo cholecystectomy, diabetes, obesity, alcohol abuse or primary sclerosing cholangitis prior to prostate cancer diagnosis.

[c] More exposed to oestrogen

9.4.3 Risk of cancers of the extra-hepatic bile ducts and the Ampulla

The median follow-up time in the more exposed and the less exposed groups was 3.3 and 4.0 years, respectively. In all, 13 cancers of the extra-hepatic bile ducts were observed in the more exposed group, compared to the expected 14.4 (SIR 0.91, 95% CI 0.48 to 1.55). In the

less exposed group, 23 cases were observed in contrast to the expected 31.3 (SIR 0.74, 95% CI 0.47 to 1.11). There were no major changes in the sensitivity analyses (Table 8). The decreased risk estimate remained when the two groups were analyzed together (OR 0.79, 95% CI 0.55 to 1.10).

The latency time analyses showed an increased risk of cancer for men in the more exposed group with a latency time of more than 5 years, but the association was not statistically significant (SIR 1.20, 95% CI 0.55 to 2.28). There were no clear differences in the less exposed group between subjects with different latency time.

9.5 STUDY V

9.5.1 Study subjects

The final matched cohort consisted of more than 1.1 million women, of which 290,186 women were ever exposed to menopausal hormone therapy. A total of 154,198 women were treated with combination regimens and 135,988 women were treated with regimens containing estrogen only (Table 9). The median duration of hormone treatment was 1.8 years (IQR: 0.7-4.3). The median age at inclusion was 57 years (52-66) and the mean follow-up time in the exposed and unexposed were 5.6 (Standard deviation[SD]: 2.3) and 7.3 (SD 1.8) years, respectively.

The unmatched cohort included more than 3.6 million unexposed women and the mean follow-up time in the unexposed was 7.5 years (SD: 1.2). The total follow-up time was 27,009,996 person-years.

Table 9. Characteristics of exposed and unexposed individuals in Study V.

Variable	Exposed subjects	Unexposed subjects (matched cohort)	Unexposed subjects (unmatched cohort)
Total (%)	290,186 (100)	870,165 (100)	3,625,136 (100)
Matching and/or confounding variables (%)			
Delivery	117,861 (40.6)	353,282 (40.6)	.
Thrombotic events	40,316 (13.9)	120,931 (13.9)	.
Hysterectomy	51,811 (17.9)	155,138 (17.8)	237,380 (6.7)
Diabetes	15,936 (5.5)	48,422 (5.6)	69,607 (2.0)
Obesity	5,146 (1.8)	15,526 (1.8)	.
Smoking-related disease	13,601 (4.7)	40,994 (4.7)	.
Alcohol abuse	7,293 (2.5)	21,455 (2.5)	.
Osteoporosis	8,256 (2.9)	22,764 (2.6)	.
Cholecystectomy	25,553 (8.6)	49,818 (5.7)	176,444 (4.9)
Gallstone disease	29,102 (10.0)	79,933 (9.2)	245,581 (6.8)
Type of hormonal regimen (%)			
Estrogen only	135,988 (46.9)	.	.
Esotrogen/progestin combination	154,198 (53.1)	.	.

9.5.2 Menopausal hormone therapy and gallbladder cancer

In the matched cohort, a total of 47 and 168 gallbladder cancers were identified in the exposed and the unexposed group, respectively. There was an inverse association between menopausal hormone therapy and gallbladder cancer, however the finding was not statistically significant (OR 0.84, 95% CI 0.60 to 1.15). The OR for combination regimens was 0.73 (95% CI 0.43 to 1.23), and 0.91 (95% CI 0.62 to 1.35) for regimens containing only estrogen.

In the unmatched cohort, a total of 714 gallbladder cancers were identified in the unexposed group. The associations between menopausal hormone therapy and gallbladder cancer were similar to those observed in the matched cohort (Table 10).

Table 10. Menopausal hormone therapy (MHT) and risk of biliary tract cancer by subsite, expressed as odds ratios (OR) $^{\alpha}$ with 95 % confidence intervals (CI) or hazard ratios (HR) $^{\beta}$ and 95% CI.

Matched cohort			
	All MHT	**Estrogen only**	**Estrogen and progestin combination**
	OR (95 % CI)	**OR (95 % CI)**	**OR (95 % CI)**
Gallbladder cancer	0.84 (0.60 - 1.15)	0.91 (0.62 - 1.35)	0.73 (0.43 - 1.23)
Cancer of the extra-hepatic bile duct and the Ampulla	0.83 (0.61 - 1.15)	0.74 (0.48 - 1.14)	0.97 (0.63 - 1.51)
Gallstone disease	6.95 (6.64 - 7.28)	7.18 (6.79 – 7.59)	6.83 (6.47 – 7.21)
Unmatched cohort			
	All MHT	**Estrogen only**	**Estrogen and progestin combination**
	HR (95% CI)	**HR (95% CI)**	**HR (95% CI)**
Gallbladder cancer	0.88 (0.69-1.13)	0.94 (0.64-1.40)	0.84 (0.61-1.15)
Cancers of the extra-hepatic bile ducts and the Ampulla	1.02 (0.76-1.37)	0.94 (0.64-1.38)	1.16 (0.76-1.77)

α Adjusted for the matching variables and osteoporosis.
β Adjusted for age, diabetes, primary sclerosing cholangitis, hysterectomy and gallstone disease.

9.5.3 Menopausal hormone therapy and cancers of the extra-hepatic bile ducts

A total of 48 cancers of the extra-hepatic bile ducts were identified in the exposed group. The corresponding number in the unexposed group was 174. There was no clear association with menopausal hormone therapy (OR 0.83, 95% CI 0.61 to 1.15). In women exposed to combination regimens, the OR was 0.97 (95% CI 0.63 to 1.51), whereas the OR was 0.74 (95% CI 0.49 to 1.14) in women exposed to regimens containing estrogen only.

A total of 428 cancers of the extra-hepatic bile duct or Ampulla were identified among the unexposed in the unmatched cohort. The slightly decreased risk of cancer observed in the matched cohort was attenuated in the unmatched cohort (HR 1.02, 95% CI 0.76 to 1.37). However, there were no statistically significant results in either cohort (Table 10).

9.5.4 Menopausal hormone therapy and gallstone disease

In the matched cohort, there was a positive association between menopausal hormone therapy and gallstone disease in women without a history of gallstones prior to exposure to hormone therapy (OR 6.95, 95% CI 6.64 to 7.28).

10 METHODOLOGICAL CONSIDERATIONS

10.1 STUDY DESIGN

Most research may be readily categorized as either experimental or observational. One may consider the difference between the two by the way the study participants receive the exposure of interest. In the experimental setting, the researcher imposes the exposure (intervention) on the study participant, much like in any other kind of experiment. Whereas in the observational setting, the researcher simply observes the exposure, without influencing the form of exposure or even the decision to actually expose any subject. In the modern evidence-based world of medicine, the experimental design is favored because of the possibility to use randomization in order to achieve a "confounding-free" study. However, many exposures are not suitable for the experimental study design because of ethical or practical considerations. For example, random allocation of parity or menopausal hormone therapy (Studies III and V respectively) is not feasible, and randomization of prostate cancer treatment to study biliary tract cancer (Study IV) is impossible. Furthermore, all types of study design have strengths and weaknesses which must be considered before choosing the most appropriate design for the research question. The most common types of observational designs are the cohort design, the case-control design, the cross-sectional design and the ecological design. Studies I, II, IV and V are examples of cohort studies, while Study III was a case-control study.

In cohort studies, the study subjects are categorized according to exposure status (exposed vs unexposed) and followed over time for an outcome (biliary tract cancer for example). It is desired that the exposed and the unexposed individuals are as alike as possible in every aspect, except for the exposure variable. Study V included a matched cohort using a rather extensive matching procedure similar to a propensity score matching procedure. The purpose of such a procedure is to make the groups (exposed and unexposed) as similar as possible to mimic the effect of a randomization.(177) Therefore, factors not necessarily associated with both exposure and outcome were included in the matching procedure to capture influence from unmeasured factors by making exposed and unexposed individuals as alike as possible. Cohort studies are generally regarded superior to other observational designs in the hierarchy of evidence. The cohort design is effective when more than one exposure is to be investigated and the outcome is not too rare.

In the case-control design, the study subjects are instead grouped by their outcome status, as cases and not cases. Control subjects are then selected from the "not case" group for

comparison with the cases. The exposure status is then collected for the both the cases and controls, making case-control studies essentially retrospective in nature. Thus, one may consider that the case-control study actually investigates the likelihood of being exposed depending on outcome status. The case-control design is efficient when the outcome is rare because the cost and effort needed to collect a reasonable sample size may be kept at minimum. However, great care must be taken in the sampling of controls. It is important that the control group accurately reflects the prevalence of the exposure in the population from which the cases were identified to avoid selection bias, which may otherwise severely limit the value of a case-control study. Furthermore, the manner in which the controls are sampled is of importance. Also, the number of controls per case must be considered because the optimal ratio may vary depending on the study parameters and research question. In Study III, 10 controls per case were selected from the same source population that was used to identify the cases, at random at the time the corresponding case was classified as a case. This type of sampling is referred to as density-based sampling. A smaller number of controls (e.g. 5 per case) could have been used without compromising statistical power much.

10.2 VALIDITY

10.2.1 Internal validity

The results of an internally valid study accurately reflect what the study was meant to reflect. That is, the study measures what it was intended to measure. A study lacking internal validity may be flawed or poorly performed in such a way that the results of the study are dubious or even incorrect and is therefore a major concern for the inference of the study. Error in a study will affect the internal validity and may be introduced on many levels.

10.2.2 External validity

The extent to which the inference of a study may be extrapolated to other populations than the study population, or in other words, the generalizability, is often termed external validity. With this in mind, external validity is not normally calculated or estimated, (nor is internal, for that matter) but is more an analysis of thought based on aspects such as study design. Generally, the external validity does not impose limits in the actual results of the study, only the generalizability. However, for a study to be generalizable, a good internal validity is necessary. All studies included in this thesis are population-based, which is one method that may be used to improve the external validity of any study.

10.3 ERROR

10.3.1 Random error

The precision of a study is inversely correlated with the degree of random error. Random error, as the term implies, will not drive results in a specific direction but rather gives rise to a less precise estimate. The level of random error can be estimated using hypothesis testing and is most often expressed as a confidence interval or a p-value related to the null-hypothesis. The null hypothesis is often the opposite of the research question, perhaps that there is no association between exposure and outcome. In Studies II-V, a 95% CI indicated an interval in which the estimate would be found, 95% of the times, if the study where to be repeated under the exact same conditions. Analogously, the p-value is the probability that the observed difference, or greater, between two groups is based solely on chance if the null-hypothesis is actually true. Hypothesis testing aims to either reject or accept the null hypothesis. Consequently, the null-hypothesis may be rejected even though it is true, referred to as Type-I error. That is, an association between two variables may be observed even though there is none. Furthermore, the null-hypothesis may be accepted even though it is false, which is referred to as Type-II error. That is, no association between two variables is observed, even though there actually is one. To minimize random error in this thesis, predefined hypotheses were used to reduce multiple testing. Also, exposures and outcomes were clearly defined and the population-based design entailed the inclusion of the entire Swedish population as the source population to increase sample size. Studies II-V, all used 95% CI to assess the level of present random error.

10.3.2 Systematic error (bias)

Contrary to random error, systematic error skew the results in a specific direction and is also called bias. In theory, a randomized experimental study should be the best way of limiting the influence of bias. Bias can be categorized, but always represents a major concern for the validity of any observational study, regardless of the type of bias.

Selection bias is the result of a systematically incorrect or invalid sampling of subjects so that the groups under study differ systematically. Even though selection bias is mostly just a potential issue in case-control studies, cohort studies can also be hampered by an incorrect sampling of unexposed subjects. In cohort studies, selection bias may occur e.g. if the loss of follow-up is different in the exposed and unexposed group. To minimize selection bias in Study III, the controls were sampled in a random fashion, and from the same population-based source population from which the cases were derived. The follow-up in all studies

included in this thesis was register-based, which counteracts selection bias due to loss of follow-up.

Information bias, or misclassification, is the result of inaccuracies or errors in the classification/measurement of a variable. In register-based research, the correctness of diagnosis codes used for categorizing a variable is a common source of misclassification. For example, routines in coding may differ between practitioners and some diseases may be more easily identified and coded, introducing misclassification. In some settings, the misclassification of a variable may differ between groups in the study, termed differential misclassification, and may skew the results of the study in either direction. However, if the error is similar between groups, a non-differential (random) misclassification is present, resulting in an estimate skewed towards null. It is possible that some non-differential misclassification is present in the studies of this thesis due to the register-based design. The classification of the exposure and confounding variables in Studies III-V may be subjected to non-differential misclassification. The categorization of the outcome in Studies III-V was based on the Cancer Register, which should limit the effect of misclassification of the outcome due to the high quality of the data in the register. Misclassification as a result of coding errors in the registers is likely not dependent on the exposure and outcome in Studies III-V, and should thus only introduce non-differential misclassification, if any. The exposure variable in Study IV, prostate cancer, was used as a proxy for exogenous hormone exposure. A proxy was used out of necessity because actual sex hormone exposure information (hormones administered to the patient) was not available for analysis. This is a limitation of Study IV and should be considered. However, the rationale behind the use of prostate cancer as a proxy for hormone exposure in men is scientifically sound.

In many settings, a separate factor, associated with both exposure and outcome, may influence the result of a study. This type of bias is labeled confounding. If this separate factor is not within the causal pathway between exposure and outcome, the factor can distort or confound the association. Confounding may be examined using Directed Acyclic Graphs (DAG), in which a diagram of the factors of interest are visualized.(178) In this thesis, the inclusion of important confounding factors such as age, sex, primary sclerosing cholangitis, educational level and diabetes, as covariates in the multivariable analyses of Studies III and V should limit the effect of cofounding on the observed associations. Furthermore, the use of sensitivity analyses in Study IV allowed for quantification of the confounding effect. Gallstone disease is an important risk factor for biliary tract cancer. Furthermore, gallstone disease may be associated with the exposures in Studies III (parity) and V (menopausal

hormone therapy). However, because gallstone disease is an intermediary step between exposure (parity) and outcome (biliary tract cancer), it may be considered a mediator rather than a confounding factor. Adjustment for a mediating factor (gallstone disease) will affect the strength of the association between exposure and outcome, and the effect size corresponds to the strength of the pathway through the mediator. However, if the goal is to assess the association without the pathway of the mediator, adjustment for the mediating factors can be performed. Despite efforts to reduce the effect of confounding in this thesis, a residual effect of lifestyle factors, such as obesity and other unmeasured factors is possible.

11 GENERAL DISCUSSION

11.1 STUDY I – UNDER-REPORTING OF BILIARY TRACT CANCER

There is substantial under-reporting of biliary tract cancer to the Cancer Register, i.e. 44% of cases diagnosed with biliary tract cancer are not reported. The rate of non-reporting is higher in the elderly and in cases diagnosed in more recent time periods. The proportion of cases with unreported (to the Cancer Register) biliary tract cancer more often have a non-biliary tract cancer as the cause of death compared to reported cases. Furthermore, the clear majority of biliary tract cancer cases die within one year of diagnosis and the survival is best for cases identified in both the Cancer Register and the Patient Register.

The reason for the under-reporting is not entirely understood. The change in diagnostic tools available for diagnosing biliary tract cancer may play a role, however. Cancer diagnoses in elderly patients with advanced disease are often based on radiology only.(179) This could be an explanation for the finding that elderly patients and those diagnosed in a later time period are less likely to be reported to the Cancer Register. Some previous studies have demonstrated under-reporting of other cancers to the Cancer Register, but no previous study has investigated biliary tract cancer specifically.(7, 180)

Biliary tract cancer is less often reported as the cause of death in unreported compared to reported cases. Furthermore, the concordance between the Cancer Register/Patient Register and the Causes of Death Register increased with the number of hospital discharges. These findings suggest that misclassification of biliary tract cancer in the Patient Register could be more common in the unreported. Such a misclassification could lead to an overestimation of the under-reporting to the Cancer Register because of a potentially incorrect biliary tract cancer diagnosis in the Patient Register. However, it is unlikely that misclassification fully explains the relatively large differences between the registers.

The better survival for cases reported to the Cancer Register and the Patient Register is likely an effect of the selection of patients reported to the Cancer Register, e.g., the elderly may less often be suited to a curative treatment, which will greatly affect survival. (5)

11.2 STUDY II – THE INCIDENCE OF BILIARY TRACT CANCER

The incidence of biliary tact cancer, especially gallbladder cancer, has decreased from 1970 until 2010. However, in comparing the Cancer Register with the Patient Register and the Causes of Death Register, an overestimation of the decreasing trends is likely.

The incidence of biliary tract cancer based on the Cancer Register reveals decreasing trends from the mid 1980's onwards. The trend is particularly strong in gallbladder cancer; a decrease of approximately 75% from 1985 to 2010 was observed in both men and women. Similar developments have been reported from other parts of Europe.(112, 115) Furthermore, accounts based on data from the US have shown analogous findings.(107-109) However, some studies have shown a more stable trend of cancers of the extra-hepatic bile ducts specifically, but the findings have been contradictory.(112-114)

The reasons behind the decreasing incidence trends are unclear. There has been no dramatic elimination of the few risk factors identified for biliary tract cancer to explain these findings. The increasing use of cholecystectomy with the advent of the laparoscopic approach has been suggested as an explanation, but available research has failed to substantiate that argument.(181) Moreover, obesity and diabetes, both risk factors for biliary tract cancer, have rather increased world-wide.(182, 183) Furthermore, the increasing age in the population should increase the incidence also in rare tumors such as biliary tract cancer.(184)

The more stable trend found in the Patient Register, suggests that under-reporting of biliary tract cancer to the Cancer Register may obscure the actual incidence trends. The first study of this thesis showed that under-reporting increased with time period. Such a situation could explain the decreasing incidence trends observed in Study II, at least in part. Although these results could be explained by misclassification of biliary tract cancer in the Patient Register, it is unlikely that such misclassification has increased with time to fully explain the observed findings.

Furthermore, the mortality rate of biliary tract cancer decreased less markedly and was higher than the incidence rate based on the Cancer Register during the second half of the study period. This finding further supports the impression that progressively increasing under-reporting to the Cancer Register is a likely explanation, at least in part, for the otherwise mysteriously decreasing incidence trends of biliary tract cancer in Sweden.

11.3 STUDY III – REPRODUCTIVE FACTORS AND BILIARY TRACT CANCER

Study III did not find evidence to support a role of reproductive factors in the etiology of biliary tract cancer as a whole. However, for gallbladder cancer specifically, the hormonal hypothesis could not be completely rejected. For cancers of the extra-hepatic bile ducts and ampullary cancers, the odds of biliary tract cancer increased with increasing number of children and decreased with increasing age at the birth of the first child. However, the associations between the exposures and biliary tract cancer were similar in men and women suggesting an effect of unmeasured confounding rather than a biological effect. For example, parity has been correlated with obesity in women.(185) A limited body of evidence suggests that paternal weight gain may also occur during pregnancy, but large systematic studies are lacking.(186) The analyses were adjusted for educational level but no adjustment for obesity was possible. Life style factors, such as obesity, could explain the similar findings in men and women. A hormonal explanation to the findings seems implausible.

Concerning gallbladder cancer specifically, there was a positive association between parity and cancer, but only in post-menopausal (aged >50 years at cancer diagnosis) women. There was no clear association between parity and gallbladder cancer in pre-menopausal women. Furthermore, an inverse association between increasing age at the birth of the first child and cancer risk in women was seen. In men, the odds of gallbladder cancer also increased with increasing parity, though, the age-stratified analysis revealed that the effect was restricted to men aged 50 years or more. However, a man's age at the birth of the first child did not affect the odds of gallbladder cancer however. A potential effect of sex hormone exposure in gallbladder cancer etiology cannot be excluded, yet, the results presented herein do not strongly support the hormonal hypothesis.

To the authors' knowledge, this is the first study to examine the association between reproductive factors and biliary tract cancer and include men in the analyses as an extra level of control. Available literature suggests an association between reproductive factors and gallbladder cancer in women.(70, 142, 146, 147) However, these studies did not include men as a global control group. Furthermore, the findings in the present study underline the importance of differentiating gallbladder cancer from other biliary tract cancers in etiological research using sex hormones as the exposure.

11.4 STUDY IV – EXOGENOUS ESTROGEN AND BILIARY TRACT CANCER IN MEN

Estrogen exposure in men may not be an important risk factor for biliary tract cancer. The results of Study IV showed a decreased risk of biliary tract cancer in prostate cancer patients. To the author's knowledge, this study is the first to evaluate if exogenous hormone exposure in men may affect the risk of biliary tract cancer. However, an inverse association between prostate cancer and biliary tract cancer has been reported previously.(187)

The explanation to this finding is interesting, but elusive. The first year of follow-up was excluded to account for potential surveillance bias. Also, though under-reporting of biliary tract cancer is time dependent, any such misclassification should be non-differential because the background population from the same time period was used as comparison. The sensitivity analyses showed a somewhat further reduced risk of biliary tract cancer when subjects with known risk factors were excluded. This observation suggests an influence of confounding in the analyses, but the association was rather modest and does not explain the decreased risk of cancer that was observed.

In subjects with prolonged exposure (time between prostate cancer and biliary tract cancer), the risk of biliary tract cancer was increased in the more exposed group, however, the association was weak. In the less exposed group though, there were no latency time-dependent differences. A prolonged hormone exposure in men could potentially contribute to a carcinogenic process in the biliary tract. Nevertheless, further research is needed to investigate this hypothesis and to address the potentially protective effect of exogenous hormone exposure in male biliary tract cancer.

11.5 STUDY V – MENOPAUSAL HORMONE THERAPY AND BILIARY TRACT CANCER

Study V did not show any clear association between menopausal hormone therapy and biliary tract cancer and there was no clear difference between hormone regimens. Yet, a non-significant, protective effect against gallbladder cancer was suggested. In addition, the study could confirm the previously reported increased risk of gallstone disease in women exposed to menopausal hormone therapy.

Some previous studies have reported a positive association between menopausal hormone therapy and biliary tract cancer, whereas others have found opposite results. However, the studies that indicated an increased risk did not adjust for gallstone disease and it is unclear how cholecystectomy was handled in the studies. (149, 150) It is possible that gallstone disease influenced the results in those studies. One study, where adjustment for gallstone disease was performed, indicated a borderline reduced risk of gallbladder cancer specifically, similar to what was seen in this study.(145) Some of the older studies did not differentiate between biliary tract and liver cancer, making comparison to the present study inappropriate.(151, 153)

Study V adjusted for some potentially important confounding factors such as age, diabetes, and prior hysterectomy. Furthermore, the study was analyzed using two different approaches and the results were similar, strengthening the reliability of the observations. The matched cohort was constructed in an effort to account for unmeasured factors, such as dietary patterns and other lifestyle factors, that may be similar in women with other similarities. The unmatched approach using survival analyses was employed in parallel to verify the observed associations.

Gallstone disease was accounted for to assess any influence of menopausal hormone therapy on the risk of biliary tract cancer without the gallstone mediated pathway. The increased risk of gallstone disease in women exposed to hormone therapy is well documented.(135) Furthermore, obesity is also associated with gallstone disease.(188) However, obesity may also be more prevalent in women using menopausal hormones compared to women who do not.(189) Even though the matched cohort design was employed to address an influence of unmeasured potentially confounding factors, the study may not reliably account for life style factors such as obesity, dietary pattern etc. and residual confounding cannot, therefore, be completely ruled out. Based on the results of the present study and the results of the previous

literature, there is insufficient data to support the hypothesis that menopausal hormone therapy increases the risk of biliary tract cancer.

12 CONCLUSIONS

- A substantial proportion of biliary tract cancers are not reported to the Swedish Cancer Register. The under-reporting increases with increasing patient age and later time period.

- The decreasing incidence trend of biliary tract cancer in Sweden is likely over-estimated.

- Reproductive factors are associated with biliary tract cancer in both women and men and a hormonal mechanism in biliary tract cancer is thus not supported. A role of sex hormone exposure in gallbladder cancer etiology specifically cannot be ruled out.

- Men with prostate cancer might have a generally decreased risk of biliary tract cancer, but any potential risk increase in indivuduals with prolonged estrogen exposure seems to be small. Further reserach is needed to investigate a protective effect of estrogen exposure on the development of biliary tract cancer in men.

- Menopasusal hormone therapy does not seem to increase the risk of biliary tract cancer in women.

13 FUTURE RESEARCH

The incidence and time trends of any cancer are important to correctly understand the disease and to help guide future research. Furthermore, the robustness of academic research based on a register will always depend on the quality of the data. There is a great need to evaluate the Swedish health-care registers from a disease-specific point of view. This thesis shows that the elderly are less likely to be included in the Cancer Register. This raises the question as to whether this applies to other cancers as well and to what extent? One other interesting research question would be if there are differences in the rate of reporting depending on geography or type of health-care provider diagnosing the cancer. In biliary tract cancer specifically, an investigation into the correctness of diagnoses in the Patient Register would be of interest to understand to what extent the reported incidence rates are under-estimated.

Concerning sex hormones and the risk of developing biliary tract cancer, there is a need for further investigations. The need to adequately distinguish between gallbladder cancer and other extra-hepatic lesions is clear, however. One general difficulty in studying endogenous sex hormones as an exposure is to assess the exposure over time. Future research should focus on how to better measure cumulative estrogen exposure over time and then use that information to investigate the risk of biliary tract cancer further. Additionally, to approach the problem from another angle, investigations of biliary tract cancer risk in hormone deficient subjects such as women subjected to oophorectomy/hysterectomy (removal of the ovaries/uterus) are rare and population-based studies may shed some light on the problem.

14 POPULÄRVETENSKAPLIG SAMMANFATTNING

14.1 Bakgrund

Gallvägscancer utanför levern är en cancerform med mycket dålig prognos och hög dödlighet. Det är en generellt ovanlig tumörform och drabbar ungefär 350 personer per år i Sverige. I Sverige beräknar vi cancerförekomst genom det svenska Cancerregistret, dit alla läkare som diagnosticerar cancer har en skyldighet att rapportera nyupptäckta fall. De senaste åren har man noterat att antalet nyinsjuknade i gallvägscancer sjunker. Patienter som får gallvägscancer märker som regel inte av något specifikt symtom, vilket också gör diagnosen svår. De flesta patienter söker vård först när tumören har spridit sig lokalt eller till andra delar av kroppen vilket medför att bot i princip inte är möjlig. De vanligaste symtomen är trötthet, viktnedgång, illamående och buksmärta. Gulsot kan tillstöta om gallvägen stängs av helt. Den enda botande behandlingen är en operation där man tar bort all cancervävnad. Ingreppet är ofta omfattande och bara ca 25 % av patienterna som får gallvägscancerdiagnos lämpar sig för operation. Orsakerna till att man får gallvägscancer är inte helt kända, men man vet att en andel av tumörerna (gallblåsecancer) är betydligt vanligare bland kvinnor. Denna avhandling syftar till att utreda om förekomsten av gallvägscancer verkligen minskar samt om kvinnliga könshormoner påverkar risken att utveckla gallvägscancer.

14.2 Metoder och resultat

Rapporter från de sista årtiondena har visat att gallvägscancer blir allt mindre vanligt. Syftet med Studie I och Studie II var att undersöka om patienter med gallvägscancer verkligen rapporteras till det svenska Cancerregistret och att beräkna nyinsjuknandet och dödligheten i gallvägscancer. Studierna baserades på data från de stora folkhälsoregistren: Cancerregistret, Patientregistret och Dödsorsaksregistret mellan 1990 och 2010. Registren jämfördes med varandra för att se om de patienter som vårdats för gallvägscancer rapporterats till Cancerregistret och om dödsorsaken också stämde. Knappt hälften (44 %) av alla patienter som vårdats för gallvägscancer rapporterades inte till Cancerregistret. Vidare blev andelen som rapporterades till Cancerregistret än mindre med stigande patientålder och under senare tidsperioder. Dödligheten för patienter som inte rapporterats till Cancerregistret var också sämre än för de patienter som fanns med i Cancerregistret. Studie II visade att nyinsjuknandet i gallvägscancer förmodligen minskar i Sverige från 1980-talet och framåt, men att den kraftiga nedgång som tidigare rapporterats är överdriven. Dödligheten i gallvägscancer har också minskat, men inte i den utsträckning nyinsjuknandet har minskat. Då andelen patienter

som botas är relativt oförändrad över tiden, talar resultaten för att Cancerregistret underskattar antalet nyinsjuknade gallvägscancerpatienter i Sverige.

Studie III undersökte om risken att få gallvägscancer ökar om man får många barn eller om man får barn tidigt. Studien baserades på hälsoregisterdata. En ökad risk för gallvägscancer noterades bland kvinnor med många barn och bland kvinnor som fått barn tidigt. Emellertid sågs samma effekt hos män vilket talar emot en hormonell effekt. En möjlig orsak till fynden kan vara en påverkan av livsstilsfaktorer, såsom övervikt och rökning, som Studie III inte kunnat mäta. För gallblåsecancer specifikt, sågs en ökad risk hos kvinnor med lägre ålder vid första barnet utan motsvarande risk hos män.

Studie IV undersökte risken att utveckla gallvägscancer hos män med prostatacancer. Prostatacancer behandlades tidigare med mycket höga doser av östrogen vilket gör att prostatacancerpatienter är en av få manliga populationer som utsatts för tillsatt östrogen. Man såg ingen tydligt ökad risk av gallvägscancer överlag utan snarare än lägre risk. För de patienter som fått högst dos av östrogen kunde en möjligt ökad risk dock ses.

Den sista studien baserades också den på registerdata och undersökte om risken att utveckla gallvägscancer är förhöjd bland kvinnor som behandlas med hormonpreparat för övergångssymtom. Studie V visade en ökad risk för gallstenssjukdom bland hormonbehandlade kvinnor men kunde inte visa någon tydligt ökad risk av gallvägscancer. En möjligt minskad risk kunde ses bland hormonexponerade kvinnor, men resultaten kunde inte justeras för livsstilsfaktorer, såsom övervikt, vilket skulle kunna påverka fynden.

14.3 Diskussion

Studie I och II visade att många fall av gallvägscancer inte rapporteras till Cancerregistret samt att äldre patienter och patienter som fått diagnos i senare tidsperiod rapporteras än mindre ofta. En konsekvens av detta är att nyinsjuknande i gallvägscancer underskattas i Sverige. Förmodligen minskar nyinsjuknandet av gallvägscancer, men sannolikt inte alls lika mycket som man tidigare misstänkt, eller är oförändrat över tid.

De tre sista studierna visar att könshormoners påverkan på risken att utveckla gallvägscancer är osäker, åtminstone verkar risken inte vara starkt ökad. För gallblåsecancer specifikt kan det finnas en ökad risk för kvinnor som fött många barn eller som fått barn tidigt. Vidare sågs en ökad förekomst av gallstenssjukdom bland kvinnor som fått hormonbehandling för övergångssymtom. Sannolikt behöver läkare inte ta hänsyn till risken att utveckla gallvägscancer när man förskriver hormonpreparat till kvinnor i övergångsåldern.

15 ACKNOWLEDGEMENTS

There are a number of people who have contributed to the making of this thesis and I want to thank you all for your participation and express my gratitude to each and every one of you:

Omid Sadr-Azodi, my principal supervisor and dear friend. Thank you for your optimism, support and knowledge. Thank you for the pushes when I needed them and for the slack time when I needed it! It has been an incredible journey and I thank you for bringing me on board all those years ago!

Jesper Lagergren, my co-supervisor and research group leader. Thank you for your never-ending energy and speedy responses! Thank you for an exemplary leadership and for making the group into the stimulating place it is!

Rickard Ljung, my co-supervisor. Your hard-core epidemiology knowledge has been a motivator for me throughout these years. Thank you for the inspiration!

Yunxia Lu, my co-supervisor, for your expertise in statistics and support.

Olle Bernell, my mentor. Thank you for pointing me in the surgical direction!

Fredrik Mattsson, thank you for your extensive expertise in biostatistics, programing and your table tennis forehand drives!

Margrete Gellervik, for the welcoming attitude and all the help.

Hanna Johans, for all the help with all the things I didn't know about!

Lizzy Lubczanski, for timely language checks even on short notice.

Dear former and current colleagues in the research unit: **Anna Schandl, Anna Lindam, Asif Johar, Dag Holmberg, John Maret Ouda, Karin Vikström, Karl Wahlin, Lovisa Backemar, Maartje van der Schaaf, Maryam Derogar, Nele Brusselaers, Pernilla Lagergren, Poorna Anandavadivelan, Wenjing Tao, and Ylva Hellstadius**

To all **my colleagues at St. Görans Hospital** for the discussions and many hours of learning in the clinic and for all the good fun outside of the hospital.

All my friends. For your support and for making my life what it is!

My family, **father Ulf Kilander, mother Charlotte Palme Kilander, brothers Claes Kilander and Henrik Kilander** for excellent parenthood and brotherly love and skepticism.

Christina, my wife and joy! Thank you for all the support and the over-dose of fun and laughter during these years. I hope for many more to come in our life together!!

Lastly, I would like to express my gratitude to the Karolinska Institute for an insightful education.

16 REFERENCES

1. Marsh W et al. Comprehensive review of the diagnosis and treatment of biliary tract cancer 2012. Part I: diagnosis-clinical staging and pathology. J Surg Oncol. 2012;106(3):332-8.

2. National Board of Health and Welfare. "Cancer incidence in Sweden 2014". 2015, Stockholm.

3. National Board of Health and Welfare: Cancer Statistics Database [Internet]. [cited 2016-10-02]. Available from: http://www.socialstyrelsen.se/statistik/statistikdatabas.

4. Randi G et al. Epidemiology of biliary tract cancers: an update. Ann Oncol. 2009;20(1):146-59.

5. Regional Cancer Center West. Nationell kvalitetsrapport för diagnosår 2014 från Svenska registret för cancer i lever och gallvägar. 2015.

6. Barlow L, Westergren K, Holmberg L, Talback M. The completeness of the Swedish Cancer Register: a sample survey for year 1998. Acta Oncol. 2009;48(1):27-33.

7. Luo J et al. Interpreting trends of pancreatic cancer incidence and mortality: a nation-wide study in Sweden (1960-2003). Cancer Causes Control. 2008;19(1):89-96.

8. Randi G, Franceschi S, La Vecchia C. Gallbladder cancer worldwide: geographical distribution and risk factors. Int J Can 2006;118(7):1591-602.

9. WHO. World Cancer Report 2008. Lyon, France; 2008.

10. de Groen PC et al. Biliary tract cancers. N Engl J Med. 1999;341(18):1368-78.

11. Tominaga S, Kuroishi T. Biliary tract cancer. Cancer surv. 1994;19-20:125-37.

12. Musser JH. Primary Cancer of the Gall-bladder and Bile-Ducts. Boston Medical and Surgical Journal. 1889;22(121):525-9.

13. Sako KS, Garside E. Carcinoma of the extrahepatic bile ducts; review of the literature and report of six cases. Surgery. 1957;41(3):416-37.

14. Taylor G. The radical surgery of cancer of the lower end of the common bile duct and adjacent pancreas. BMJ. 1942;2:119-21.

15. Whipple AO, Parsons WB, Mullins CR. Treatment of Carcinoma of the Ampulla of Vater. Ann Surg. 1935;102(4):763-79.

16. Marsh W et al. Comprehensive review of the diagnosis and treatment of biliary tract cancer 2012. Part II: multidisciplinary management. J Surg Oncol. 2012;106(3):339-45.

17. Pitt HA, Dooley WC, Yeo CJ, Cameron JL. Malignancies of the biliary tree. Current problems in surgery. 1995;32(1):1-90.

18. Karaliotas CB, C.E. Liver and Biliary Tract Surgery. Habib NA. Athens, Greece: Springer; 2006.

19. Khan SA, Thomas HC, Davidson BR, Taylor-Robinson SD. Cholangiocarcinoma. Lancet. 2005;366(9493):1303-14.

20. Steinert R, Nestler G, Sagynaliev E, Muller J, Lippert H, Reymond MA. Laparoscopic cholecystectomy and gallbladder cancer. J Surg Oncol. 2006;93(8):682-9.

21. Kwon AH, Imamura A, Kitade H, Kamiyama Y. Unsuspected gallbladder cancer diagnosed during or after laparoscopic cholecystectomy. J Surg Oncol. 2008;97(3):241-5.

22. Liu KJ, Richter H et al. Carcinoma involving the gallbladder in elderly patients presenting with acute cholecystitis. Surgery. 1997;122(4):748-54.

23. Miyazaki M et al. Clinical practice guidelines for the management of biliary tract cancers 2015: the 2nd English edition. Journal of hepato-biliary-pancreatic sciences. 2015;22(4):249-73.

24. Lazcano-Ponce EC et al. Epidemiology and molecular pathology of gallbladder cancer. CA Can J Clin. 2001;51(6):349-64.

25. Heimbach JK, Sanchez W, Rosen CB, Gores GJ. Trans-peritoneal fine needle aspiration biopsy of hilar cholangiocarcinoma is associated with disease dissemination. HPB. 2011;13(5):356-60.

26. Chapman WC, Sharp KW, Weaver F, Sawyers JL. Tumor seeding from percutaneous biliary catheters. Ann Surg. 1989;209(6):708-13; discussion 13-5.

27. Hennedige TP, Neo WT, Venkatesh SK. Imaging of malignancies of the biliary tract- an update. Cancer imaging. 2014;14:14.

28. Rodriguez-Fernandez A et al. Application of modern imaging methods in diagnosis of gallbladder cancer. J Surg Oncol. 2006;93(8):650-64.

29. Gandolfi L, Torresan F, Solmi L, Puccetti A. The role of ultrasound in biliary and pancreatic diseases. European journal of ultrasound. 2003;16(3):141-59.

30. Petrowsky H et al. Impact of integrated positron emission tomography and computed tomography on staging and management of gallbladder cancer and cholangiocarcinoma. J Hepatol. 2006;45(1):43-50.

31. Yoshimitsu K et al. Helical CT of the local spread of carcinoma of the gallbladder: evaluation according to the TNM system in patients who underwent surgical resection. AJR Am J Roentgenol. 2002;179(2):423-8.

32. Domagk D et al. Endoscopic retrograde cholangiopancreatography, intraductal ultrasonography, and magnetic resonance cholangiopancreatography in bile duct strictures: a prospective comparison of imaging diagnostics with histopathological correlation. Am J Gastroenterol. 2004;99(9):1684-9.

33. Barish MA, Yucel EK, Ferrucci JT. Magnetic resonance cholangiopancreatography. N Eng J Med. 1999;341(4):258-64.

34. Park HS et al. Preoperative evaluation of bile duct cancer: MRI combined with MR cholangiopancreatography versus MDCT with direct cholangiography. AJR Am J Roentgenol. 2008;190(2):396-405.

35. Ponchon T et al. Value of endobiliary brush cytology and biopsies for the diagnosis of malignant bile duct stenosis: results of a prospective study. Gastrointestinal endoscopy. 1995;42(6):565-72.

36. Chen YK, Pleskow DK. SpyGlass single-operator peroral cholangiopancreatoscopy system for the diagnosis and therapy of bile-duct disorders: a clinical feasibility study (with video). Gastrointestinal endoscopy. 2007;65(6):832-41.

37. Garrow D et al. Endoscopic ultrasound: a meta-analysis of test performance in suspected biliary obstruction. Clinical gastroenterology and hepatology. 2007;5(5):616-23.

38. Williams DB et al. Endoscopic ultrasound guided fine needle aspiration biopsy: a large single centre experience. Gut. 1999;44(5):720-6.

39. Kim HJ et al. A new strategy for the application of CA19-9 in the differentiation of pancreaticobiliary cancer: analysis using a receiver operating characteristic curve. Am J Gastroenterol. 1999;94(7):1941-6.

40. Harder J et al. Prognostic relevance of carbohydrate antigen 19-9 levels in patients with advanced biliary tract cancer. Cancer Epidemiol Biomarkers Prev. 2007;16(10):2097-100.

41. Edge SB, American Joint Committee on Cancer. AJCC cancer staging manual. 7th ed. New York: Springer; 2010. xiv, 648 p. p.

42. Sobin LH, Gospodarowicz MK, Wittekind C, International Union against Cancer. TNM classification of malignant tumours. 7th ed. Chichester, West Sussex, UK ; Hoboken, NJ: Wiley-Blackwell; 2010.

43. Chang L, Stefanidis D, Richardson WS, Earle DB, Fanelli RD. The role of staging laparoscopy for intraabdominal cancers: an evidence-based review. Surgical endoscopy. 2009;23(2):231-41.

44. Lee YM, Kaplan MM. Primary sclerosing cholangitis. N Engl J Med. 1995;332(14):924-33.

45. Broome U et al. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. Gut. 1996;38(4):610-5.

46. Molodecky NA et al. Incidence of primary sclerosing cholangitis: a systematic review and meta-analysis. Hepatology. 2011;53(5):1590-9.

47. Boonstra K, Beuers U, Ponsioen CY. Epidemiology of primary sclerosing cholangitis and primary biliary cirrhosis: a systematic review. J Hepatol. 2012;56(5):1181-8.

48. Razumilava N, Gores GJ, Lindor KD. Cancer surveillance in patients with primary sclerosing cholangitis. Hepatology. 2011;54(5):1842-52.

49. Ren HB, Yu T, Liu C, Li YQ. Diabetes mellitus and increased risk of biliary tract cancer: systematic review and meta-analysis. Cancer Causes Control. 2011;22(6):837-47.

50. Schlesinger S al. Diabetes mellitus, insulin treatment, diabetes duration, and risk of biliary tract cancer and hepatocellular carcinoma in a European cohort. Ann Oncol. 2013;24(9):2449-55.

51. Wideroff L et al. Cancer incidence in a population-based cohort of patients hospitalized with diabetes mellitus in Denmark. J Natl Cancer Inst. 1997;89(18):1360-5.

52. Alberti KG et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120(16):1640-5.

53. Shebl FM et al. Diabetes in relation to biliary tract cancer and stones: a population-based study in Shanghai, China. Br J Cancer. 2010;103(1):115-9.

54. Schlesinger S et al. Abdominal obesity, weight gain during adulthood and risk of liver and biliary tract cancer in a European cohort. Int J Can. 2013;132(3):645-57.

55. Larsson SC, Wolk A. Obesity and the risk of gallbladder cancer: a meta-analysis. Br J Cancer. 2007;96(9):1457-61.

56. Hsing AW, Sakoda LC, Rashid A, Chen J, Shen MC, Han TQ, et al. Body size and the risk of biliary tract cancer: a population-based study in China. Br J Cancer. 2008;99(5):811-5.

57. Chow WH, McLaughlin JK, Menck HR, Mack TM. Risk factors for extrahepatic bile duct cancers: Los Angeles County, California (USA). Cancer Causes Control. 1994;5(3):267-72.

58. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med. 2003;348(17):1625-38.

59. Carey MC. Pathogenesis of gallstones. Am J surg. 1993;165(4):410-9.

60. Hsing AW, Gao YT, Han TQ, Rashid A, Sakoda LC, Wang BS, et al. Gallstones and the risk of biliary tract cancer: a population-based study in China. Br J Cancer. 2007;97(11):1577-82.

61. Nordenstedt H, Mattsson F, El-Serag H, Lagergren J. Gallstones and cholecystectomy in relation to risk of intra- and extrahepatic cholangiocarcinoma. Br J Cancer. 2012;106(5):1011-5.

62. Khan ZR, Neugut AI, Ahsan H, Chabot JA. Risk factors for biliary tract cancers. Am J Gastroenterol. 1999;94(1):149-52.

63. Stinton LM, Myers RP, Shaffer EA. Epidemiology of gallstones. Gastroenterol Clin North Am. 2010;39(2):157-69, vii.

64. Ekbom A, Hsieh CC, Yuen J, Trichopoulos D, McLaughlin JK, Lan SJ, et al. Risk of extrahepatic bileduct cancer after cholecystectomy. Lancet. 1993;342(8882):1262-5.

65. Everhart JE, Khare M, Hill M, Maurer KR. Prevalence and ethnic differences in gallbladder disease in the United States. Gastroenterology. 1999;117(3):632-9.

66. Heaton KW, Braddon FE, Mountford RA, Hughes AO, Emmett PM. Symptomatic and silent gall stones in the community. Gut. 1991;32(3):316-20.

67. Scragg RK, McMichael AJ, Seamark RF. Oral contraceptives, pregnancy, and endogenous oestrogen in gall stone disease--a case-control study. Br Med J. 1984;288(6433):1795-9.

68. Braverman DZ, Johnson ML, Kern F, Jr. Effects of pregnancy and contraceptive steroids on gallbladder function. N Engl J Med. 1980;302(7):362-4.

69. Kern F, Jr., Everson GT, DeMark B, McKinley C, Showalter R, Erfling W, et al. Biliary lipids, bile acids, and gallbladder function in the human female. Effects of pregnancy and the ovulatory cycle. J Clin Invest. 1981;68(5):1229-42.

70. Lambe M et al. Parity and cancers of the gall bladder and the extrahepatic bile ducts. Int J Can. 1993;54(6):941-4.

71. Stinton LM, Shaffer EA. Epidemiology of gallbladder disease: cholelithiasis and cancer. Gut and liver. 2012;6(2):172-87.

72. Callea F et al. Precancerous lesions of the biliary tree. J Surg Oncol Supplement. 1993;3:131-3.

73. Shin HR et al. Hepatitis B and C virus, Clonorchis sinensis for the risk of liver cancer: a case-control study in Pusan, Korea. Int J Epidemiol. 1996;25(5):933-40.

74. Rajagopalan V, Daines WP, Grossbard ML, Kozuch P. Gallbladder and biliary tract carcinoma: A comprehensive update, Part 1. Oncology. 2004;18(7):889-96.

75. Lepage C et al. Survival in patients with primary liver cancer, gallbladder and extrahepatic biliary tract cancer and pancreatic cancer in Europe 1999-2007: Results of EUROCARE-5. Eur J Can. 2015.

76. Albores-Saavedra J, Schwartz AM, Batich K, Henson DE. Cancers of the ampulla of vater: demographics, morphology, and survival based on 5,625 cases from the SEER program. J Surg Oncol. 2009;100(7):598-605.

77. Coupland VH et al. Incidence and survival for hepatic, pancreatic and biliary cancers in England between 1998 and 2007. Cancer epidemiology. 2012;36(4):e207-14.

78. Lai CH, Lau WY. Gallbladder cancer--a comprehensive review. The surgeon. 2008;6(2):101-10.

79. Miyakawa S et al. Biliary tract cancer treatment: 5,584 results from the Biliary Tract Cancer Statistics Registry from 1998 to 2004 in Japan. J HPB Surg. 2009;16(1):1-7.

80. de Aretxabala XA et al. Curative resection in potentially resectable tumours of the gallbladder. Eur J Surg. 1997;163(6):419-26.

81. Cho JY et al. Laparoscopic approach for suspected early-stage gallbladder carcinoma. Arch Surg. 2010;145(2):128-33.

82. Chijiiwa K et al. Surgical treatment of patients with T2 gallbladder carcinoma invading the subserosal layer. Journal of the American College of Surgeons. 2001;192(5):600-7.

83. Todoroki T et al. Treatment of gallbladder cancer by radical resection. Br J Surg. 1999;86(5):622-7.

84. Goetze TO, Paolucci V. Benefits of reoperation of T2 and more advanced incidental gallbladder carcinoma: analysis of the German registry. Ann Surg. 2008;247(1):104-8.

85. Reid KM, Ramos-De la Medina A, Donohue JH. Diagnosis and surgical management of gallbladder cancer: a review. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 2007;11(5):671-81.

86. Jang JY et al. Actual long-term outcome of extrahepatic bile duct cancer after surgical resection. Ann Surg. 2005;241(1):77-84.

87. Qiao QL et al. Carcinoma of the ampulla of Vater: factors influencing long-term survival of 127 patients with resection. World J Surg. 2007;31(1):137-43; discussion 44-6.

88. Kondo S et al. Guidelines for the management of biliary tract and ampullary carcinomas: surgical treatment. J HPB Surg. 2008;15(1):41-54.

89. Nishio H et al. Aggressive surgery for stage IV gallbladder carcinoma; what are the contraindications? J HPB Surg. 2007;14(4):351-7.

90. Welsh FK et al. Increased intestinal permeability and altered mucosal immunity in cholestatic jaundice. Ann Surg. 1998;227(2):205-12.

91. Nagino et al. Two hundred forty consecutive portal vein embolizations before extended hepatectomy for biliary cancer: surgical outcome and long-term follow-up. Ann Surg. 2006;243(3):364-72.

92. Ebata T et al. Portal vein embolization before extended hepatectomy for biliary cancer: current technique and review of 494 consecutive embolizations. Digestive surgery. 2012;29(1):23-9.

93. McMasters KM et al. Neoadjuvant chemoradiation for extrahepatic cholangiocarcinoma. Am J Surg. 1997;174(6):605-8.

94. Kato A et al. Surgical resection after downsizing chemotherapy for initially unresectable locally advanced biliary tract cancer: a retrospective single-center study. Ann Surg Oncol. 2013;20(1):318-24.

95. Glazer ES, Liu P, Abdalla EK, Vauthey JN, Curley SA. Neither neoadjuvant nor adjuvant therapy increases survival after biliary tract cancer resection with wide negative margins. Journal of gastrointestinal surgery. 2012;16(9):1666-71.

96. Eckel F, Brunner T, Jelic S, Group EGW. Biliary cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2011;22 Suppl 6:vi40-4.

97. Horgan AM, Amir E, Walter T, Knox JJ. Adjuvant therapy in the treatment of biliary tract cancer: a systematic review and meta-analysis. J Clin Oncol. 2012;30(16):1934-40.

98. Glimelius B et al. Chemotherapy improves survival and quality of life in advanced pancreatic and biliary cancer. Ann Oncol. 1996;7(6):593-600.

99. Prat F et al. Predictive factors for survival of patients with inoperable malignant distal biliary strictures: a practical management guideline. Gut. 1998;42(1):76-80.

100. Abraham NS, Barkun JS, Barkun AN. Palliation of malignant biliary obstruction: a prospective trial examining impact on quality of life. Gastrointestinal endoscopy. 2002;56(6):835-41.

101. Shepherd HA et al. Endoscopic biliary endoprosthesis in the palliation of malignant obstruction of the distal common bile duct: a randomized trial. Br J Surg. 1988;75(12):1166-8.

102. Valle J et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. N Engl J Med. 2010;362(14):1273-81.

103. Agostinis P al. Photodynamic therapy of cancer: an update. CA: Can J Clin. 2011;61(4):250-81.

104. Shinohara ET, Mitra N, Guo M, Metz JM. Radiotherapy is associated with improved survival in adjuvant and palliative treatment of extrahepatic cholangiocarcinomas. Int J Radiat Oncol Biol Phys. 2009;74(4):1191-8.

105. Rothman J. Modern Epidemiology. 3rd ed: Lippincott Williams and Wilkins; 2008.

106. Welzel TM et al. Impact of classification of hilar cholangiocarcinomas (Klatskin tumors) on the incidence of intra- and extrahepatic cholangiocarcinoma in the United States. J Natl Cancer Inst. 2006;98(12):873-5.

107. Goodman MT, Yamamoto J. Descriptive study of gallbladder, extrahepatic bile duct, and ampullary cancers in the United States, 1997-2002. Cancer Causes Control. 2007;18(4):415-22.

108. Castro FA, Koshiol J, Hsing AW, Devesa SS. Biliary tract cancer incidence in the United States-Demographic and temporal variations by anatomic site. Int J Can. 2013;133(7):1664-71.

109. Yang JD et al. Biliary tract cancers in Olmsted County, Minnesota, 1976-2008. Am J Gastroenterol. 2012;107(8):1256-62.

110. Rostain F et al. Trends in incidence and management of cancer of the ampulla of Vater. World J Gastroenterol. 2014;20(29):10144-50.

111. WHO World Cancer Report 2008. 2008.

112. West J et al. Trends in the incidence of primary liver and biliary tract cancers in England and Wales 1971-2001. Br J Cancer. 2006;94(11):1751-8.

113. Lepage C et al. Trends in the incidence and management of biliary tract cancer: a French population-based study. J Hepatol. 2011;54(2):306-10.

114. Jepsen P et al. Incidence rates of intra- and extrahepatic cholangiocarcinomas in Denmark from 1978 through 2002. J Natl Cancer Inst. 2007;99(11):895-7.

115. Pinter M et al. Incidence and mortality trends for biliary tract cancers in Austria. Liver international. 2014;34(7):1102-8.

116. Grundmann RT, Meyer F. Gender-specific influencing factors on incidence, risk factors and outcome of carcinoma of the liver, gallbladder, extrahepatic bile duct and pancreas. Zentralbl Chir. 2014;139(2):184-92.

117. Hsing AW, Gao YT, Devesa SS, Jin F, Fraumeni JF, Jr. Rising incidence of biliary tract cancers in Shanghai, China. Int J Can. 1998;75(3):368-70.

118. The National Board of Health and Welfare. Cancerregistret Kodningsinstruktion 2010. 2010, Stockholm.

119. Simpson E et al. Estrogen, a fundamental player in energy homeostasis. J Steroid Biochem Mol Biol. 2005;95(1-5):3-8.

120. Cunningham FL, K.; Bloom, S.; Hauth, J.; Rouse, D.; Spong, C. Williams Obstetrics. 23 ed. The United State of America: The McGraw-Hill Companies; 2010.

121. Loriaux DL, Ruder HJ, Knab DR, Lipsett MB. Estrone sulfate, estrone, estradiol and estriol plasma levels in human pregnancy. J Clin Endocrinol Metab. 1972;35(6):887-91.

122. Cauley JA et al. The epidemiology of serum sex hormones in postmenopausal women. Am J Empidemiol. 1989;129(6):1120-31.

123. Luoto R, Kaprio J, Uutela A. Age at natural menopause and sociodemographic status in Finland. Am J Empidemiol.. 1994;139(1):64-76.

124. Jacobsen BK, Heuch I, Kvale G. Age at natural menopause and all-cause mortality: a 37-year follow-up of 19,731 Norwegian women. Am J Empidemiol.. 2003;157(10):923-9.

125. Bengtsson C, Lindquist O, Redvall L. Menstrual status and menopausal age of middle-aged Swedish women. A population study of women in Goteborg 1968--69 and 1974--75. Acta obstetricia et gynecologica Scandinavica. 1981;60(3):269-75.

126. Early Breast Cancer Trialists' Collaborative G. Tamoxifen for early breast cancer. Cochrane Database. 2001(1):CD000486.

127. Crawford ED. Hormonal therapy in prostate cancer: historical approaches. Reviews in urology. 2004;6 Suppl 7:S3-S11.

128. Gatta G et al. Prostate cancer treatment in Europe at the end of 1990s. Acta Oncol. 2009;48(6):867-73.

129. Medical Products Agency. Behandling med HRT - Behandlingsrekommendationer. Information från Läkemedelverket. 2004;15(3).

130. Hoffmann M et al. Changes in women's attitudes towards and use of hormone therapy after HERS and WHI. Maturitas. 2005;52(1):11-7.

131. Santen RJ et al. Postmenopausal hormone therapy: an Endocrine Society scientific statement. J Clin Endocrinol Metab. 2010;95(7 Suppl 1):s1-s66.

132. Shapiro S, Farmer RD, Stevenson JC, Burger HG, Mueck AO. Does hormone replacement therapy cause breast cancer? An application of causal principles to three studies. Part 4: the Million Women Study. J Fam Plann Reprod Health Care. 2012;38(2):102-9.

133. Grodstein F, Newcomb PA, Stampfer MJ. Postmenopausal hormone therapy and the risk of colorectal cancer: a review and meta-analysis. Am J Med. 1999;106(5):574-82.

134. Lindblad M, Garcia Rodriguez LA, Chandanos E, Lagergren J. Hormone replacement therapy and risks of oesophageal and gastric adenocarcinomas. Br J Cancer. 2006;94(1):136-41.

135. Cirillo DJ et al. Effect of estrogen therapy on gallbladder disease. JAMA. 2005;293(3):330-9.

136. Carriaga MT, Henson DE. Liver, gallbladder, extrahepatic bile ducts, and pancreas. Cancer. 1995;75(1 Suppl):171-90.

137. Moerman CJ, Berns MP, Bueno de Mesquita HB, Runia S. Reproductive history and cancer of the biliary tract in women. Int J Can. 1994;57(2):146-53.

138. Bahmanyar S et al. Parity and risk of stomach cancer by sub-site: a national Swedish study. Br J Cancer. 2008;98(7):1295-300.

139. Adami HO et al. Parity, age at first childbirth, and risk of ovarian cancer. Lancet. 1994;344(8932):1250-4.

140. Kvale G, Heuch I, Nilssen S. Parity in relation to mortality and cancer incidence: a prospective study of Norwegian women. Int J Epidemiol. 1994;23(4):691-9.

141. Tavani A, Negri E, La Vecchia C. Menstrual and reproductive factors and biliary tract cancers. Eur J Cancer Prev. 1996;5(4):241-7.

142. La Vecchia C, Negri E, Franceschi S, Parazzini F. Long-term impact of reproductive factors on cancer risk. Int J Can. 1993;53(2):215-9.

143. Shukla VK, Chauhan VS, Mishra RN, Basu S. Lifestyle, reproductive factors and risk of gallbladder cancer. Singapore Med J. 2008;49(11):912-5.

144. Plesko I et al. Parity and cancer risk in Slovakia. Int J Can. 1985;36(5):529-33.

145. Pandey M, Shukla VK. Lifestyle, parity, menstrual and reproductive factors and risk of gallbladder cancer. Eur J Can. 2003;12(4):269-72.

146. Zatonski WA et al. Epidemiologic aspects of gallbladder cancer: a case-control study of the SEARCH Program of the International Agency for Research on Cancer. J Natl Cancer Inst. 1997;89(15):1132-8.

147. Andreotti G et al. Reproductive factors and risks of biliary tract cancers and stones: a population-based study in Shanghai, China. Br J Cancer. 2010;102(7):1185-9.

148. Khan ZR, Neugut AI, Ahsan H, Chabot JA. Risk factors for biliary tract cancers. Am J Gastroenterol. 1999;94(1):149-52.

149. Jain K et al. Risk factors for gallbladder cancer: a case-control study. Int J Can. 2013;132(7):1660-6.

150. Gallus S et al. Post-menopausal hormonal therapy and gallbladder cancer risk. Int J Can. 2002;99(5):762-3.

151. Fernandez E et al. Hormone replacement therapy and cancer risk: a systematic analysis from a network of case-control studies. Int J Can. 2003;105(3):408-12.

152. Adami HO, Persson I, Hoover R, Schairer C, Bergkvist L. Risk of cancer in women receiving hormone replacement therapy. Int J Can. 1989;44(5):833-9.

153. The WHO Collaborative Study of Neoplasia and Steroid Contraceptives. Combined oral contraceptives and gallbladder cancer. Int J Epidemiol. 1989;18(2):309-14.

154. Persson I, Yuen J, Bergkvist L, Schairer C. Cancer incidence and mortality in women receiving estrogen and estrogen-progestin replacement therapy--long-term follow-up of a Swedish cohort. Int J Can. 1996;67(3):327-32.

155. Bennion LJ, Ginsberg RL, Gernick MB, Bennett PH. Effects of oral contraceptives on the gallbladder bile of normal women. N Engl J Med. 1976;294(4):189-92.

156. Hulley S et al. Noncardiovascular disease outcomes during 6.8 years of hormone therapy: Heart and Estrogen/progestin Replacement Study follow-up (HERS II). JAMA. 2002;288(1):58-66.

157. The coronary drug project research group. Gallbladder disease as a side effect of drugs influencing lipid metabolism. Experience in the Coronary Drug Project. N Engl J Med. 1977;296(21):1185-90.

158. Alvaro D et al. Estrogens and the pathophysiology of the biliary tree. World J Gastroenterol. 2006;12(22):3537-45.

159. Alvaro D et al. Estrogens and insulin-like growth factor 1 modulate neoplastic cell growth in human cholangiocarcinoma. Am J Pathol. 2006;169(3):877-88.

160. Sampson LK, Vickers SM, Ying W, Phillips JO. Tamoxifen-mediated growth inhibition of human cholangiocarcinoma. Cancer research. 1997;57(9):1743-9.

161. Gupta P, Agarwal A, Gupta V, Singh PK, Pantola C, Amit S. Expression and clinicopathological significance of estrogen and progesterone receptors in gallbladder cancer. GCR. 2012;5(2):41-7.

162. Srivastava A, Sharma KL, Srivastava N, Misra S, Mittal B. Significant role of estrogen and progesterone receptor sequence variants in gallbladder cancer predisposition: a multi-analytical strategy. PloS one. 2012;7(7):e40162.

163. Park SK, Andreotti G, Sakoda LC, Gao YT, Rashid A, Chen J, et al. Variants in hormone-related genes and the risk of biliary tract cancers and stones: a population-based study in China. Carcinogenesis. 2009;30(4):606-14.

164. Ludvigsson JF, Otterblad-Olausson P, Pettersson BU, Ekbom A. The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research. Eur J Epidemiol. 2009;24(11):659-67.

165. National Board of Health and Welfare. Ett halvt sekel med svenska cancerregistret. 2008, Stockholm.

166. Klint A, Talback M, Holmberg L. [Cancer Registry reporting can be improved]. Lakartidningen. 2009;106(11):752-3.

167. Mattsson B, Wallgren A. Completeness of the Swedish Cancer Register. Non-notified cancer cases recorded on death certificates in 1978. Acta radiologica Oncology. 1984;23(5):305-13.

168. Kilander C, Mattsson F, Ljung R, Lagergren J, Sadr-Azodi O. Systematic underreporting of the population-based incidence of pancreatic and biliary tract cancers. Acta Oncol. 2013.

169. National Board of Health and Welfare. Kvalitet och innehåll i patientregistret - Utskrivningar från slutenvården 1964–2007 och besök i specialiserad öppenvård (exklusive primärvårdsbesök) 1997–2007. 2009, Stockholm.

170. Ludvigsson JF et al. External review and validation of the Swedish national inpatient register. BMC public health. 2011;11:450.

171. National Board of Health and Wellfare. Dödsorsaksstatistik. Historik, produktionsmetoder och tillförlitlighet. http://www.socialstyrelsen.se/; 2010.

172. Ludvigsson J et al. Registers of the Swedish total population and their use in medical research. Eur J Epidemiol. 2016;31(2):125-36.

173. Ekbom A. The Swedish Multi-generation Register. Methods Mol Biol. 2011;675:215-20.

174. Wettermark B et al. The new Swedish Prescribed Drug Register--opportunities for pharmacoepidemiological research and experience from the first six months. Pharmacoepidemiology and drug safety. 2007;16(7):726-35.

175. Wang MH, Shugart YY, Cole SR, Platz EA. A simulation study of control sampling methods for nested case-control studies of genetic and molecular biomarkers and prostate cancer progression. Cancer Epidemiol Biomarkers Prev. 2009;18(3):706-11.

176. Sjolander A, Greenland S. Ignoring the matching variables in cohort studies - when is it valid and why? Stat Med. 2013;32(27):4696-708.

177. Austin PC. A critical appraisal of propensity-score matching in the medical literature between 1996 and 2003. Stat Med. 2008;27(12):2037-49.

178. Shrier I, Platt RW. Reducing bias through directed acyclic graphs. BMC Med Res Methodol. 2008;8:70.

179. Nilsson M, Tavelin B, Axelsson B. A study of patients not registered in the Swedish Cancer Register but reported to the Swedish Register of Palliative Care 2009 as deceased due to cancer. Acta Oncol. 2014;53(3):414-9.

180. Salehi S et al. Reporting and incidence trends of hydatidiform mole in Sweden 1973-2004. Acta Oncol. 2011;50(3):367-72.

181. Le MD, Henson D, Young H, Albores-Saavedra J. Is gallbladder cancer decreasing in view of increasing laparoscopic cholecystectomy? Ann Hepatol. 2011;10(3):306-14.

182. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract. 2010;87(1):4-14.

183. WHO. Obesity: preventing and managing the global epidemic. 2004, Geneva.

184. Modig K, Drefahl S, Andersson T, Ahlbom A. The aging population in Sweden: can declining incidence rates in MI, stroke and cancer counterbalance the future demographic challenges? Eur J Epidemiol. 2012;27(2):139-45.

185. Heliovaara M, Aromaa A. Parity and obesity. J Epidemiol Community Health. 1981;35(3):197-9.

186. Garfield CF, Duncan G, Gutina A, Rutsohn J, McDade TW, Adam EK, et al. Longitudinal Study of Body Mass Index in Young Males and the Transition to Fatherhood. Am J Mens Health. 2016;10(6):NP158-NP67.

187. Su Y, Ahsan H, Neugut AI. The association between biliary tract cancers and cancers of other sites. Am J Gastroenterol. 1999;94(8):2256-62.

188. Stampfer MJ, Maclure KM, Colditz GA, Manson JE, Willett WC. Risk of symptomatic gallstones in women with severe obesity. Am J Clin Nutr. 1992;55(3):652-8.

189. Matthews KA, Kuller LH, Wing RR, Meilahn EN, Plantinga P. Prior to use of estrogen replacement therapy, are users healthier than nonusers? Am J Epimdemiol. 1996;143(10):971-8.