Surgical practice patterns and outcomes in T2 and T3 gallbladder cancer: insights from a population based study

by

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Abstract

**Background:** Gallbladder cancer (GBC) is a lethal malignancy. Surgery remains the only option for cure. Our study aimed to evaluate practice patterns in patients with stage T2 and T3 GBC and describe the association between the extent of surgical resection and overall survival.

**Methods:** This was a population-based cohort study from 2002-2012 including all cases of GBC in Ontario identified using the Ontario Cancer Registry (OCR). Those who underwent surgical resection were identified using linked administrative datasets and their pathology reports were abstracted to identify T2 and T3 GBCs. Type of surgical resection was classified as ‘extended’ (cholecystectomy + liver resection and/or bile duct resection) or ‘simple’ (cholecystectomy only). The association between type of surgical resection and OS was explored using Cox proportional hazards regression models.

**Results:** 232 cases of T2 and 138 cases of T3 GBC were identified with 24% (56/232) of T2 cases and 37% (51/138) of T3 cases receiving extended resection. Unadjusted overall 5-year survival for simple vs extended resection was 39.7% vs 49.5% for T2 GBC (p =0.03) and 13.5% vs 22.8% for T3 GBC respectively (p=0.05). In T2 adjusted analysis, extended resection was associated with improved overall survival (OS) (HR = 0.51; 95% CI 0.30 -0.97, P = 0.01), while poor differentiation (HR = 3.42; 95% CI 1.92 -6.08, P = 0.0001), presence of lymphovascular invasion (HR = 1.75; 95% CI 1.16 -2.64, P = 0.03), and positive lymph nodes (HR = 1.78; 95% CI 1.03 -3.08, P = 0.03) was associated with worse OS. In T3 adjusted analysis, only female sex (HR = 0.66; 95% CI 0.43-1.00, P = 0.05) was a predictor of improved OS, while older age (HR = 1.04; 95% CI 1.02-1.04, P = 0.0005) was associated with worse OS. On stratified analysis, extended resection demonstrated a trend towards improved survival in node negative cases only (HR=0.20; CI 0.03-1.06, P=0.07).

**Conclusions:** The use of extended resection for T2 and T3 GBC in Ontario is modest. Extended resection is associated with improved OS in all T2 disease and node negative T3 disease.

**Keywords:** gallbladder cancer, extended resection, population-based study, overall survival
Co-Authorship

This thesis is the work of Senthuran Tharmalingam in collaboration with co-supervisors, Dr. Sulaiman Nanji and Dr. Harriet Richardson and his thesis committee member, Dr. Jennifer Flemming. The study was designed by Senthuran Tharmalingam with input from Dr. Nanji, Dr. Flemming and Dr. Richardson. All data collection/abstraction was performed by Senthuran Tharmalingam using an electronic template created with the help of Ms. Tina Dyer at Cancer Care and Epidemiology (CCE). Data linkage was performed by Senthuran Tharmalingam. Work by Ms. Jina-Zhang Salomons at CCE was used to obtain the median household income (MHI) at the level of the dissemination area (DA) from Statistics Canada. Data preparation and statistical analysis was performed by Senthuran Tharmalingam with input and guidance from Dr. Nanji, Dr. Richardson and Dr. Flemming. This thesis was written by Senthuran Tharmalingam with feedback and revisions with respect to wording and content provided by Dr. Nanji, Dr. Richardson and Dr. Flemming.
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Thank you.
Statement of Originality

I hereby certify that all of the work described within this thesis is the original work of the author. Any published (or unpublished) ideas and/or techniques from the work of others are fully acknowledged in accordance with the standard referencing practices.

(Senthran Tharmalingam)

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<td>Canadian Institute for Health Information</td>
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Chapter 1

Introduction

Background

Gallbladder cancer (GBC) is a rare but lethal malignancy. The median survival of all stages of gallbladder cancer is 6 months (1). In Ontario, the age standardized incidence rate is 11.4 per 100 000 person-years and the mortality rate in this cohort for GBC was 6.4 per 100 000 person years and has remained stable over the last 20 years (1). The etiology of GBC remains unknown, however, various factors, both genetic and environmental, have been postulated to play a role (2). Treatment options are limited with surgery offering the only option for cure. The role for adjuvant treatment (chemotherapy and/or radiation) in GBC is unclear as there is no existing evidence-based clinical study to demonstrate any benefit (3). The surgical management is dictated by the T stage of cancer. The earliest stage, T1a GBC, is managed with a simple cholecystectomy while the most advanced stage T4 GBC is considered palliative. The extent of resection needed for stage T1b, T2 and T3 cancers is unclear.

Rationale

Given the rarity of GBC, most studies in the literature describe small, single center observational experiences in surgical management (4). Given the lack of level one evidence, there are currently no consensus guidelines on the surgical management of gallbladder cancers, except for the earliest stage. Therefore, there remains considerable debate about the surgical management of T1b to T3 cancers where the benefit of aggressive resection in comparison to simple cholecystectomy has not been well established. Two previous population based studies using the Surveillance, Epidemiology and End Results (SEER) data from the US lack key oncologic variables such as margin status of the resected tumor and information on adjuvant treatment (5, 6). Furthermore, despite the quality assurance measures taken by the SEER registries, reliability in coding for rare histologies is not always consistent and other biliary tract cancers, such as cholangiocarcinoma, often get inadvertently included (7). Finally, there is no
large study from Canada, a population with access to universal healthcare and a potentially distinct
distribution of prognostic factors. Therefore, there is a need to study the clinical epidemiology of
gallbladder cancer in the Canadian population and assess the effectiveness of treatment modalities being
practiced in Canada.

**Objectives**

The objectives of this study are to:

1. To describe patient characteristics and treatment patterns including surgical management
   for stage T2 and T3 gallbladder cancer (GBC) in Ontario between 2002-2012.
2. To determine the independent association between extended surgical resection and
   overall survival in patients with stage T2 GBC, controlling for important prognostic
   factors.
3. To determine the independent association between extended surgical resection and
   overall survival in patients with stage T3 GBC, controlling for important prognostic
   factors.

**Thesis organization**

This thesis is organized into 5 chapters. Chapter 2 provides a review of the literature that is
relevant for this study, including: the epidemiology and prognosis of GBC, anatomy of the gallbladder
and liver, types of surgical resection, GBC staging, curative treatment options, including the available
evidence for surgery and adjuvant treatment and concludes with a comprehensive rationale. Chapter 3
outlines the methods used for this study, including more detailed objectives, the study design and cohort
population, exposure, outcome and covariate definitions, and strategies for statistical analysis. Chapter 4
presents the results from the statistical analyses in the form of a manuscript for submission to *Annals of
Surgery*. A discussion of the findings is described in Chapter 5, contextualizing the results with respect to
the current literature, along with strengths and limitations of the study and implications of this research.
References


Chapter 2

Literature Review

2.1 Introduction

Gallbladder cancer (GBC) is a rare but highly lethal malignancy. Five-year survival rates for gallbladder cancer vary from 0-12% in most reported series. The median survival is 6.4 months (1).

The diagnosis of GBC is often late, when the disease is at an advanced stage (2). Historically, it had been deemed a terminal illness with extremely poor prognosis and the surgical treatment of GBC has traditionally been “viewed with nihilism” due to poor survival results (2). However, for the last 2 decades, this has changed considerably for two main reasons. First, aggressive liver surgery has become safer through advances in anesthesia and improved peri-operative care (3). Second, with the uptake of laparoscopic surgery and relative ease of laparoscopic cholecystectomy for gallstone disease, the detection of early stage incidental gallbladder cancers have increased (3). This in turn has revived interest in improving outcomes in this disease.

2.2 Epidemiology

A recent population based study of GBC from Ontario showed an age standardized incidence rate of 11.4 per 100,000 person-years in 2012 (1). The mortality rate in this cohort for GBC was 6.4 per 100,000 person years and has remained stable over the last 20 years (1).

Gallbladder cancer rates tend to increase with advancing age. The median age was 67 years in a Memorial Sloan-Kettering report of 435 gallbladder cancer patients (4). US data from 2010 reveals that age-adjusted incidence rates in 2010 rose from 0.16/100,000 (for those 20−49 years) to 1.47/100,000 (for those 50−64 years), to 4.91/100,000 (65−74 years), and to 8.69/100,000 for individuals over the age of 75 years. This corresponded with mortality rates of 0.08/100,000 (for those 20−49 years), 0.77/100,000 (50−64 years), 2.68/100,000 (65−74 years) and 5.05/100,000 for individuals over the age of 75 (4).

There is a wide variability in the geographic pattern for GBC, unlike other tumors of the biliary tract. The incidence rates are extraordinarily high in Latin America and Asia, relatively high in some
countries in eastern and central Europe (eg, Hungary, Germany, and Poland), yet low in the United States and most western European countries. GBC tends to particularly afflict indigenous populations. South American Amerindian populations, in particular from Chile exhibit the highest rate of GBC: 12.3/100,000 for males and 27.3/100,000 for females. Indeed, gallbladder cancer is the leading cause of death in Chilean women, exceeding breast, lung and cervical cancers. Amerindians in New Mexico, USA, follow, with an average annual rate of 8.9/100,000 (4).

2.3 Etiology

The etiology of GBC remains unknown. However, various factors, both genetic and environmental, have been postulated to play a role. As stated previously, certain ethnic populations have a higher predisposition to the disease. The higher predisposition is thought to be due to as yet unknown genetic factors and environmental factors such as diet that can predispose to gallstone disease.

Indeed, gallstones represent an important risk factor for this malignancy, being present in most patients (~85%) with gallbladder cancer. The basis for this relationship likely resides in gallstones creating local mucosal irritation and chronic inflammation (4).

Obesity also plays an important role. In the Cancer Prevention Study II Nutrition Cohort, comprising 84,000 men and 97,000 women, the relative risk of gallbladder cancer was 1.8 in overweight (BMI > 25) men and 2.1 in overweight women, when compared to men and women of normal BMI, respectively (4).

Various environmental exposures have been hypothesized to contribute to gallbladder cancer. A case cohort study from a university hospital in western India comparing 38 patients with GBC and 58 patients with simple gallstone disease, showed that the mean biliary concentrations of cadmium, chromium, and lead were significantly higher in patients with carcinoma of the gall bladder than in those with gall stones (31). Another case cohort study from Nepal consisting of 50 cases and 100 controls, showed current smoking status to be a significant risk factor (OR - 2.42, CI 1.005-5.86) (32).
2.4 Anatomy and Physiology

2.4.1 Gallbladder

The gallbladder is about 7 to 10 cm long and about a 2.5 cm wide.

The gallbladder is made up of layers of tissue (Figure 1):

2. mucosa: consists of a thin inner layer of epithelial cells (epithelium) and lamina propria (connective tissue).
3. a muscular layer: a layer of smooth muscle
4. perimuscular (subserosa) layer: a layer of connective tissue that covers the muscular layer.
5. serosa: the outer covering of the gallbladder. It wraps around the gallbladder everywhere except where the gallbladder is attached to the liver.
The gallbladder, liver and small intestine are interconnected by a series of ducts. Together, they form the hepatobiliary system (Figure 2). This system is involved in the production, storage and transportation of bile from liver to the small intestine. The gallbladder is involved in the storage and concentration of bile (6).

The common hepatic duct drains bile from the liver through the left and right hepatic ducts. The cystic duct joins the gallbladder to the common bile duct. The common bile duct is where the common hepatic duct and cystic duct meet. The common bile duct then drains into the small intestine.

Figure 2: Hepatobiliary system

2.4.2 Liver

Given the intimate association of the gallbladder with the liver, many of the proposed surgical resections require partial liver resections. Liver resections are commonly carried out in anatomical planes defined by the branching patterns of the hepatic artery, portal vein and bile duct. The branching results in successive division of the liver into 2 hemi-livers (right and left), 4 sections (right anterior section, right posterior section, left medial section and left lateral section), and 8 segments (Figure 3).
Tumors of the gallbladder that extend into the liver can either be excised by anatomical resection or non-anatomical resection. Anatomic resection is based on our understanding of the segmental anatomy of the liver, where each segment has its own hepatic artery, portal vein and biliary drainage. Resection of lesions in the liver can thus be planned and carried out according to the segmental distribution. This carries the potential advantage of less bleeding as it avoids major vessels and also reduces the likelihood of leaving ischemic liver tissues behind, since the blood supply to the remnant liver is preserved. Non-anatomical or wedge resection are also commonly performed. They have a place for peripheral tumors, as may be the case in GBC or in situations where the preservation of liver substance is important. Wedge resections may reduce the amount of liver tissue removed but may lead to increased blood loss and possibility of leaving ischemic liver behind (7).
2.4.3 Lymph nodal system

The lymphatic drainage of the gallbladder descends down around the bile duct involving cystic node, pericholedocohal nodes and nodes around the proper hepatic artery (see Figure 4). Together, these comprise the “N1” nodal station (8).

From there connections are made to nodes posterior to pancreas, portal vein and common hepatic artery. Finally, the flow reaches the outer celiac artery, peri-pancreatic nodes, aorta and vena cava nodes. Together, these form the next nodal level, the “N2” station. Figure 4 demonstrates the peri-pancreatic and celiac nodes of the N2 station (9).

![Figure 4: Gallbladder nodal stations](image)

2.5 Definitions of types of surgical resection in gallbladder cancer

Simple cholecystectomy: The procedure involves removal of gallbladder with part or all of the cystic duct.

The extended resections:

A variety of operations for gallbladder cancer have been proposed. The reason for the need of an extended resection maybe due to direct extension of gallbladder tumor into liver, as may be the case in T3 disease (see “Staging” section below) or the belief that even in earlier stages of disease, as in T1b and T2 disease
(See “Staging” section below), which may be grossly confined to gallbladder, there may be microscopic disease extension into adjacent liver parenchyma. Different types/extent of “extended resection” are described and vary from institution to institution. Given the paucity of data, there is no known advantage of one type of extended resection from another. Therefore, the term radical or extended resection can mean any of the following and/or their combinations:

6. **Cholecystectomy + wedge resection:** A cholecystectomy and a non-anatomic resection of the liver surrounding the GB is performed. Typically, 2cm rim of liver beyond where the GBC invades the liver is removed. Depending on the size of the mass, a varying extent of liver is removed as a wedge. Since this is a non-anatomic liver resection, there is a risk of increased blood loss and a higher rate of a positive surgical margin. However, there is preservation of more liver tissue with this approach.

7. **Cholecystectomy + extended right hepatectomy:** This involves removal of gallbladder and anatomic liver resection including segments 4, 5, 6, 7 and 8. Typically, this larger, anatomic liver resection is used in cases where there is vascular involvement of the blood vessels supplying the right lobe of the liver by the tumor, hence necessitating removal of the entire right lobe together with the GBC.

8. **Cholecystectomy + common bile duct:** This involves removal of part or all of the bile duct. Typically, this is the case when the tumor is shown to involve the cystic duct margin. However, some surgeons recommend routine resection of the common bile duct, even in those without involvement of the cystic duct as it permits better clearance of the pericholedochal lymph nodes of the N1 station and other tissues in this area such as nerves and connective tissue that may contain “occult” metastatic disease (9).
2.6 Gallbladder cancer staging

2.6.1 TNM stages of GBC

TNM stands for Tumor, Node, and Metastasis. It describes the degree of invasion of the gallbladder (T), whether cancer cells have spread to the lymph nodes (N) and whether the cancer has spread to a different part of the body (M).

**T-stage**

There are 4 stages of tumor invasion in the current TNM classification (T1 - T4) depending on the depth of invasion of the GBC into the wall.

T1 indicates that the tumor has started to grow into the wall of the gallbladder. T1 is divided into 2 further subgroups, T1a and T1b. T1a implies that the cancer has grown into the lamina propria (connective tissue) layer underneath the mucosa (inner lining) of the gallbladder wall. T1b implies that the cancer has started to grow into the muscle layer (below the lamina propria).

T2 denotes that the cancer is still contained in the gallbladder but has grown through the main muscle layer of the wall into the subserosa (another layer of connective tissue).

T3 means the cancer has grown right through the gallbladder wall. It has grown through the outer layer (serosa) and into one other nearby organ such as the stomach, bowel or pancreas. Alternatively, it may have grown directly into the liver.

T4 denotes that the cancer has grown into one of the nearby main blood vessels into the liver (the portal vein or hepatic artery) or that it has grown into 2 or more organs outside of the liver.

**N-stage**

There are 3 main stages of lymph node involvement in cancer of the gallbladder. N0 means there is no spread to lymph nodes. As previously discussed, N1 means there are cancer cells in one or more nearby lymph nodes, notably the node involving the cystic duct, nodes along the bile duct and nodes
along the main blood vessels to the liver (hepatic artery nodes). N2 means there are cancer cells in lymph nodes further away from the gallbladder, specifically around the aorta, inferior vena cava or celiac artery (8).

**M-stage**

M0 means there is no distant spread. M1 means the cancer has spread to another part of the body far away from the gallbladder, such as the brain or lungs.

**Stages**

Together the T, N and M stages give a complete description of the overall stage of GBC. A summary of the TNM stages is presented in figure 1. There have been many changes between the 6th and 7th edition in the staging of GBC from the American Joint Committee on Cancer (AJCC) (10).

**Stage 1**: This is the earliest stage of invasive cancer. The tumor is above the muscle and subserosal layer. It has not spread to nearby tissues, lymph nodes or other organs. Stage 1 corresponds to T1, N0, M0.

**Stage 2**: Stage 2 corresponds to T2, N0, M0.

**Stage 3**: Stage 3 is subdivided into 3a and 3b. 3a corresponds to T3, N0, M0. 3b can represent T1-T3 disease with metastasis to N1 nodal station.

**Stage 4**: Stage 4 is further subdivided into 4a and 4b. 4a corresponds to corresponds to T4 disease with or without nodal involvement. 4b can either represent any T stage with N2 disease or distant metastatic disease.
The R classification is sometimes used along with the TNM staging to denote the presence or absence of residual tumor after treatment. The R classification describes the residual tumor as microscopic or macroscopic in amount. Although, it is typically used to describe the tumor after surgical resection, it can be used after non-operative treatment such as chemo or radiotherapy. The R categories are defined as follows:

R0: No residual tumor after treatment.

Prognosis

The American Joint Committee on Cancer “Cancer Staging Manual” assessed 10,000 patients diagnosed with gallbladder cancer from 1989 to 1996. The 5-year survival rates start at 50% for stage I, then progressively fall to 28% for stage II, 8% for stage IIIa, 7% for stage IIIb, 4% for stage IV (11).
The primary treatment of gallbladder cancer is surgery (1, 2, 3). However, the optimal type of resection for the different T-stages remains controversial (1, 2, 3).

2.7 Controversies in treatment

2.7.1 Surgical controversies

While surgery offers the only potential for cure in gallbladder cancer, the extent of resection required in cases of advanced gallbladder cancer varies. Given the rarity of the disease, most studies in the literature describe small, single center observational experiences (8). Given the lack of experimental evidence, there are currently no consensus guidelines on the surgical management of gallbladder cancers, except for the earliest stage (T1a) (12).

The management of gallbladder cancer is determined by the clinical stage, in particular the T stage, of disease.

T1a tumors

For treatment of early gallbladder tumors, confined to the lamina propria (Tis or T1a), simple cholecystectomy is curative (12). This is the only stage where there is agreement as to the surgical management.

T1b tumors

The management of tumors that invade the muscularis propria (T1b) is controversial. Many groups including Wagholikar et al. advocate more aggressive, radical resection than T1a disease given the relative increased predisposition for lymph node metastases (up to 15% of cases) (9, 10, 13). However, the necessity for aggressive surgery has been questioned by Wakai et al, who examined 25 patients with T1b tumors, 13 underwent simple cholecystectomy and 12 had a radical cholecystectomy and lymph node dissection (14). There was no evidence of metastatic disease in 147 lymph nodes examined. The overall survival was 87% and was similar in both groups.
**T2 tumors**

T2 lesions have a higher rate of liver and lymph node involvement. Several retrospective cohort studies have compared survival for tumors after simple cholecystectomy and radical resection with an approximate doubling of survival after more aggressive surgery. Wakai et al., with a sample of 13 patients, demonstrated a 5-year survival rate for T2 tumors of 50% and 100%, for simple cholecystectomy vs extended resection, respectively (14). De Aretxabala et al. observed an increase in 5-year survival from 20% to 70% in 109 patients with extended resections (15) and Fong et al, with a cohort of 53 patients observed an increase in 5-year survival from 19% to 61%, similar to Chijiba et al who also observed an increase in 5-year survival from 19% to 61% for T2 tumors in 28 patients with extended resections (15, 16). Although these and other studies have demonstrated an improvement in survival with extended resection, once again, these observations are not universal and the definition of radical surgery varies from one study to the other. Suzuki and colleagues demonstrated a 5-year survival rate of 77% in a series of T2 gallbladder cancer following simple cholecystectomy or extended resection with no overall survival difference between surgical treatments (17). Toyoaga and colleagues reported a series of 43 patients with T2 disease (17). Almost half of this group had undergone simple cholecystectomy and the other half an extended resection. The overall survival rate was 54% and was not statistically different in the two groups. However, they did observe that patients with positive tumor margins from a simple cholecystectomy showed a significant improvement in survival after undergoing an extended resection. Therefore, the large difference in survival results from these small single institution studies maybe due to effects such as margin status and other unmeasured pathologic and clinical factors.

**T3 tumors**

The management of T3 also varies. Japanese centers have generally been aggressive with an anatomic extended liver resection, removal of the common bile duct and in some cases partial removal of the pancreas and duodenum (12). The reported 5-year survival rates vary from 38% to 63%. However, North American centers have been less enthusiastic to universally adopt such an aggressive surgical approach due to poor survival results and prefer to be more selective in whom they offer such aggressive
resection (12). Fong et al. from Memorial Sloan Kettering reported 5-year survival of 21% in 8 patients undergoing radical resection (ranging from wedge resection, right hepatectomy to extended right hepatectomy with common bile duct resection) for T3 cancers (16). Dixon et al. from the University of Toronto report improved survival in 28 patients with at least T3 cancers, undergoing radical resection compared to simple cholecystectomy (18). However, a large population based study by Jensen et al, did not find an improvement in overall survival between simple cholecystectomy and extended resection for T3 cancers (19).

Therefore, there remains considerable debate in the surgical management of T1b to T3 cancers where the benefit of aggressive resection in comparison to simple cholecystectomy has not been well established. Of those with radical resection, the extent of hepatic resection is also not well established.

Given the difficulty in interpreting the results of these small retrospective studies, population based studies have been undertaken to address the question of optimal surgical resection.

### 2.7.2 Population based studies

There are currently 4 large cohort studies that have attempted to address some of these challenging issues using a population based method (19, 20, 21, 22). One of the studies is from a national registry in Chile and simply examines the effect of pathologic prognostic factors on survival (20). Three other studies have all used the Surveillance, Epidemiology and End Results (SEER) registry in the United States (19, 21, 22). Of the 3, only the studies by Jensen et al (2009) and Coburn et al. (2008) address the question of extent of resection and survival (19, 21).

Jensen et al. identified 4,631 patients who underwent surgery for gallbladder cancer from 1988 through 2004. Of these patients, 4,188 (90.4%) underwent cholecystectomy alone and 443 (9.6%) underwent radical surgery including hepatic resection. The proportion of patients having radical surgery for T1b, T2, and T3 cancers was 4.5%, 5.6%, and 16.3%, respectively. For patients with T1b/T2 cancer, radical resection was associated with significant improvement in overall cancer (median overall survival of 123 months vs 23 months, respectively). For patients with T3 cancers, there was no improvement in overall survival (10 months for radical resection vs. 6 months for simple cholecystectomy (19).
Coburn et al. looked at extent of resection (simple cholecystectomy vs en bloc resection) and extent of lymphadenectomy (>3 nodes or <3 nodes) on survival of T1-T3 cancers. They again used the SEER database from 1988-2005 and identified 2835 patients. Once again, they remark on the paucity of patients with T2-T3 cancers who underwent en bloc resection (8.6%). Their analysis concludes that en bloc resection has improved survival in patients with T2 cancers (median survival of 25 months vs 19 months, respectively), but did not improve survival in T3 cancers (medial survival of 11 months vs 10 months). With regards to lymphadenectomy, a survival benefit was conferred when 3 or more lymph nodes were assessed (21).

Both population based studies are limited by information with regards to key oncologic variables such as margin status and adjuvant treatment data. Furthermore, confounding by indication, is a primary threat to the validity of using observational data to estimate benefits of treatment. No information related to patient comorbidity, nutritional status or performance status is available. These variables can act as a source of bias by influencing a physician’s clinical judgment in who receives aggressive extended resection.

2.7.3 Lymphadenectomy

Removal of lymph nodes is a vital part of cancer surgery. However, agreement with regards to benefits of lymphadenectomy in GBC was not always evident.

In 1998, Beonist and associates reported that there were no long-term survivors with N1 (node positive) disease among 21 patients who underwent radical resection with portal lymph node dissection and concluded that radical resection should only be considered in the absence of regional lymph node metastasis (23). In 2005, Dixon et al documented an improvement in survival in 99 patients with gallbladder cancer who underwent curative extended resection, however there were no 5-year survivors with N1 disease in the series, suggesting that nodal dissection very rarely achieves long-term survival in patients with N1 disease (18).

Despite initial nihilism from the West with regards to lymphadenectomy, Japanese groups continued to suggest that N1 patients may survive for 5 years after a potentially curative, margin negative
(R0) resection with extended portal lymphadenectomy. In recent years, Western groups have also reported some long term survivors with N1 disease undergoing extended lymphadenectomy (23).

Therefore, while it is accepted today that an extended lymphadenectomy may confer a survival advantage, the extent of lymphadenectomy for gallbladder cancer remains controversial (7). In 2006, Shirai et al. showed that extended portal lymphadenectomy is effective against up to three positive lymph nodes, provided an R0 resection is achieved (37). This suggests that there is a survival benefit in patients with a “modest” degree of N1 disease (3 or less).

However, other groups have recently argued that it is not the presence of positive nodes that affects survival, but rather the harvesting of 1, 3, 6 or even 8 nodes (regardless of whether they are negative or positive nodes) that affects survival (7).

Brinbaum et al. have explored this concept further and report that the lymph node ratio (number of metastatic lymph nodes to number of retrieved lymph nodes) is a better prognostic indicator than total lymph nodes harvested or number of positive nodes (23). However, Kim et al. have challenged the benefit of lymph node ratio as a prognostic factor, particularly in T3 disease (24).

Therefore, while there is mounting evidence for the benefit of lymphadenectomy, the extent of disease and the benefits of sampling non-metastatic nodes remain unclear. Despite this knowledge, based on population-based studies in the United States, the average nodal harvest for GBC remains low at 1-2 nodes (21, 22). The Canadian experience is not known.

2.7.4 Adjuvant treatment

The role of adjuvant treatment (chemotherapy and/or radiation) in GBC is unclear and there is no existing evidence-based clinical study to demonstrate any benefit in gallbladder cancer (29). Available reports on adjuvant treatment contain small numbers of patients with incomplete reporting of treatment data, and are strongly biased by patient selection. Furthermore, these studies frequently group together other biliary tract tumors such as intrahepatic or extrahepatic cholangiocarcinoma. Despite the lack of evidence, chemoradiotherapy is often proposed ad hoc after resection because of the intrinsic poor prognosis in this patient population (25).
A retrospective study from the MD Anderson cancer center in 2012 analyzed the impact of neoadjuvant or adjuvant chemotherapy in 63 GBC patients having undergone surgical resection. Neoadjuvant treatment was given to 20% of patients, which delayed surgical resection by a mean of 6.8 months compared to those managed with a surgery-first approach, and this did not prolong survival from the date of diagnosis. Furthermore, patients treated with adjuvant chemotherapy had a poorer median overall survival (3.8 vs. 5.8 years) (26). The study is limited by lack of information on extent of surgery, and pathologic stage of disease. Furthermore, the cohort combined GBC patients and patients with cholangiocarcinoma, in order to increase the study power, but in doing so, baseline differences in the two disease entities were ignored.

There are some reports exploring the role of adjuvant therapy following aggressive surgical resection with negative margins. One example is from the institution in Korea with 166 patients that underwent surgical resection with curative intent. Of these, almost a quarter received some form of adjuvant therapy. Neither adjuvant radiotherapy, chemotherapy or chemoradiotherapy had any impact on disease free survival (25).

However, another study from Korea by Cho et al that included 68 patients reported somewhat different results. All 68 patients had T2 or T3 disease that underwent margin negative resection and adjuvant chemoradiotherapy. Their results show that presence of positive lymph nodes (N1 disease) was associated with improvement in disease free survival with adjuvant chemoradiotherapy (p<0.05). The survival difference was not seen in the case of lymph node negative (N0) disease (p=0.45). The result held true for both T2 and T3 disease. There was no benefit in patients without nodal metastasis (33).

Finally, a phase 3 multicenter prospective randomized controlled trial from Japan looked at effect of chemotherapy + extended surgery (treatment group, n=69 patients) compared to extended surgery alone (control group, n=43 patients). The 5-year survival was significantly better in the chemotherapy group compared to surgery alone group (26.6vs 14.5%). However, the majority of the patients (46% in treatment group and 60% in control group) had stage 4 metastatic disease (27).
Therefore, the evidence for benefit of chemoradiotherapy in GBC remains elusive. Results of studies in the literature range from improvement from adjuvant chemoradiotherapy to no survival benefit and to even harmful effect on survival. Most of these studies are hampered by lack of information on important pathologic factors and are not stratified by specific T stage. Studies comparing chemoradiotherapy to surgery alone need to be controlled for several factors that are thought to influence prognosis, notably T-stage, N stage, margin status and adequacy of surgery.

2.8 Prognostic factors for survival in gallbladder cancer

Other than surgical resection, several factors have been previously reported to affect survival in gallbladder cancer. These factors can be divided into patient related (age, sex, and socioeconomic status), treatment related (type/extent of surgery and adjuvant treatment) and pathologic factors (margin status, presence of perineural invasion, presence of lymphovascular invasion, number of lymph nodes harvested and presence of positive nodes). Role of extent of surgery, lymphadenectomy and adjuvant treatment have previously been discussed. The following section will assess the other remaining risk factors.

Age: The mean age at diagnosis of gallbladder cancer, from a systematic review of 29 series, was 65.2 years. When stratified by age, the incidence of gallbladder carcinoma was 0.3% in those under 50 years of age, 3.8% in those over 50 years old, and 8.8% in those older than 65 years of age (4). In multiple single institution studies, age has been shown to be an important risk factor for gallbladder cancer survival after controlling for other confounders. The hazard ratio for age ranges from 1.45 to 5.17. However, the large population based study by Coburn et al. failed to demonstrate an effect of age on multivariate analysis for T2 and T3 cancers, although it existed for T1 disease (22).

Sex: There is very compelling evidence that shows that gallbladder cancer occurs more than twice as often in women. Gallstones and gallbladder inflammation are important risk factors for gallbladder cancer and are also much more common in women than men. It is hypothesized that the underlying mechanism is female sex hormones that adversely influence bile secretion and gallbladder function (28).

Socio-economic status (SES): The large population-based study from Chile had identified patients of low SES and low schooling as being at higher risk of death from gallbladder cancer (19). While the effect of
SES on treatment and survival should not be affected in a country with universal healthcare such as Canada, it remains an important covariate that has not been previously studied in developed countries.

**Margin status:** Achieving an adequate margin around a tumor (i.e. not leaving any tumour in situ) is important for any oncologic surgery with curative intent. Gallbladder cancer is no different. Margin status has been reported to be an important prognostic factor in a number of single institution studies. Indeed, some have suggested that more than extent of resection or any other factor, achieving negative margins (R0) should be the goal of GBC treatment. The hazard ratio of a margin positive (non R0) resection ranges from 1.2 to 13.5 (25)

**Tumor grade:** In the population-based registry study from Chile (n=1366) assessing pathologic prognostic factors in gallbladder cancer, tumor grade was found to have a statistically significant and independent effect on survival (21). The study is limited by the authors failing to account for type of surgery and adjuvant treatment in their multivariate analysis. Nevertheless, several single institution studies are concordant with these results. However, the SEER study by Coburn et al. failed to show any effect of grade on survival in multivariate analysis (21).

**Perineural and perivascular invasion:** The Chilean study also showed perivascular invasion as a statistically significant predictor of survival (20).

### 2.9 Rationale for current study

While there has been much recent interest in gallbladder cancer and its treatment, the existing literature is inconsistent and often times contradictory. Only two stages of gallbladder cancer have universal agreement with regards to treatment: simple cholecystectomy for the earliest stage (T1a) and palliative treatment for T4 disease. There is disagreement/ insufficient evidence on extent of resection, extent of lymphadenectomy, and use of adjuvant treatment for stages T1b, T2 and T3. Furthermore, if extended liver resection is important, the extent of this resection (wedge resection vs formal hepatectomy) and need for resection of adjacent structures like the bile duct remains a matter of debate.

Results from large population based studies such as the SEER registry must be analyzed with caution. Information in the SEER registry is done by abstracting for particular variables from a set
Despite the quality assurance measures taken by the SEER registries, reliability in coding for rare histologies may not always be consistent and other biliary tracts cancers, such as cholangiocarcinomas, often get inadvertently included (30). Secondly, survival data from SEER registry are difficult to analyze given the lack of pathologic data such as margin status, which has been shown to be an important prognostic factor in many cancers (22). Thirdly, SEER does not account for any adjuvant chemotherapy data (30). With regards to radiation treatment, the sensitivity of the SEER database in correctly identifying all patients is only 80% (30).

Single institution studies, while specific about type of resections and pathologic factors, often suffer from selection biases and smaller cohorts. Comparison of these single institution studies is also difficult given their differing surgical techniques, definition of extent of resection, and adjuvant treatment modalities used.

Finally, since East Asian countries are at the forefront of aggressive gallbladder surgery, much of the literature comes from Japan and Korea. There is no large study from Canada, a population with a distinct genetic makeup and risk factors. Therefore, there is a need to study the epidemiology of gallbladder cancer in the Canadian population and assess the effectiveness of treatment modalities being practiced.
References

Chapter 3

Methods

3.1 Study Objectives

1. To describe patient characteristics and treatment patterns including surgical management for stage T2 and T3 gallbladder cancer (GBC) in Ontario between 2002-2012.

2. To determine the independent association between extended surgical resection and overall survival in patients with stage T2 GBC, controlling for important prognostic factors.

3. To determine the independent association between extended surgical resection and overall survival in patients with stage T3 GBC, controlling for important prognostic factors.

3.2 Study design

This is a retrospective population based cohort study. As described by Booth & Mackillop, population based cohort studies can provide insight into practice patterns and the impact of a change in practice on outcomes (1). For these reasons, population-based outcome studies are becoming increasingly recognized as central in the generation and dissemination of medical evidence and have become an important tool for conducting comparative-effectiveness research. We undertook this population-based cohort study to evaluate whether the extent of surgical resection (exposure variable) had an impact on overall survival (outcome measure) of T2 and T3 GBC patients at a population level, while controlling for potential confounders. The extent of surgical resection was classified as: a) simple cholecystectomy b) extended (cholecystectomy + liver and/or bile duct resection).
3.3 Study population

From January 1, 2002 to March 31, 2012, patients > 18 years of age at the time of diagnosis of GBC were identified from the OCR and included in the initial cohort. A total of 1055 patients were identified. We excluded any patients misclassified as a GBC (N=28) and those with only a biopsy confirmation of GBC (N=500). 527 patients underwent complete gallbladder resections for cancer between Jan 1 2002 to March 31 2012, and of these we excluded 1) any cases of gallbladder cancers that were not adenocarcinomas or adenosquamous carcinomas (ex. Neuroendocrine tumor, small cell carcinoma, sarcomas were excluded), 2) any cases with unknown or indeterminate T stage or 3) metastatic (Stage 4) gallbladder cancer at initial surgery. Figure 6 outlines the cohort development.
Figure 6 – Identification of patients with GBC who underwent surgical resection in Ontario 2002-2012

All pts with procedural codes for GBC, n = 1055

Exclude: Biopsy only, n = 500

All pts with surgical resection for GBC, n = 555

Exclude: Non GBC, n = 28

All pts with gallbladder resection, n = 527

Exclude: Nonadenocarcinoma histology, n = 14

Pts with gallbladder adenocarcinomas, n = 513

Exclude: unknown/indeterminate T stage, n = 23

Pts with GBC and known T stage, n = 490

Exclude: metastatic gallbladder cancer and T4 stage, n = 38

T1a, T1b, T2, T3, n = 452

Exclude: T1a and T1b, n = 82

T2, n = 232

T3, n = 138
3.3.1 Ontario Cancer Registry

Since 1995, investigators at the Queen's University Division of Cancer Care and Epidemiology (CCE) have been using the Ontario Cancer Registry (OCR) to describe the management and outcomes of cancer in Ontario. The OCR is a passive, population-based cancer registry that captures diagnostic and demographic information regarding at least 98% of all incident cases of cancer diagnosed in Ontario, which has an estimated population of 13.2 million and represents 39% of the Canadian population. The OCR provided the following information: International Classification of Diseases (ICD) 9th and 10th revision codes, the International Classification of Diseases for Oncology (ICD-O) histology code, age, sex, date of diagnosis, and date of death. Patients were identified as having GBC based on ICD-9 topography code 1560 and ICD-10 topography code C23, with ICD-O histology codes 8000, 8001, 8010, 8012, 8020, 8021, 8041, 8046, 8070, 8071, 8140, 8144, 8160, 8260, 8261, 8480, 8481, 8490, 8560, and 9990. OCR data on individuals are formed through a series of probabilistic linkages from four different sources: cancer center records, Canada Institute of Health Information (CIHI) discharge data, pathology records and death certificates. Unique cases are assigned a Group ID number and all contributing source data are assigned this Group ID. This allows linkage and cross-referencing of information of data from cancer records, pathology records and death certificates.

3.3.2 Pathology reports & abstraction

After identification of patients from the OCR eligible for pathology report abstraction, the pathology reports were transferred electronically from CCO to CCE for primary data abstraction. The pathology report cohort also included all related reports for cases identified as having gallbladder cancer, including those for other related or nonrelated diagnoses for that subject. All GBC reports from January 1, 2002 to December 31, 2012 were collected. Reports prior to 2002 were not electronically recorded by the OCR and were of generally poorer quality. A total of 1564 reports were identified.

An electronic data abstraction tool was created with the help of Ms. Tina Dyer at CCE. The data abstraction tool was created in Microsoft Access 2010. Variables were carefully selected by myself and the study team, after a thorough literature review. The following data was abstracted from pathology
reports: age at operation, hospital where surgery performed, type of surgery, extent of liver resection, American Joint Committee on Cancer 7th edition (AJCC) T-category, tumor location on gallbladder (body, neck, fundus), N-stage (location of nodes, number of nodes, and number of positive nodes), surgical resection margin status (any positive vs all negative), location of margin (cystic duct and/or liver), closest distance of tumor from margin, tumor grade (1, well-differentiated; 2, Moderately differentiated; 3, poorly differentiated; 4, undifferentiated), presence of perineural and/or perivascular invasion.

3.3.4 Adjuvant treatment data

Using the unique identifier for each patient in the OCR, CCE links the cancer diagnosis to chemotherapy and radiotherapy administration data from the 14 Ontario cancer centers. A radiotherapy and chemotherapy database was assembled at Queen’s Division of Cancer Care and Epidemiology (CCE) with permission from all 14 Ontario cancer centers.

The intent of radiotherapy in the OCR is classified as curative, adjuvant or palliative. Only patients with curative and adjuvant intent were considered as ‘adjuvant” treatment in the analysis for this thesis. For those patients in whom no information was available regarding intent of treatment, only treatment received within 3 months before or after the surgical intervention date was deemed to be adjuvant. A 3 month end point was chosen after discussion with oncologists at Kingston General Hospital. Information regarding radiotherapy was only available until December 31 2011.

Chemotherapy data did not identify intent of treatment and therefore treatment within 3 months before or after the surgical intervention date was deemed to be adjuvant. Information regarding chemotherapy was available until December 31 2012.

The radiotherapy database was assembled at the Queen’s Division of Cancer Care and Epidemiology (CCE) with permission from all nine provincial cancer centers open during the period under study. Information includes body site treated with radiation, treatment intent
(palliative or curative), dose of radiation, fractionation of radiation and dates of all treatments. The centers have kept electronic radiation therapy records since 1982. This database was assembled at the Queen’s Division of Cancer Care and Epidemiology (CCE).

3.3.4 Socioeconomic status

For the purpose of our study, we estimated socioeconomic status (SES) using an ecologic measure: patient’s neighborhood median household income (MHI). MHI has been used in a number of provincial studies of oncology health services. It is well correlated with other ecological measures of SES, including poverty, education, occupation, unemployment rate, and marital status.

Information from Statistics Canada census data on MHI of the Canadian population by region has allowed linkage to this information at CCE at the census enumeration level to the patient’s postal code as listed in the OCR.

Work by Ms. Jina-Zhang Salomons was used to obtain the median household income (MHI) at the level of the dissemination area (DA) from Statistics Canada. The MHI from the 2001 census was assigned to the cases diagnosed in 2002. The MHI from the 2006 census was assigned to the cases diagnosed from 2003-2012. The DAs were grouped into quintiles based on their MHI, with the 5th quintile (Q5) representing the communities where the wealthiest 20% in Ontario resided and the 1st quintile (Q1) representing the communities where the poorest 20% resided.

Using this information, cancer and income data were linked by the patient’s postal code, and each patient was assigned an SES quintile based on the MHI of the DA in which they resided at the time of diagnosis. This was done by utilizing the Postal Code Conversion File (PCCF+), which provided the correspondence between postal codes and census DA. PCCF+ automatically assigns a range of Statistics Canada’s standard geographic areas and other geographic identifiers based on Postal Codes. The PCCF+ uses population-weighted random allocation for Postal Codes that link to more than one geographic area.
3.3.5 Vital Statistics

The OCR database is linked to the provincial death registry, providing highly complete documentation of date of death. Complete information regarding vital status in the OCR was available up to December 31, 2012, whereas information concerning cause of death was available up to December 31, 2010. To account for possible miscoding of the cause of death, cancer-specific survival included death from any cancer.

**Figure 7 - Data sources**

| OCR: Pathology reports (type of surgery and histology); Postal code; Date of birth; Date of diagnosis; Date and cause of death |
| Gallbladder cancer Database |
| CCE: Adjuvant chemo/radiotherapy from 14 Ontario cancer centers |
| Census (Statistics Canada): Median Neighborhood income |

*Legend:*

CCO – Cancer Care Ontario; OCR – Ontario Cancer Registry; CCE – Cancer Care and Epidemiology
3.4 Censoring and follow-up

Censoring occurred on December 31 2012, the last day of follow-up available in OCR. Given that the study cohort was comprised of patients having undergone surgical resection up until March 31, 2012, this allowed for at least 9 months of follow-up, which is just above the median survival of 6.4 months for all GBC patients (2). The unadjusted median survival among the study cohort was 20.4 months, with a mean of 31 months. The minimum survival was 0.3 months and maximum of 129.9 months. Follow-up time was defined from date of first surgery.

3.5 Inter-rater reliability

To assess the degree of pathology abstraction consistency and validity, the inter-rater reliability results were examined. To ensure reliability in the abstraction of the reports, a pathologist, Dr. David Hurlbut, randomly and independently, abstracted in duplicate 7% of reports. This was done iteratively throughout and ensured high quality abstraction.

The error rate was calculated on a scale of 10 points for each study subject calculated on the correct abstraction of the following variables: Date of diagnosis, type of surgery, T stage, Number of lymph nodes, number of positive lymph nodes harvested, presence of absence of perineural invasion, presence/absence of lymphovascular invasion, margin status, histologic type an grade of differentiation. An error rate of 3.5% was noted.

3.6 Creating a useable database

After the data was collected, the information obtained from the pathology reports, vital statistics from OCR, adjuvant therapy data from CCE and MHI quintiles (SES) from Statistics Canada were merged to obtain a single entry for each subject using their study identification number. The final database was entered into Excel spreadsheets, where it was formatted prior to being transferred to SAS 9.4.
3.7 Potential confounders

Potential confounders in the relationship between the exposure variable (extent of surgical resection) and outcome (overall survival) were explored. These covariates were classified into patient factors, disease factors, and treatment related factors.

3.7.1 Patient factors

Patient factors included age, sex and socioeconomic status. Age and sex are the only \textit{a priori} confounders in this study. Age is a continuous variable and sex is dichotomous. Socioeconomic status is a categorical variable based on the 5 levels/quintiles of the neighborhood median household income: Q1 (highest) to Q5 (lowest).

3.7.2 Disease factors

Disease factors include number of lymph nodes harvested, presence of positive lymph nodes, grade of differentiation, perineural invasion, and lymphovascular invasion.

1) Number of lymph nodes: The harvested number of nodes ranged from 0-16. Therefore, it was transformed into a 4 level categorical variable: 0, 1, 2 or 3+ nodes. The cut-off of 3 nodes was chosen from the AJCC 7th edition, which states at least 3 nodes need to be sampled to ensure adequate oncologic staging.

2) Positive lymph nodes: Categorized into a dichotomous variable, presence or absence of positive nodes.

3) Grade of differentiation: Grade of differentiation is defined as how different the cancer cells look from normal cells and also measures how frequently they are dividing. It is divided into a 3 level categorical variable: well, moderate, or poor. Undifferentiated is often used as the worst grade of
differentiation, however, given the small number in this group, they were considered together with the poorly differentiated.

4) Perineural invasion: Perineural invasion is defined as cancer cell invasion in, around and through nerves or the finding of tumor cells within any of the layers of the nerve sheath. Presented as a categorical variable: present, absent or unknown.

5) Lymphovascular invasion: Lymphovascular invasion is defined as spread of cancer into the microscopic blood vessels or lymphatics within the wall of the gallbladder. Presented as a categorical variable: present, absent or unknown.

3.7.3 Treatment factors:

Adjuvant treatment was considered as a potential confounder. Adjuvant treatment includes both chemotherapy and/or radiotherapy. Receiving chemo and/or radiotherapy was considered as a single data point. It was used as a dichotomous variable, receipt or no receipt of adjuvant “chemo-radiotherapy” treatment.

As previously stated, only adjuvant treatment that were explicitly stated as “curative or adjuvant intent” were included. Palliative intent treatment was not considered. Occasionally, the intent of chemo-radiotherapy was not explicitly stated. Therefore, keeping in mind the need to balance the need to exclude chemo-radiation treatment for palliation with the need to capture as many patients as possible who truly received adjuvant chemo-radiation, we set our definition of adjuvant chemo-radiation as treatment within 3 months of surgery. From a clinical standpoint, it is highly unlikely that an oncologist would offer adjuvant chemo-radiation any longer than this after surgery for GBC.
3.8 Analysis methodology

All analysis was conducted using SAS 9.4.

3.8.1 Objective 1: Identify prognostic factors associated with overall survival in surgically treated patients.

Pearson correlation and multicollinearity

Exploratory bivariate correlation analysis was initially conducted to explore the relationship between all potential prognostic variable combinations, including the exposure variable (type of surgery). Pearson correlations were computed. A very strong correlation was observed between type of surgery and number of lymph nodes harvested, suggesting a likely collinearity effect. Collinearity between number of nodes harvested and surgery is clinically explained by the simple fact that a more extended resection would involve the liver +/- bile duct and would thus lead to increased number of nodes, compared to a simple cholecystectomy, whereby the nodal harvest would expectedly be low. Therefore, number of lymph nodes was removed as a potential confounder and prognostic factor.

Survival analysis techniques were used to model the time to death, with censoring occurring on December 31, 2012. Cox regression models were used to measure the relationship between gallbladder cancer and survival, controlling for important confounders/prognostic factors.

Cox proportional hazards regression analysis was used to generate crude (unadjusted) hazard ratios (HRs) for surgery and for potential confounders and overall survival (and cancer specific 5-year survival). Independent variables that met the screening criteria (Wald p<0.25) were considered for inclusion in the final multivariable models. For the multivariable models, a backward elimination procedure, using a liberal p-value of 0.20, was employed to identify factors associated with survival. The following covariates that were assessed for potential confounding included: age, sex, socioeconomic status, presence/absence of perineural invasion, presence/absence of positive lymph nodes, and adjuvant treatment.
Socioeconomic status was not retained in the final models. In contrast, age was kept in all the models because we were interested in age as a precision variable despite its weak association with survival in the crude models.

3.8.2 Objective 2: Examine if extent of surgical resection is associated with overall survival in accordance to stages T2 and T3, using an Ontario population based cohort from 2002-2012.

Five-year overall survival rates for the entire study period was determined using the Kaplan-Meier method and compared using the log-rank test. Median 5-year survival for simple and extended cholecystectomy was calculated for T2 and T3 GBC, respectively.

To explore the hazard ratio associated with type of surgery, a Cox proportional hazards model was performed. A Cox proportional hazards model was computed using the exposure variable and confounding variables identified in objective 1 above. The computed model was assessed for its adherence to the proportional hazards assumption using plots of the natural logarithm of the cumulative baseline hazard rates.

3.9 Effect modification

No a priori effect modifiers were identified, but exploratory analysis were considered. Models with multiplicative interaction terms between the exposure variable (type of surgery) and all potential confounders were calculated. Given the smaller sample size, any interaction term approaching significance with p<0.15 was considered a potential effect modifier. If an effect modifier was identified, the analysis/results was stratified on the effect modifier.
3.10 Power calculation

The previous population based study by Coburn et al showed a significantly unequal group size between the simple choelcystetcomy and extended surgery groups, with a proportion of was 1:20 for T2 and 1:8 for T3 GBC (3. Given these proportions, Coburn et al, showed a significant HR reduction of 0.40 for T2 extended surgery and no reduction in HR reduction for T3 extended surgery. We postulate a higher proportion of cases in the extended surgery group among both the T2 and T3 cohort. In the table below, study power has been calculated for potential hazard ratios with an alpha of 0.05, assuming a cohort of 1:20 and 1:10 for T2 GBC (n=232) and 1:8 and 1:5 for T3 GBC (n=138). The power calculations were obtained using the online survival analysis calculator from San Diego State University (4).

Table A – Table of potential HRs and associated study power, $\alpha = 0.05$

<table>
<thead>
<tr>
<th>Potential HR</th>
<th>Power (1-β)</th>
<th>T2 (n=232)</th>
<th>T2 (n=232)</th>
<th>T3 (n=138)</th>
<th>T3 (n=138)</th>
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<td>1:10</td>
<td>1:8</td>
<td>1:5</td>
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<td>98.7%</td>
<td>94.5%</td>
<td>99.0%</td>
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<tr>
<td>0.50</td>
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3.11 Ethical consideration

The retrospective design of this study precluded any direct impact on the care of any patient in the study. Confidentiality was strictly maintained. The identity of the patient was known only by the student and the 2 reviewers. Each patient was given a unique study number that was used in the study. The patient's name was never abstracted from the chart. In fact, none of the electronic data include personal identifiers. The data from the chart review was stored on a secure system at CCE. Access was restricted to authorized personnel through a password sign-on system. The study received ethics clearance from Queen’s Research Ethic Board (DMED-1651-13).
References


Chapter 4

Surgical practice patterns and outcomes in T2 and T3 gallbladder cancer: insights from a population based study
ABSTRACT

Background: Gallbladder cancer (GBC) is a lethal malignancy. Surgery remains the only option for cure. Our study aimed to evaluate practice patterns in patients with T2 and T3 GBC and describe the association between the extent of surgical resection and overall survival.

Methods: A population-based cohort study from 2002-2012 including all cases of GBC in Ontario was identified using the Ontario Cancer Registry (OCR). Those who underwent surgical resection were identified using linked administrative datasets and their pathology reports were abstracted to identify T2, and T3 GBCs. Type of surgical resection was classified as ‘extended’ (cholecystectomy + liver resection and/or bile duct resection) or ‘simple’ (cholecystectomy only). 5-year overall survival (OS) based on type of resection and stage of GBC was determined using the Kaplan-Meier technique and the log-rank test. The association between type of surgical resection and OS was explored using Cox proportional hazards regression models.

Results: A total of 232 cases of T2 and 138 cases of T3 GBC were identified with 24% (56/232) of T2 cases and 37% (51/138) of T3 cases receiving extended resection. Unadjusted overall 5-year survival for simple vs extended resection was 39.7% vs 49.5% for T2 GBC (p =0.03) and 13.5% vs 22.8% for T3 GBC respectively (p=0.05). In adjusted analysis of T2 cases, extended resection was associated with improved overall survival (HR = 0.51; 95% CI 0.30 -0.97, P = 0.01), while poor differentiation (HR = 3.42; 95% CI 1.92 -6.08, P = 0.0001), presence of lymphovascular invasion (HR = 1.75; 95% CI 1.16 - 2.64, P = 0.03), and positive lymph nodes (HR = 1.78; 95% CI 1.03 -3.08, P = 0.03) led to worse OS. In adjusted analysis of T3 cases, only female sex (HR = 0.66; 95% CI 0.43-1.00, P = 0.05) was a predictor of improved OS, while older age (HR = 1.04; 95% CI 1.02-1.04, P = 0.0005) was associated with worse OS. On subgroup analysis, extended resection demonstrated a trend towards improved survival in node negative cases only (HR=0.20; CI 0.03-1.06, P=0.07).

Conclusions: The use of extended resection for T2 and T3 GBC in Ontario is modest. Extended resection is associated with improved OS in all T2 disease and node negative T3 disease.
Keywords: gallbladder cancer, extended resection, lymphadenectomy, population-based study, overall survival
4.1 Introduction

Gallbladder cancer (GBC) is a rare but highly lethal malignancy. 5-year survival rates for GBC vary from 0%-12% in most reported series with a median survival of 6.4 months (1). The diagnosis of GBC is often late when the disease is at an advanced stage (2). Historically, it had been deemed a terminal illness with extremely poor prognosis and the surgical treatment of GBC has traditionally been viewed with skepticism due to poor survival results (2). However, for the last 2 decades, this has changed considerably for two main reasons. First, aggressive liver surgery has become safer through advances in anesthesia, peri-operative care and surgical techniques (3). Second, the uptake of laparoscopic surgery and relative ease of laparoscopic cholecystectomy for gallstone disease has resulted in the increased detection of early stage incidental gallbladder cancers (3). This in turn has revived interest in improving outcomes.

While surgery offers the only potential for cure in GBC, the extent of resection required in cases of advanced GBC is controversial (2, 4, 5, 9). There is support for doing extended resection in the case of T2 and T3 GBC and there are those who believe an extended resection does not provide any further survival benefit (4, 5, 7, 8, 9, 10). Given the rarity of the disease, most of these studies describe small, single center observational experiences with inherent selection biases. Secondly, two previous population based studies from the United States (US) on GBC, using the Surveillance, Epidemiology, and End Results (SEER) Program data lack key variables including use of adjuvant treatment and surgical margin status required to properly evaluate the relationship between the extent of surgery and GBC outcomes (4,5). Therefore, there is currently no consensus on the surgical management of GBC except for the earliest stage (T1a) (2, 9).

The aims of the current study are to describe the association between the extent of surgical resection and overall survival (OS) in patients with T2 and T3 GBC in addition to identifying other histopathologic factors associated with improved overall survival.
4.2 Study methods

4.2.1 Study Population and Data Sources

This is a population-based, retrospective cohort study of all surgically resected GBCs in Ontario, Canada. Ontario has a population of approximately 13.5 million people and a single-payer universal health insurance program. To identify the study cohort we used the Ontario Cancer Registry (OCR), which is a passive, population-based cancer registry that captures diagnostic and demographic information on at least 98% of all incident cases of cancer in the province of Ontario (20).

From January 1, 2002 to March 31, 2012, patients > 18 years of age at the time of diagnosis of GBC were identified using the International Classification of Disease (ICD-9) code for gallbladder cancer (ICD-9 code 156.0) from the OCR. We then linked these cases to the Canadian Institute for Health Information (CIHI) records to identify those who had received procedural codes for hepatobiliary surgical procedures (see Appendix 1) after their date of diagnosis. These pathology reports were then reviewed and abstracted. The following data was abstracted from pathology reports: age at operation, type of surgery, extent of liver resection, American Joint Committee on Cancer 7th edition (AJCC) T-category, N-stage (number of nodes, and number of positive nodes), surgical resection margin status (any positive vs all negative), tumor grade (1, well-differentiated 2. Moderately differentiated; 3, poorly differentiated; 4, undifferentiated), presence of perineural and/or perivascular invasion. The OCR also provides information about vital status and cause of death. Complete information regarding vital status in the OCR was available up to December 31, 2012, whereas information concerning cause of death was available up to December 31, 2010. We excluded all patients having only undergone liver and/or gallbladder biopsies without a gallbladder resection, any cases of GBC with histology other than adenocarcinomas or adenosquamous carcinomas, cases with unknown or indeterminate T stage, as well as all T1a and T1b cases, and metastatic GBC at initial surgery. Patients having undergone a cholecystectomy and subsequent re-resection were identified based on having 2 separate pathology reports on different dates – one for cholecystectomy and another for an extended resection.
4.2.2 Adjuvant treatment

Radiotherapy and chemotherapy databases are assembled at Queen’s Division of Cancer Care and Epidemiology (CCE) with permission from all 14 Ontario cancer centers. Using the unique identifier for each patient in the OCR, CCE links the cancer diagnosis from the OCR to chemotherapy and radiotherapy administration data from the 14 Ontario cancer centers. Only curative intent adjuvant treatment was included. Intent of adjuvant treatment was often listed in the CCE database as curative or palliative intent; when the intent of adjuvant treatment was unknown, only treatment administered within 3 months of surgery was considered curative intent.

4.2.3 Socioeconomic status

For the purpose of our study, we estimated socioeconomic status (SES) using an ecologic measure: patient’s neighborhood median household income (MHI). MHI at the level of the dissemination area (DA) was obtained from Statistics Canada. Postal Code Conversion File (PCCF+) provided the correspondence between patient postal codes and census DA. PCCF+ automatically assigns a range of Statistics Canada’s standard geographic areas and other geographic identifiers based on Postal Codes. The MHI from the 2001 census was assigned to the cases diagnosed in 2002. The MHI from the 2006 census was assigned to the cases diagnosed from 2003-2012. The DAs were grouped into quintiles based on their MHI, with the 5th quintile (Q5) representing the communities where the wealthiest 20% in Ontario resided and the 1st quintile (Q1) representing the communities where the poorest 20% resided.

4.2.4 Statistical analysis

All analysis was conducted using SAS 9.4. Survival analysis techniques were used to model the time to death, with censoring occurring on December 31, 2012 for the primary end-point, overall survival (OS) and December 31, 2010 for the secondary outcome, cancer specific survival (CSS). Factors associated with OS and CSS were evaluated using the Cox proportional hazards regression model. Prior to building the Cox model, bivariate analysis was conducted on the following potential confounding variables: age, sex, socioeconomic status, presence/absence of perineural invasion, presence/absence of positive lymph nodes, and adjuvant treatment. On bivariate analysis, only socioeconomic status and
adjuvant treatment failed to achieve a P-value <0.20 on both T2 and T3, and were therefore excluded from the full Cox model. Margin status was not included in the Cox model as margin is not a true confounding variable and is a surrogate marker for the type of surgery (exposure variable). Effect of margin status was assessed post-hoc.

4.3 Results

4.3.1 Demographic Characteristics

Linked administrative data-sets identified 1055 potentially eligible patients who underwent surgical resection for GBC from 2002 to 2012 (Figure 1). Complete gallbladder resections were performed on 50% of cases, with the other 50% being other cancers misclassified as GBC or having only had biopsies done. Following exclusion criteria, 370 patients comprised the study cohort.

Details regarding patient, treatment and pathologic findings are shown in Table 1. Among the T2 cases, there were 176 (75.9%) simple cholecystectomies and 56 (24.1%) extended resection. On average patients undergoing simple cholecystectomy were older (69.8 years), than those undergoing extended resection (63.4 years). Upon further subdivision, 24/56 cases underwent upfront extended resection and 32/56 cases underwent a delayed extended resection. Delayed resections took place at a mean of 2.9 months from initial cholecystectomy with a range of 1 to 5 months.

Among the T3 cases, there were 87 (63.0%) simple cholecystectomies and 51 (37.0%) extended resections. On average patients undergoing simple cholecystectomy for T3 disease were also older (70 years), than those undergoing extended resection, (66.3 years). There were 37 upfront extended resections and 14 delayed extended resections. Delayed resections took place at a mean of 2.2 months from initial cholecystectomy with a range of 1.2 to 5 months.

4.3.2 Overall Survival in T2 and T3 GBC

Survival outcomes are presented for T2 and T3 disease in table 2.

For T2 disease, the 30-day postoperative mortality rate was comparable with 1.7% deaths in both the simple cholecystectomy and extended resection groups. However, there was a 2% 30-day
postoperative mortality rate in the T3 simple cholecystectomy group but no deaths in the T3 extended resection group.

In T2 disease, the 5-year unadjusted OS for extended resection was 49.5% vs. 39.7% for simple cholecystectomy (P = 0.01) (figure 8a). The 5-year CSS was 49.9% and 41.3% for extended resection and simple cholecystectomy, respectively (P= 0.03). The median OS was 23 months for simple resection, versus > 60 months for extended resection (P = 0.08). Among the extended resections, the 5-yr overall survival for the upfront extended resection group was 58.9% and 42.1% for the delayed extended resection group, although it was not statistically significant (P = 0.66).

In T3 disease, the 5-year unadjusted OS was 22.8% for extended resection and 13.5% for simple cholecystectomy (P = 0.05) (figure 8b); the 5-year CSS was 24.4% and 17.0% for extended resection and simple cholecystectomy, respectively (0.06). Median 5-year OS was 21.5 months for extended resection vs 10.5 months for simple cholecystectomy (P = 0.001). Among the extended resections, the 5-yr overall survival for the upfront extended resection group was 37.3% and 18.1% for the delayed extended resection group, although it was not statistically significant (P = 0.19).

4.3.3 Clinical Factors Associated with Survival and T2 GBC

Table 3 presents the Cox model results for T2 disease. In multivariate analysis, after adjustment for confounders, extended resection was associated with improved overall survival compared to simple resection (HR = 0.51; 95% CI 0.30 - 0.97, P= < 0.001). Furthermore, patients with high grade of differentiation (HR = 3.42; 95% CI 1.92 -6.08, P = 0.03), presence of lymphovascular invasion (HR = 1.75; 95% CI 1.16 - 2.64, P=0.03) and presence of tumor positive lymph nodes (HR= 1.77; 95% CI 1.03 - 3.08, P=0.03) had worse OS. Female sex showed a trend towards improved OS for T2 cancers (HR = 0.70; 95% CI 0.49 - 1.01, P=0.06). Presence of perineural invasion showed a trend towards worse OS (HR = 1.58; 95% CI 0.98 - 2.54, P = 0.09) for T2 cancers.
4.3.3 Clinical Factors Associated with Survival and T3 GBC

In patients with T3 tumors, the full model Cox proportional hazards analysis did not confer a benefit in overall survival for extended resection (HR= 0.98; 95% CI 0.58 - 1.14, P = 0.78) (Table 4). In the full model, older age (HR= 1.04; 95% CI 1.02-1.06, P = 0.005) showed worse prognosis but female sex was protective (HR = 0.66; 95% CI 0.43-1.00, P=0.05). In addition, interaction terms between the type of surgery (exposure variable) and the other potential confounders were tested. The results showed a trend towards significant multiplicative interaction between lymph node status (negative, positive or unknown) and type of surgery (p=0.13). Therefore, the model was stratified by lymph node status. Table 5 presents the Cox model results stratified by lymph node status. There was a trend towards improved survival among T3 cancer patients with extended surgery and negative nodal status (HR=0.20; CI 0.03-1.06, P = 0.07). However, it is important to remark that among the 27 T3 cases with negative nodal status, there were less than 6 cases that underwent a simple cholecystectomy and 23 had an extended resection. No statistically significant survival benefit was seen in T3 patients with positive lymph node status or unknown node status. Indeed, among unknown node status patients, extended resection was associated with worse (HR = 2.06; 95% CI 0.78-5.09, P = 0.14). However, again it is important to note that among the 65 T3 cases with unknown nodal status, 60 had simple cholecystectomy and less than 6 cases had extended resection. No other pathologic factor showed an association with survival for node positive or node negative T3 disease. Female sex was suggestive of a protective effect in node positive T3 disease (HR = 0.47; CI 0.21-1.04, P = 0.06).

4.3.5 Effect of margin status

Effect of margin status was not examined in the Cox proportional model. We sought to explore the relationship between type of resection and survival depending on the margin status of incidental GBC patients. To do so, only patients with simple cholecystectomies and those with simple cholecystectomies followed by a delayed extended resection were analyzed. Results are graphically depicted in Figure 9.

For T2 cancers, there were a total of 111 simple cholecystectomies with a negative resection margin (R0). Among these, 21 patients underwent a subsequent extended resection, whereas 90 did not
receive any further surgery. As demonstrated in Figure 9a, there is no difference in overall survival between patients undergoing simple cholecystectomy with an R0 resection and those who had an initial R0 resection but still went on to get further liver resection (P = 0.59). Conversely, there were a total of 50 simple cholecystectomies with a positive resection margin (R1/2). Among these, 42 did not undergo any further resection but 8 underwent a subsequent extended resection with a final R0 margin. Figure 9b depicts a trend towards improved survival in patients undergoing subsequent re-resection for an initial positive margin (P = 0.14).

A similar analysis was performed for T3 cancers. Figure 9c demonstrates no difference in OS between 22 patients who had a simple cholecystectomy with an R0 resection and the less than 5 patients who had an initial R0 resection but still underwent further resection (P = 0.89). Of the 64 simple cholecystectomies with an R1 resection, 9 went on to undergo a delayed extended resection (7 with a final negative resection and 2 with a persistent positive margin). Figure 9d shows a trend towards a difference in survival favouring patients undergoing re-resection for an initial positive resection margin at cholecystectomy (P = 0.12).

4.4 Discussion

In this study, we describe the surgical practice of T2 and T3 gallbladder cancer patients using a population based cohort design. Several important findings have emerged. First, the use of extended resection for T2 and T3 gallbladder cancers is modest in Ontario. Only 24% of all T2 cases and 37% of T3 cases underwent an extended resection. Second, the overall survival of T2 cancer patients undergoing extended resection is significantly improved compared to simple cholecystectomy, with a hazard ratio of 0.51. Thirdly, the need for an extended resection in both T2 and T3 cases may be negated by a negative margin on simple cholecystectomy. Finally, in T3 gallbladder cancers, the benefit in extended resection is best noted among node negative cases.

In T2 GBC, only 56 patients (24%) underwent an extended resection. The proportion of extended resections was only modestly higher in T3 GBC (37% of patients). Although modest, these proportions
are still higher than in a population based study in the US, where 5.2% of T2 and 13.3% of T3 cases underwent an extended resection (5).

For T2 cancers treated with an extended resection, there was a marked association with improved survival based on the Kaplan-Meier analysis and there is a near 50% reduction in the hazard for death in those with extended resections (HR=0.51). Several single institution studies have found a similar survival advantage for extended resections compared to simple cholecystectomy for T2 GBC. De Aretxabala et al. demonstrated an increase in 5-year survival from 20% for simple cholecystectomy to 70% for extended resection in 109 patients (7). Fong et al, with a cohort of 53 patients observed an increase in 5-year survival from 19% for simple cholecystectomy to 61% for extended resection; and Chijiba et al observed an increase from 19% for simple cholecystectomy to 61% for extended resection for T2 tumors in 28 patients (8). However, these observations are not universal and the definition of radical surgery varies from study to study. The population based study by Coburn et al using the Surveillance Epidemiology and End Results registry, showed a significant improvement in survival for extended vs simple cholecystectomy on Kaplan Meier analysis, although this effect was attenuated in their multivariate model. Suzuki and colleagues demonstrated a 5-year survival rate of 77% in a series of T2 gallbladder cancer following simple cholecystectomy or extended resection with no overall survival difference between surgical treatments (9). Toyonaga and colleagues reported a series of 73 incidental gallbladder cancers and a 5-year survival rate of 54% for 43 patients with T2 disease (10). They did not see any survival difference between those treated with simple cholecystectomy and those with an extended resection. However, patients with positive margins or tumor within 5 mm of the margin did show a significant survival benefit with more radical surgery. The observation of repeat resection in the setting of positive margin status was corroborated by the results of our study.

Our study is the first to assess the interplay of margin status and the need for extended resection on incidental cases of GBC using a population based cohort. In a sub-analysis of our T2 disease cohort, it was noted a simple cholecystectomy with negative margins was associated with similar overall survival to those who had an initial simple cholecystectomy with clear margins but still underwent a delayed
extended resection. This is supported by a number of single institution series that purport a negative margin is the most important prognostic factor (17, 19, 20, 21). Indeed, some have suggested that more than extent of resection and any other factor, achieving negative margins (R0) should be the goal of GBC treatment (17). Previous studies report that the hazard ratio of a margin positive (R1/2) resection ranges from 1.2 to 13.5.

With regards to T3 GBC, on unadjusted Kaplan Meier analysis, there was improved OS for extended resection compared to simple cholecystectomy. However, the difference in the benefit of extended resection was not observed in the adjusted Cox PH analysis for T3 disease. Fong et al. from Memorial Sloan Kettering reported 5-year survival of 21% in 8 patients undergoing radical resection for T3 cancers (10). Dixon et al. from the University of Toronto report improved survival in 28 patients with at least T3 cancers, undergoing radical resection compared to simple cholecystectomy (11). However, 2 large population based studies in the US, using the same SEER data did not find an improvement in overall survival between simple cholecystectomy and extended resection for T3 cancers (4,5). Coburn et al, using the SEER data failed to show a significant benefit for extended resection on both Kaplan Meier analysis (P= 0.80) and in their multivariate model. In fact, their multivariate model suggested a trend toward extended resection being associated with worse survival (HR = 1.20; 95% CI: 0.98-1.45).

On subgroup analysis, after stratification by lymph node status for T3 disease, a trend towards improved OS with extended resection was seen in node negative patients, compared to node positive disease or unknown nodal status, albeit a small sample size. Indeed, of the 27 patients with node negative T3 disease, there were less than 6 patients in the simple cholecystectomy group and 23 in the extended resection group. Nevertheless, there is support for this result among previous single institution studies. In 1998, Beonist and associates reported that there were no long-term survivors with node positive disease among 21 patients who underwent radical resection with portal lymph node dissection and concluded that radical resection should only be considered in the absence of regional lymph node metastasis (12). In 2005, Dixon et al documented an improvement in survival in 99 patients with gallbladder cancer who underwent curative extended resection. However, there were no 5 year survivors with node positive
disease in the series, suggesting that aggressive surgery very rarely achieves long term survival in patients who are node positive (11). Despite the nihilism from western literature, extended lymphadenectomy for gallbladder cancer is aggressively pursued in Korean and Japanese centers. However, their results are highly nuanced. For instance, in 2006, Shirai et al. showed that extended portal lymphadenectomy is effective against up to three positive lymph nodes, provided an R0 resection is achieved (37), suggesting that there is a survival benefit in patients with a “modest” degree of N1 disease (3 or less). Although our study does not have the sample size to validate their result, it should be addressed in future studies, specifically with respect to T3 disease (13).

In our cohort of 46 patients with node positive T3 disease, there did not appear to be an improved survival among patients with extended resection (n=23) compared to simple cholecystectomy (n=23), although the point estimate of the hazard ratio (HR = 0.63) still favored an extended resection.

It is also important to note that in our cohort of T3 disease with unknown node status (n=65), there was also no difference in survival among extended resection (n < 6) and simple cholecystectomy (n=60). In fact, in this group, there was a trend toward extended resection being associated with decreased survival on adjusted analysis. There are 2 possible explanations for this finding. Firstly, 2 patients with extended resection and unknown lymph node status, were still left with a positive margin. Similar to T2 results, in the post-hoc analysis of effect of margin (Figure ) for T3 cancers, we have shown that a negative margin is a key predictor of survival and can preclude the need for an extended resection. Secondly, it is plausible that other patient comorbidities could eliminate any survival benefit in this highly aggressive disease. Indeed, Hoehn et al, in their analysis of 6690 GBC patients using the American College of Surgeons National Cancer Database, have shown that patients were less likely to have their lymph nodes examined if they had any significant comorbidities (22).

For T3 disease, increasing age was shown to confer worse survival. In multiple single institution studies, age has been shown to be an important risk factor for gallbladder cancer survival after controlling for other confounders. The hazard ratio for age ranges from 1.45 to 5.17 (16). However, the large
population based study by Coburn et al. failed to demonstrate an effect of age on multivariate analysis for T2 and T3 cancers, although it existed for T1 disease (5).

There are some limitations to our investigation. First, it is a retrospective database with a modest sample size, both a result and reflection of the rarity of GBC. Furthermore, the database does not contain variables related to the patient’s comorbidities, similar to previous SEER based population level studies. However, in an aggressive disease such as GBC, the effect of other comorbidities may be marginal. Nevertheless, to mitigate for the effects of comorbidity, GBC cancer specific survival outcomes were calculated in the subset of the population where this data was available (2002-2010). More than 95% of this cohort had cancer as the cause of death. The median and 5-year survival data are concordant with the overall survival results. The strength of our study, in comparison to previous population based studies, lies in the availability of key oncologic variables, primarily adjuvant treatment data and margin status.

4.5 Conclusion

Our data shows that extended resection, along with well differentiated tumors, absence of node positive disease and absence of lymphovascular invasion are associated with improved overall survival. The benefits of an extended resection for T2 disease appears to be negated by a negative margin on initial cholecystectomy. T3 GBC results suggest that aggressive extended resections may be most beneficial in node negative disease.

Therefore, our study adds to the existing literature on the benefits of an extended resection, while underscoring the importance of margin status and nodal status in the decision to pursue aggressive surgery. Nevertheless, the use of extended resection in gallbladder cancer is modest in Ontario for both T2 and T3 disease, highlighting a need to increase the awareness in the surgical community of the treatment options and outcomes.
References

Figure 1 – Identification of patients with GBC who underwent surgical resection in Ontario 2002-2012

All pts with procedural intervention for GBC, n = 1055

All pts with surgical resection for n = 555

All surgical pts with gallbladder cancer, n = 527

Pts with gallbladder adenocarcinomas n = 513

Pts with GBC and known T stage n = 490

T1a, T1b, T2, T3 n = 452

T2 n = 232

T3 n = 138

Exclude: Biopsy, n =500

Exclude: Non GBC, n =28

Exclude: Non-adenocarcinoma histology, n =14

Exclude: unknown/indeterminate T stage, n =23

Exclude: metastatic gallbladder cancer and T4 stage, n =38

Exclude: T1a and T1b, n =82
Table 1 – Characteristics of patients with GBC who underwent surgical resection in Ontario 2002-2012

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| Sex               |             |                |                 |             |               |                 |
| Female            | 156 (67.2%) | 116 (65.9%)    | 40 (71.4%)      | 99 (71.7%)  | 59 (67.8%)    | 40 (78.4%)      |
| Male              | 75 (32.3%)  | 60 (34.1%)     | 15 (26.8%)      | 39 (28.2%)  | 28 (32.2%)    | 11 (21.6%)      |
| Unknown           | 1           | 1              |                 |             |               |                 |

| Socioeconomic status (Quintiles) |             |                |                 |             |               |                 |
| Q1 (lowest)        | 48 (20.7%)  | 41 (23.3%)     | 7 (12.5%)       | 23 (16.7%)  | 15 (17.2%)    | 8 (15.7%)       |
| Q2                 | 45 (19.4%)  | 37 (21.0%)     | 8 (14.3%)       | 31 (22.4%)  | 20 (23.0%)    | 11 (21.6%)      |
| Q3                 | 47 (20.3%)  | 32 (18.2%)     | 15 (26.8%)      | 28 (16.7%)  | 18 (20.7%)    | 10 (19.6%)      |
| Q4                 | 32 (13.8%)  | 22 (12.5%)     | 10 (17.9%)      | 20 (14.5%)  | 11 (12.6%)    | 9 (17.6%)       |
| Q5 (highest)       | 37 (15.9%)  | 28 (15.9%)     | 9 (16.1%)       | 17 (12.3%)  | 12 (8.7%)     | 5 (9.8%)        |
| Unknown            | 23 (9.9%)   | 16 (9.1%)      | 7 (12.5%)       | 19 (13.8%)  | 11 (12.6%)    | 8 (15.7%)       |

| Surgery            |             |                |                 |             |               |                 |
| Simple             | 176 (75.6%) | 176            | -               | 87 (63.0%)  | 87            | -               |
| Extended           | 56 (24.1%)  | -              | 56 (37.0%)      | -           | 51            |                 |
| Upfront            | 23 (9.9%)   | -              | 23 (26.1%)      | -           | 36            |                 |
| Delayed            | 33 (14.2%)  | -              | 33 (15.0%)      | -           | 14            |                 |

56
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<td></td>
</tr>
<tr>
<td>No</td>
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<td>164 (93.2%)</td>
<td>39 (69.6%)</td>
<td>119 (86.2%)</td>
<td>77 (88.5%)</td>
<td>42 (75.0%)</td>
</tr>
<tr>
<td>Yes</td>
<td>29 (12.5%)</td>
<td>12 (6.8%)</td>
<td>17 (30.4%)</td>
<td>19 (13.8%)</td>
<td>10 (11.5%)</td>
<td>9 (16.1%)</td>
</tr>
<tr>
<td><strong>Grade</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>67 (28.9%)</td>
<td>48 (27.7%)</td>
<td>19 (33.9%)</td>
<td>56 (40.6%)</td>
<td>38 (43.7%)</td>
<td>18 (35.3%)</td>
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<tr>
<td>Moderate</td>
<td>106 (45.7%)</td>
<td>82 (46.6%)</td>
<td>24 (13.6%)</td>
<td>53 (38.4%)</td>
<td>35 (42.5%)</td>
<td>18 (35.3%)</td>
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<tr>
<td>Well</td>
<td>45 (19.4%)</td>
<td>33 (18.8%)</td>
<td>12 (6.8%)</td>
<td>21 (15.2%)</td>
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<tr>
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<td>8 (5.8%)</td>
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</tr>
<tr>
<td><strong>Perineural invasion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>10 (19.6%)</td>
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<tr>
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<td>81 (34.9%)</td>
<td>59 (25.4%)</td>
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<td>25 (49.0%)</td>
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<tr>
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<td>71 (30.5%)</td>
<td>4 (2.9%)</td>
<td>55 (39.9%)</td>
<td>39 (44.8)</td>
<td>16 (31.4%)</td>
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<tr>
<td><strong>Lymphovascular invasion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>95 (40.9%)</td>
<td>72 (40.9%)</td>
<td>23 (38.3%)</td>
<td>39 (28.3%)</td>
<td>27 (31.0)</td>
<td>8 (15.7%)</td>
</tr>
<tr>
<td>Absent</td>
<td>89 (38.3%)</td>
<td>64 (36.4%)</td>
<td>25 (46.7%)</td>
<td>62 (45.0%)</td>
<td>35 (40.2)</td>
<td>31 (60.8%)</td>
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<tr>
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<td>48 (20.7%)</td>
<td>40 (22.7%)</td>
<td>8 (15.0%)</td>
<td>37 (26.8%)</td>
<td>25 (28.8)</td>
<td>12 (23.5%)</td>
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<td>142 (61.2%)</td>
<td>89 (50.9%)</td>
<td>53 (94.6%)</td>
<td>59 (42.8%)</td>
<td>22 (25.3)</td>
<td>37 (72.6%)</td>
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<tr>
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<td>43 (18.5%)</td>
<td>42 (24.0%)</td>
<td>1 (1.8%)</td>
<td>69 (50.0%)</td>
<td>55 (63.2)</td>
<td>14 (27.4%)</td>
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<tr>
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<td>46 (19.8%)</td>
<td>44 (25.1%)</td>
<td>2 (3.6%)</td>
<td>10 (7.2%)</td>
<td>10 (11.5)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Lymph node status</strong></td>
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<td>50 (21.6%)</td>
<td>29 (16.5%)</td>
<td>21 (38.3%)</td>
<td>27 (19.6%)</td>
<td>4 (4.6%)</td>
<td>23 (45.1%)</td>
</tr>
<tr>
<td>Node +</td>
<td>63 (27.6%)</td>
<td>31 (17.6%)</td>
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<td>23 (26.4%)</td>
<td>28 (54.9%)</td>
</tr>
<tr>
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<td>65 (47.1%)</td>
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<td>11 (19.6%)</td>
<td>35 (25.4%)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>15</td>
<td>6</td>
<td>12</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>&gt;=3</td>
<td>36</td>
<td>1</td>
<td>35</td>
<td>24</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>Mean</td>
<td>1.31 +/- 2.3</td>
<td>0.4 +/- 0.7</td>
<td>4.1 +/- 3.2</td>
<td>1.6 +/- 2.8</td>
<td>0.3 +/- 0.6</td>
<td>3.7 +/- 3.8</td>
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</table>
Table 2 – Outcomes of T2 and T3 GBC by Kaplan-Meier analysis

<table>
<thead>
<tr>
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<th>T2 GBC</th>
<th></th>
<th>T3 GBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Simple chole.</td>
<td>All extend</td>
<td>Upfront extend</td>
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<tr>
<td>30-day postoperative mortality</td>
<td>1.7%</td>
<td>1.7%</td>
<td>0</td>
</tr>
<tr>
<td>5- year overall survival</td>
<td>39.7%</td>
<td>49.5%</td>
<td>58.9%</td>
</tr>
<tr>
<td>5-year cancer specific survival</td>
<td>41.3%</td>
<td>49.9%</td>
<td>61.7%</td>
</tr>
<tr>
<td>Median survival (months)</td>
<td>23</td>
<td>---</td>
<td>---</td>
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</tbody>
</table>
Table 3 – Univariate and Multivariate Cox Proportional Hazards Analysis of Factors Prognostic of Overall Mortality in T2 GBC (n = 232)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple</td>
<td>1.0</td>
<td>0.39-1.03</td>
</tr>
<tr>
<td>Extended</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.0</td>
<td>0.56-1.12</td>
</tr>
<tr>
<td>Female</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.01</td>
<td>0.99-1.03</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 (lowest)</td>
<td>1.0</td>
<td>0.99</td>
</tr>
<tr>
<td>Q2</td>
<td>1.07</td>
<td>0.63-1.80</td>
</tr>
<tr>
<td>Q3</td>
<td>0.97</td>
<td>0.56-1.66</td>
</tr>
<tr>
<td>Q4</td>
<td>0.78</td>
<td>0.40-1.51</td>
</tr>
<tr>
<td>Q5 (highest)</td>
<td>1.03</td>
<td>0.59-1.81</td>
</tr>
<tr>
<td>Adjuvant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.0</td>
<td>0.38</td>
</tr>
<tr>
<td>Yes</td>
<td>1.26</td>
<td>0.75-2.13</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>1.0</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mod</td>
<td>2.34</td>
<td>1.32-4.13</td>
</tr>
<tr>
<td>Poor</td>
<td>3.51</td>
<td>1.95-6.31</td>
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</tbody>
</table>
### Perineural invasion

<table>
<thead>
<tr>
<th>Status</th>
<th>OR (95% CI)</th>
<th>p Value</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>1.0</td>
<td>0.006</td>
<td>1.0</td>
<td>0.09</td>
</tr>
<tr>
<td>Present</td>
<td>2.12</td>
<td>1.33-3.38</td>
<td>1.58</td>
<td>0.98-2.54</td>
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<td>Unknown</td>
<td>1.49</td>
<td>0.92-2.42</td>
<td>1.08</td>
<td>0.65-1.78</td>
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</table>

### Lymphovascular invasion

<table>
<thead>
<tr>
<th>Status</th>
<th>OR (95% CI)</th>
<th>p Value</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>1.0</td>
<td>0.03</td>
<td>1.0</td>
<td>0.03</td>
</tr>
<tr>
<td>Present</td>
<td>1.75</td>
<td>1.16-2.64</td>
<td>1.75</td>
<td>1.16-2.64</td>
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<td>Unknown</td>
<td>1.30</td>
<td>0.78-2.16</td>
<td>1.30</td>
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</tbody>
</table>

### Lymph node status

<table>
<thead>
<tr>
<th>Status</th>
<th>OR (95% CI)</th>
<th>p Value</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Node -</td>
<td>1.0</td>
<td>0.09</td>
<td>1.0</td>
<td>0.03</td>
</tr>
<tr>
<td>Node +</td>
<td>1.71</td>
<td>1.03-2.85</td>
<td>1.78</td>
<td>1.03-3.08</td>
</tr>
<tr>
<td>Unknown</td>
<td>1.28</td>
<td>0.80-2.05</td>
<td>1.03</td>
<td>0.62-1.72</td>
</tr>
</tbody>
</table>
Table 3 – Univariate and Multivariate Cox Proportional Hazards Analysis of Factors Prognostic of Overall Mortality in T3 GBC (n =138)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple</td>
<td>1.0</td>
<td>0.70-1.04</td>
</tr>
<tr>
<td>Extended</td>
<td>0.70</td>
<td>0.47-1.04</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.0</td>
<td>0.64-0.96</td>
</tr>
<tr>
<td>Female</td>
<td>0.64</td>
<td>0.43-0.96</td>
</tr>
<tr>
<td>Age</td>
<td>1.01</td>
<td>1.01-1.05</td>
</tr>
<tr>
<td>Socioeconomic status</td>
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</tr>
<tr>
<td>Q1 (lowest)</td>
<td>1.0</td>
<td>0.98</td>
</tr>
<tr>
<td>Q2</td>
<td>1.16</td>
<td>0.63-2.12</td>
</tr>
<tr>
<td>Q3</td>
<td>1.04</td>
<td>0.55-1.94</td>
</tr>
<tr>
<td>Q4</td>
<td>0.99</td>
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</tr>
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<td>Q5 (highest)</td>
<td>1.77</td>
<td>0.58-2.36</td>
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<td>Adjuvant</td>
<td></td>
<td></td>
</tr>
<tr>
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<tr>
<td>Well</td>
<td>1.0</td>
<td>0.13-1.30</td>
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<td>Mod</td>
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<td>0.61-1.84</td>
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<td>Poor</td>
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<td>0.70-2.38</td>
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### Perineural invasion

<table>
<thead>
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<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>1.0</td>
<td>0.26</td>
<td>1.0</td>
<td>0.75</td>
</tr>
<tr>
<td>Present</td>
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<td>0.51-1.68</td>
<td>1.03</td>
<td>0.57-1.84</td>
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<td>Unknown</td>
<td>1.34</td>
<td>0.73-2.46</td>
<td>1.22</td>
<td>0.65-2.29</td>
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### Lymphovascular invasion

<table>
<thead>
<tr>
<th>Category</th>
<th>Ratio</th>
<th>95% CI</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.43</td>
<td>1.0</td>
<td>0.28</td>
</tr>
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<td>Present</td>
<td>1.22</td>
<td>0.76-1.95</td>
<td>1.07</td>
<td>0.65-1.76</td>
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<td>Unknown</td>
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<td>0.53-1.56</td>
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<td>0.42-1.27</td>
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</table>

### Lymph node status

<table>
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<th>Ratio</th>
<th>95% CI</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Node -</td>
<td>1.0</td>
<td>0.03</td>
<td>1.0</td>
<td>0.14</td>
</tr>
<tr>
<td>Node +</td>
<td>1.71</td>
<td>1.13-3.45</td>
<td>1.69</td>
<td>0.93-3.07</td>
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<td>1.14-3.34</td>
<td>1.78</td>
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Table 5 - Multivariable Cox proportional hazard analysis of prognostic factors in T3 patients who underwent surgical resection, 2002-2012, stratified by lymph node status

<table>
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<tr>
<th>Variable</th>
<th>T3 lymph node negative (N=4 simple, N=23 extended)</th>
<th>T3 lymph node positive (N=23 simple, N=23 extended)</th>
<th>T3 lymph node status unknown (N=60 simple, &lt; 6 extended)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HR 95% CI p-value</td>
<td>HR 95% CI p-value</td>
<td>HR 95% CI p-value</td>
</tr>
<tr>
<td>Surgery</td>
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<td></td>
</tr>
<tr>
<td>Simple</td>
<td>1.0 0.03-1.06 0.07</td>
<td>1.0</td>
<td>0.88</td>
</tr>
<tr>
<td>Extended</td>
<td>0.20 0.63 0.30-1.34 0.21</td>
<td>2.06 0.78-5.49</td>
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<td>Age</td>
<td>0.99 0.92-1.07 0.88</td>
<td>1.00 0.97-1.04 0.77</td>
<td>1.05 1.02-1.08 0.004</td>
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<tr>
<td>Sex</td>
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</tr>
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<td>Male</td>
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<td>1.0</td>
<td>1.0 0.37-1.34</td>
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<tr>
<td>Female</td>
<td>1.09 0.32-3.70 0.47</td>
<td>0.47 0.21-1.04 0.71</td>
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<td>Grade</td>
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</tr>
<tr>
<td>Well</td>
<td>1.0 0.70 0.07</td>
<td>1.0</td>
<td>0.84</td>
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<tr>
<td>Moderate</td>
<td>0.96 0.16-5.72 0.01</td>
<td>0.97 0.34-2.79 0.17</td>
<td>1.07 0.45-2.55</td>
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<td>2.10 0.31-14.5 0.01</td>
<td>2.22 0.83-5.94 0.06</td>
<td>1.15 0.48-2.75</td>
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<tr>
<td>Perineural invasion</td>
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<td></td>
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</tr>
<tr>
<td>Absent</td>
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<tr>
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<td>1.66 0.57-4.84 0.78</td>
<td>1.78 0.84-3.79</td>
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<tr>
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<td>0.64 0.10-4.00 0.03</td>
<td>0.90 0.24-3.34 0.48</td>
<td>0.69 0.27-1.78</td>
</tr>
<tr>
<td>Lymphvas. invasion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1.0 0.17 0.05</td>
<td>1.0</td>
<td>0.84</td>
</tr>
<tr>
<td>Present</td>
<td>1.71 0.38-7.77 0.52</td>
<td>0.52 0.14-2.02 1.78</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>0.13 0.01-1.47 0.39</td>
<td>0.39 0.08-1.79 0.69</td>
<td>0.27 0.27-1.78</td>
</tr>
</tbody>
</table>
Figure 8 – Kaplan-Meier Survival Analysis Curves

2a

5-year Overall Survival in T2 GBC

P = 0.03

2b

5-year Overall Survival in T3 GBC

P = 0.05
Figure 9 – Margin status and benefit of extended resection in incidental GBC

3a

3b

3c

3d
Chapter 5

General Discussion

The purpose of this study was to determine whether the extent of surgical resection is associated with improved survival in T2 and T3 gallbladder cancers. A retrospective cohort design of patients diagnosed with gallbladder cancer (GBC) within a 10-year period was used. Linked administrative databases from the Ontario Cancer Registry (OCR, Statistics Canada and Cancer Care and Epidemiology (CCE) at Queen’s Cancer Research Institute), enabled the identification of the study cohort, the characterization of extent of surgery and adjuvant treatment regimens, as well as the collection of relevant clinical and histopathological characteristics. A survival analysis approach was used throughout the objectives of this study. This chapter will highlight the key findings of this study in the context of the existing literature, while outlining methodological shortcomings and strengths and future directions of this research.

5.1 Key Findings

Several important findings emerged. First, the use of extended resection for T2 and T3 gallbladder cancers is modest in Ontario. Only 24% of all T2 cases and 37% of T3 cases underwent an extended resection. Second, the overall survival of T2 cancer patients undergoing extended resection is significantly improved compared to simple cholecystectomy, with a near 50% improvement in overall survival. Thirdly, this is the first population based study to show that the need for an extended resection in T2 and T3 cases may be negated by a negative margin on simple cholecystectomy. Finally, in T3 gallbladder cancers, the benefit in extended resection is best noted among node negative cases.
5.2 Literature Review and Study Findings

In this study, only 24% of T2 GBCA patients underwent simple cholecystectomy. The proportion of extended resection was only modestly higher in T3 GBCA, 37%. Although modest, comparably, they are higher than in previous population based studies in the US using the SEER database where 5.2% of T2 and 13.3% of T3 cases underwent an extended resection (5). Similarly, Jensen et al, also using the SEER database, showed that only 11% of potentially resectable cancers receive an extended resection.

For T2 cancers treated with an extended resection, there was a marked association with improved survival on Kaplan-Meier analysis. This was also seen on adjusted survival analysis. There is a near 50% reduction in the hazard for death in those with extended resections (HR=0.51). The unadjusted 5-year overall survival was 39.7% for simple cholecystectomy vs 49.5% for extended resection. Several single institution studies have found a similar survival advantage for extended resections compared to simple cholecystectomy for T2 GBC. De Aretxabala et al. demonstrated an increase in 5-year survival from 20% for simple cholecystectomy to 70% for extended resection in 109 patients (7). Fong et al, with a cohort of 53 patients observed an increase in 5-year survival from 19% for simple cholecystectomy to 61% for extended resection; and Chijiba et al observed an increase from 19% for simple cholecystectomy to 61% for extended resection for T2 tumors in 28 patients (8). However, these observations are not universal and the definition of radical surgery varies from study to study. Suzuki and colleagues observed a 5-year survival rate of 77% in a series of T2 gallbladder cancer following simple cholecystectomy or extended resection with no overall survival difference between surgical treatments (9). Toyonaga and colleagues reported a series of 73 incidental gallbladder cancers and a 5-year survival rate of 54% for 43 patients with T2 disease (10). They did not see any survival difference between those treated with simple cholecystectomy and those with an extended resection.

While, the above single institution studies have shown varied results among patients treated with simple versus extended resection, the results of our population based study corroborate observations from other population-based studies such as the cohort study by Coburn et al using the SEER registry. Similar to this study, Coburn et al. also reported a significant improvement in survival for extended vs simple
cholecystectomy. Therefore, based on the current best available evidence from population based cohort studies including this study, it would appear that there is good evidence supporting the benefits of extended resection in T2 GBCA.

Our study is the first to assess the interplay of margin status and the need for extended resection on incidental cases of GBC using a population based cohort. Margin status is an important oncologic marker. If cancerous cells are found at the edges the operation is much less likely to achieve the desired outcome. In a sub-analysis of our T2 and T3 disease cohort, it was noted a simple cholecystectomy with negative margins was associated with similar overall survival to those who had an initial simple cholecystectomy with clear margins but still underwent a delayed extended resection. Therefore, this suggests that the need for any extended resection is negated by obtaining a negative margin. This is supported by a number of single institution series that purport a negative margin is the most important prognostic factor (17, 19, 20, 21). Indeed, some have suggested that more than extent of resection and any other factor, achieving negative margins should be the goal of GBC treatment (17).

With regards to T3 GBC, after stratification by lymph node status for T3 disease, a trend towards improved OS with extended resection was seen in node negative patients, compared to when there is node positive disease or unknown nodal status, on adjusted analysis. The sample size is small. Indeed, of the 27 patients with node negative T3 disease, there were only 4 patients in the simple cholecystectomy group and 23 in the extended resection group. Nevertheless, there is support for this result among previous single institution studies. In 1998, Beonist and associates reported that there were no long-term survivors with node positive disease among 21 patients who underwent radical resection with portal lymph node dissection and concluded that radical resection should only be considered in the absence of regional lymph node metastasis (12). In 2005, Dixon et al documented an improvement in survival in 99 patients with gallbladder cancer who underwent curative extended resection, however there were no 5 year survivors with node positive disease in the series, suggesting that aggressive surgery very rarely achieves long term survival in patients who are node positive (11).
In our cohort of 46 patients with node positive T3 disease patients, there did not appear to be an improved survival among those with an extended resection (n=23) compared to simple cholecystectomy (n=23), although the point estimate of the hazard ratio (HR = 0.63) still favored an extended resection.

It is also important to note that in our cohort of T3 disease with unknown node status (n=65), there was also no difference in survival among extended resection (n=5) and simple cholecystectomy (n=60). In fact, in this group, there was a trend toward extended resection being associated with decreased survival on adjusted analysis. There are 2 possible explanations for this finding. Firstly, 2/5 patients with extended resection and unknown lymph node status, were still left with a positive margin. Secondly, it is plausible that other patient comorbidities could attenuate any survival benefit in this highly aggressive disease. However, it is important to note that Jensen et al, have also shown that unknown nodal status is a poor prognostic factor for survival in GBCA. Their results showed that T3 patients with unknown nodal status who underwent extended resection had similar survival outcome to those T3 patients receiving simple cholecystectomy only (Overall survival of 7 months vs 6 months, respectively). Their results and ours, highlight the importance of nodal harvest in the prognosis of GBCA.

5.3 Methodological strengths and limitations

Results from this study fill an important knowledge gap in the management of gallbladder cancer. As this is a retrospective, population based cohort, it is very suitable for evaluating the outcomes among a group of patients with a disease that is not very common in Canada. Adjuvant treatment data and the manual abstraction of pathology reports provide key oncologic variables that were not available in the 2 previous population based studies from the US (9, 10). Furthermore, no other study has used appropriate statistical methods to analyze this data, particularly considering the issue of effect modification. However, this study is not without its limitations.

5.3.1 Selection bias

Given that the Ontario Cancer Registry (OCR) is a retrospective provincial database that captures 98% of all incidental cancers in Ontario, it minimizes selection of participants included in the study.
However, selection biases, particularly confounding by indication, remains a primary threat to the validity of using observational data to estimate benefits of treatment. OCR does not capture information related to patient comorbidity, nutritional status or performance status. These variables are lacking in the current study and can act as a source of selection bias by influencing a physician’s clinical judgment in who receives aggressive extended resection.

Indeed, patients with comorbidity, poor nutritional and performance status are generally less likely to receive curative treatment for their cancer than those without comorbidity. This phenomenon has been reported across different health settings, cancer sites, and treatment types. Taking colorectal cancer as an example, numerous studies have found that the offer and uptake of chemotherapy among colorectal cancer patients is lower among patients with comorbidity independent of age (15). There are several reasons that may explain the impact of comorbidity on treatment uptake. Clinicians may be concerned that concomitant conditions will increase the toxicity and side effects of treatment, that treatments may be less effective in these groups, or that the life expectancy of these patients is insufficient to justify the use of potentially toxic agents. It is also possible that these patients themselves are more likely to decline treatment.

However, the relation between comorbidity and surgery is less clear, with some studies reporting no association and others showing an inverse relation between increasing comorbidity and decreasing likelihood of surgery or reduced quality of surgical care for those with comorbidity (17). This lends itself to possible selection bias with respect to who do and do not receive surgery. For gallbladder cancer, there are no studies that examine the impact of comorbidity on extent of resection. However, it can be argued that given all patients in the study underwent a form of surgery (cholecystectomy and/or extended resection), the effect of comorbidity on the decision to pursue further aggressive surgery may be minimal.

Furthermore, with regards to survival, the relative impact of comorbidity tends to be greater for cancers with a better prognosis. This is because those who have cancer associated with a high mortality rate will be more likely to die from their cancer regardless of other concomitant disease compared with
patients who have a less severe prognosis (15). Therefore, the impact of comorbidity is greater for early stage cancer as opposed to advanced and aggressive cancers, such as gallbladder cancer.

Nevertheless, to mitigate for some of the effects of comorbidity on overall survival, we examined the effect on cancer specific survival, for which data was available from 2002-2010. 96.4% of the deaths in the cohort from 2002-2010 was due to cancer. 5-year cancer specific survival data for T2 and T3 diseases was concordant with 5-year overall survival results (Chapter 4, table 2).

5.3.2 Measurement error

The availability of Ontario-wide administrative data was a tremendous advantage in attempting to answer this research question, however when using administrative data, and categorizing exposures, outcomes and covariates based on diagnostic and procedural codes, the possibility of misclassification is quite high. Adequate validity has been shown across all of the databases that were used, however, they are far from perfect measures of assessment.

Exposure:

The current study was a retrospective, population-based cohort study of all patients diagnosed with incident GBC in Ontario from 2002 through 2012 who were identified using the Ontario Cancer Registry (OCR). OCR provided the following information: International Classification of Diseases (ICD) 9th and 10th revision codes and the International Classification of Diseases for Oncology (ICD-O) histology code. The following codes were used for GBC: ICD-9 topography code 1560 and ICD-10 topography code C23, with ICD-O histology codes 8000, 8001, 8010, 8012, 8020, 8021, 8041, 8046, 8070, 8071, 8140, 8144, 8160, 8260, 8261, 8480, 8481, 8490, 8560, and 9990.

Given that the exposure variable in this study is surgery for GBC (simple cholecystectomy or extended resection), information regarding the use of surgery was determined using linked clinical data that capture all treatments provided to patients at any of the regional cancer centers in Ontario. They were identified as having received curative-intent surgical treatment if they received a Canadian Classification of Diagnostic Therapeutic and Surgical Procedures or Canadian Classification of Health Interventions.
code for a hepatobiliary procedure on or after their date of cancer diagnosis (see Appendix 1 for details on procedural codes used). Information regarding surgery was available until December 31, 2012.

The OCR captures diagnostic and demographic information regarding at least 98% of all incident cases of cancer diagnosed in Ontario. However, the possibility of misclassification exists, particularly for GBC, which can be misclassified with other biliary tract cancers, including hepatocellular carcinoma, intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma and ampullary tumors. Although no previous studies exist showing the error rate of misclassification of GBC, biliary tract cancers are sometimes lumped together as one entity.

An example of such misclassification by site of cancer and the OCR can be gleamed through Hall et al’s study assessing the OCR’s ability to correctly identify exact site assignment for head and neck cancer by comparing it to the results of their own database of all head and cancers at the Kingston regional Cancer Centre (KRCC). In their study, they reported that in the OCR 14% of the patients were coded with the wrong head and neck cancer (19).

In the present study, all pathology reports of patients were reviewed manually to ensure only patients with GBC were included. However, given the possibility of miscoding of GBC diagnosis in the OCR, our study cohort may be slightly smaller than the true number of patients diagnosed with GBC. The number of GBC cases miscoded under other biliary tract cancers is likely small, given only 28/1055 cases identified as GBC in this study were found to be other biliary tract cancers. Nevertheless, the outcomes of any gallbladder cancer patient that were excluded due to miscoding should not be different from those included in the present study. Therefore, it would be a nondifferential misclassification.

Covariate:

All histological covariates were abstracted from pathology reports. To ensure reliability and validity in the abstraction of the reports, a pathologist, Dr. David Hurlbut, randomly and independently, abstracted in duplicate 7% of reports (n=75 reports). This was done iteratively throughout and ensured high quality abstraction. The error rate was calculated on a scale of 10 points for each study subject.
calculated on the correct abstraction of the following variables: Date of diagnosis, type of surgery, T stage, Number of lymph nodes, number of positive lymph nodes harvested, presence of absence of perineural invasion, presence/absence of lymphovascular invasion, margin status, histologic type and grade of differentiation. An error rate in abstraction of 3.5% was noted. Typically, a 5% error rate is the threshold applied to designate a low error rate. A low rate of data under 5% error should have little effect in statistical analysis. Furthermore, only studies that demonstrate a small effect size with borderline significance levels are intuitively liable to the effect of abstraction errors. In our study, we demonstrated marked difference in survival and outcome between the two groups, especially in the T2 GBC cohort. Any errors in abstraction are expected to be nondifferential.

With regards to adjuvant treatment, we included adjuvant treatment that was explicitly stated as “curative or adjuvant intent;” palliative intent treatment was not considered. There were instances when the intent of adjuvant treatment was not explicitly stated. Therefore, in these cases, keeping in mind the need to balance the need to exclude adjuvant treatment for palliation with the need to capture as many patients as possible who truly received adjuvant treatment, we set our definition of adjuvant treatment within 3 months of surgery. In the latter cases, it is possible that we may have non-differentially misclassified some curative intent adjuvant treatment given after 3 months as palliative. However, its impact is likely minimal, and should not bias the results, given the vast majority of patients receiving adjuvant treatment did so within 3 months.

Outcome:
The outcome of interest is 5-year overall survival and 5-year cancer specific survival. The variables needed to calculate 5-year survival is dependent on date of surgery and vital statistics data.

Information regarding date of surgery was obtained through manual review of date of procedure recorded on pathology reports. Information on follow-up and vital statistics was obtained through OCR. OCR has excellent accuracy of vital statistics data. In their study, Hall et al. found almost 100% agreement in vital status, excellent agreement on date of death within 1 month and no statistically significant difference between the disease specific survival curves for their KRCC and OCR datasets (19).
5.3.3 Missing data bias

The construction of any prognostic model ideally requires a large database with complete information on all potential prognostic factors. However, this is often not possible in retrospective observational studies. In the present study, missing data was encountered for various clinical and pathologic prognostic covariates (See Table 1, Chapter 4), namely for socioeconomic status, presence of perineural invasion, presence of lymphovascular invasion and lymph node status. These prognostic factors were all categorical in nature. The reason for the missing data is due to incomplete pathologic reports and are missing at random.

The issue of missing data for these covariates was addressed by the creation of a missing/unknown category for each variable. The advantage of this method is the inclusion of all observed data points with no loss of statistical power.

Table 1 (Chapter 4) shows that the proportion of cases in missing/unknown category for covariates was similar between the simple cholecystectomy and extended resection groups with regards to socioeconomic status. However, there was a higher proportion in the unknown category for margin status, perineural invasion status, lymphovascular invasion status and lymph node status in the simple cholecystectomy group compared to the extended group. As such, this can lead to a potential biased point estimate of the hazard ratio of the independent effect of each of these variables on survival. However, as the results of the T2 multivariate analysis indicate, the point estimate of the HR for lymph node status, perineural invasion and lymphovascular invasion in the unknown category fall in between the point estimate of positive and negative status, respectively, and as such the bias is likely to be attenuated.

Two other potential approaches to dealing with missing data include complete cases analysis method and multiple imputation method. The complete case analysis method uses only the cases with complete data for all collected variables. This approach would lead to a significant loss of statistical power in our study. Multiple imputation is said to be the method with the least bias with regards to missing data. However, it is an advanced technique requiring advanced statistical expertise and was considered beyond the scope of this thesis.
5.4 Effect modification

Evidence of effects that modify the relationship between gallbladder cancer and survival is unknown, and therefore, the approach in this study was exploratory. Multiplicative interactions effect between gallbladder cancer and the following variables was undertaken: age, sex, grade of differentiation, lymph node status, presence of perineural invasion, and presence of lymphovascular invasion.

Although, no previous studies had looked at these variables as effect modifiers, they have been shown to be independent risk factors. In multiple single institution studies, age has been shown to be an important risk factor for gallbladder cancer survival after controlling for other confounders. Female sex has been shown to have a protective effect on survival. With regards to pathologic factors, a population-based registry study from Chile (n=1366) assessing pathologic prognostic factors in gallbladder cancer, tumor grade, perineural invasion and perivascular invasion were found to have a statistically significant and independent effect on survival (21).

Therefore, we performed exploratory multiplicative interaction analysis using these variables. A clinically apparent interaction was noted for T3 disease (See Appendix 1) In T3 disease, lymph node status showed a strong trend towards having significant interaction effect with surgery. Indeed, the initial Cox model did not confer benefit of extended resection for T3 disease. After stratification by lymph node status for T3 disease, a trend towards improved OS with extended resection was seen in node negative patients, compared to when there is node positive disease or unknown nodal status, on adjusted analysis.

The role of lymph node status as effect modifier is not surprising, given that lymph node status is a well-known prognostic factor for many cancers. In non-metastatic colorectal cancer, lymph node status is the strongest pathologic predictor of patient outcome. Approximately 70% of patients with no lymph node involvement will survive 5 years, compared with only 40% of those with lymph node metastases. Because of the high risk of tumor recurrence, patients with positive lymph nodes are routinely referred for adjuvant therapy (12). Similarly, for breast cancer, the presence or absence of tumor in the axillary lymph nodes is the most important prognostic factor (13). Breast cancer that has spread to the lymph nodes has a higher risk of recurring and a less favorable prognosis than breast cancer that has not spread to the lymph
nodes. Also in breast cancer, it is known that the number of positive lymph nodes is also an important prognostic factor; the greater the number of positive lymph nodes, the higher the risk of recurrence. The highest risk of recurrence is for breast tumours with 4 or more positive lymph nodes (13).

In T2 disease, sex was shown to have a significant interaction effect at a level of $p < 0.05$ (See Appendix -2 ). In particular, when stratified by sex, females benefitted the most from extended resection. For males, no effect was seen between simple or extended resection, although the point estimate of the hazard ratio suggested worse survival in males who underwent extended resection. From a clinical perspective, it is hard to rationalize offering surgical treatment based on patient sex. A lack of statistical power and possibility of higher comorbidity among males, likely explain the discrepancy in the sex effects of surgery. However, it is worth noting that GBC is known to be more prevalent in females. Furthermore, female sex has been shown to have an independent protective effect on survival. Coburn et al. showed that female sex had an independent protective effect for T2 GBC (HR = 0.82, CI 0.66-1.03) and for T3 GBC (HR = 082, CI 0.71-0.95) compared to males on multivariate analysis. The results of our study corroborate the finding of the protective effect of female sex in T2 (HR = 0.70, CI 0.49-1.01) and T3 (HR = 0.66, CI 0.43-1.00) GBC (See Chapter 3, table 3).

The underlying pathologic mechanism is not well understood. The sex differential in cancer occurrence is one very consistent finding in descriptive epidemiology. In Korea, a 9% lower relative risk of death for women, was reported for all solid cancers. In a Canadian study, for all cancers combined, women had a 13% lower relative risk of death. A biological advantage mediated through sex hormones has been proposed. Another possibility is that the difference may, in part, reflect women’s generally healthier attitudes and behaviors. Whether the explanation is biological or cultural, or a combination of the two, has yet to be determined. For GBCA, future studies, assessing the role of sex specific effect on treatment should be further examined.
5.5 Statistical analysis

For T2 GBC, the study was adequately powered to detect a hazard ratio of 0.50 because of the higher than anticipated (24% or 1:4) prevalence of extended surgery in this population compared to the previous population based studies (5% or 1:20). The actual study power for T2 was 99%.

The actual study power for T3 GBC was only 29%. The analysis for T3 extended surgery was underpowered due to the small sample size (n=138) and small effect size (HR = 0.78). This study was only powered to detect a HR ≤ 0.60 with 80% power (see Appendix 5).

5.6 External validity

Given that this was a population-based retrospective study over a 10 year period, and was not subject to patient selection into the study, it greatly adds to the generalizability of the findings. The average age, age distribution of the population, distribution of socioeconomic status and higher proportion of females in the study population are all concordant with previously described studies.

As discussed in the preceding section, although biases are possible in the form of selection bias due to patient comorbidity and measurement error, these are expected to be minimal.

The generalizability of the results are further supported by previous observational and population based studies that support the benefit of extended resection in T2 disease and node negative T3 disease.

5.7 Conclusion and future directions

GBC is a highly lethal malignancy with poor prognosis. There has been recent interest in improving the outcomes of this disease, in particular for locally advanced disease. At present, surgery is the only option for cure. Recent advances in anesthesia and safety of aggressive liver surgery, has encouraged pursuit of aggressive liver resection in GBC. However, given the rarity of the disease, there is paucity of evidence supporting the benefits of extended liver resection. Previous SEER based studies suggest that only 11% of patients undergo any extended resection for locally advanced GBC. Previous studies are limited to small sample, highly selective observational studies. Previous population based studies from the US lack key oncologic variables and do not use adequate statistical methods to examine this complex question.
Using one of the largest cohorts of GBC, this study highlights the benefits of an extended resection, while underscoring the importance of margin status and nodal status in the decision to pursue aggressive surgery. The next step is to increase awareness of the benefits of an extended resection in the surgical community, where the use of extended resections remains modest.

Future studies in this topic should focus on several important questions:

1) Qualify the exact type of extended resection needed, i.e. whether a simple wedge resection of the liver suffices or does a formal hepatectomy add further benefit?

2) Assess the timing of re-resection of the liver after simple cholecystectomy that optimizes survival benefit.

3) Assess whether the location of the tumor on the gallbladder (neck, body, fundus) should dictate need for simple vs extended resection.

4) Address the benefits of an extended lymphadenectomy/nodal harvest. Specifically, for T3 disease, our results showed a significant benefit in node negative disease only. Smaller observational studies have suggested benefit of T3 extended resection when there is limited node positive disease (3 or less positive nodes).

5) Develop improved adjuvant treatment directed at GBC
References

### Appendix 1. Surgical Administrative Codes from Canadian Classification of Health Intervention Codes

**Cholecystectomy**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>1.OA.87.</td>
<td>DA Using laparoscopic approach</td>
</tr>
<tr>
<td>1.OA.87.</td>
<td>LA Using open approach</td>
</tr>
<tr>
<td>1.OA.87.LA-AZ</td>
<td>Using ultrasonic aspirator device (for dissection) and open approach</td>
</tr>
<tr>
<td>1.OD.57.DA</td>
<td>Laparoscopic</td>
</tr>
<tr>
<td>1.OD.57.LA</td>
<td>Open</td>
</tr>
<tr>
<td>1.OD.89.DA</td>
<td>Alone, laparoscopic</td>
</tr>
<tr>
<td>1.OD.89.EC</td>
<td>Alone, open</td>
</tr>
<tr>
<td>1.OD.89.LA</td>
<td>With bile duct exploration and no stones extracted, laparoscopic</td>
</tr>
<tr>
<td>1.OD.89.TP</td>
<td>With bile duct exploration and no stones extracted, open</td>
</tr>
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</table>

**Bypass of bile ducts**

<table>
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</thead>
<tbody>
<tr>
<td>1.OE.76.DV</td>
<td>Choledochoenterostomy</td>
</tr>
<tr>
<td>1.OE.76.EE</td>
<td>Hepaticoenterostomy</td>
</tr>
<tr>
<td>1.OE.76.EG</td>
<td>Pancreaticoenterostomy</td>
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**Partial bile duct**

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<th>Description</th>
</tr>
</thead>
<tbody>
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<td>1.OE.84.LA</td>
<td>Construction or reconstruction of the bile ducts using open approach</td>
</tr>
<tr>
<td>1.OE.87.LA</td>
<td>Excision of the bile ducts using open approach</td>
</tr>
<tr>
<td></td>
<td>Total bile duct excision</td>
</tr>
<tr>
<td>1.OE.89.SR</td>
<td>Using open approach and choledochojejunostomy technique</td>
</tr>
<tr>
<td>1.OE.89.UF</td>
<td>Using open approach and hepaticojejunostomy technique</td>
</tr>
</tbody>
</table>

**Liver surgery**

<table>
<thead>
<tr>
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<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>62.0</td>
<td>Hepatectomy</td>
</tr>
<tr>
<td>62.1</td>
<td>Local excision or destruction of lesion or tissue of liver</td>
</tr>
<tr>
<td>62.12</td>
<td>Partial hepatectomy</td>
</tr>
<tr>
<td>62.2</td>
<td>Lobectomy of liver</td>
</tr>
<tr>
<td>62.3</td>
<td>Total hepatectomy</td>
</tr>
<tr>
<td>62.59</td>
<td>Other repair of liver</td>
</tr>
</tbody>
</table>
### Appendix 2 – Multiplicative interaction effects/Testing for Effect Modification

<table>
<thead>
<tr>
<th>Step</th>
<th>Interaction effect</th>
<th>T2 GBC</th>
<th>T3 GBC</th>
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<tr>
<td></td>
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<td>P-value</td>
<td>P-value</td>
</tr>
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<td>1</td>
<td>Type of surgery*Perineural invasion</td>
<td>0.7068</td>
<td>0.7686</td>
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<tr>
<td>2</td>
<td>Type of surgery*Grade off differentiation</td>
<td>0.7515</td>
<td>0.6195</td>
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<tr>
<td>3</td>
<td>Type of surgery*Lymphovascular invasion</td>
<td>0.4665</td>
<td>0.9732</td>
</tr>
<tr>
<td>4</td>
<td>Type of surgery*Lymph node status</td>
<td>0.6432</td>
<td>0.1336</td>
</tr>
<tr>
<td>5</td>
<td>Type of surgery*Age</td>
<td>0.4320</td>
<td>0.5455</td>
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<td>6</td>
<td>Type of surgery*Sex</td>
<td>0.0336</td>
<td>0.9396</td>
</tr>
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</table>
Appendix 3 – Testing Proportional Hazards (PH) Assumption using Survival Curve Method

The proportional hazards assumption was tested for all variables input in the Cox model. All categorical variables were tested using log-log(s(t)) curves. Categorical variables include type of surgery, grade of differentiation, sex, presence/absence of perineural invasion, presence/absence of lymphovascular invasion, and lymph node status. When we graph the survival function versus survival time, if the predictors satisfy the proportional hazard assumption then the graph of the log(-log(survival)) versus log of survival time graph should result in lines that are relatively parallel for the various levels of each predictor. If the lines are not parallel then the proportional hazard assumption is violated.

Plots were graphed for each categorical variable for T2 and T3 GBC separately. All levels of the categorical variables showed plots that were relatively parallel in both T2 and T3 GBC. Therefore, the PH assumption was upheld.

For the continuous variable age,” the PH assumption was tested by using the observed standardized score process with the associated p-value from the Kolmogorov-type supremum test for proportional hazards assumption. In this test, a significant p-value (<0.05) would indicate a PH-assumption violation. This test showed that the continuous variable “age” did not violate the PH assumption for both T2 and T3 GBC.
Testing PH assumption for covariates in T2 GBC model
Supremum test for continuous variable, Age in T2 GBC cohort. $P = 0.30$
Testing PH assumption for covariates in T3 GBC model

Evaluation of proportion hazard for variable: Lymph node status

Evaluation of PH assumption for variable: Perineural invasion

Evaluation of PH assumption for variable: Lymphovascular invasion

Evaluation of PH assumption for variable: Grade of differentiation

Evaluation of PH assumption for variable: Type of surgery

Evaluation of PH assumption for variable: Gender
Supremum test for continuous variable, Age in T3 GBC cohort. P = 0.68
Appendix 4 – Testing Proportional Hazards Assumption using the time dependent covariate method

The pitfall of the survival curve method described in Appendix 2 because the graphs can be cluttered and interpretation subjective. A more objective method is the time dependent covariate method. In this method, we generate the time dependent covariates by creating interactions of all the predictors and a function of survival time and include it in the model. If any of the time dependent covariates are significant at an alpha <0.05, then those predictors are not proportional.

For both T2 and T3 GBC, none of the time dependent covariates were significant at an alpha < 0.05.

<table>
<thead>
<tr>
<th>Time dependent covariate</th>
<th>T2 GBC P-value</th>
<th>T3 GBC P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time*Perineural invasion</td>
<td>0.5416</td>
<td>0.4320</td>
</tr>
<tr>
<td>Time*Grade off differentiation</td>
<td>0.4061</td>
<td>0.0689</td>
</tr>
<tr>
<td>Time*Lymphovascular invasion</td>
<td>0.3208</td>
<td>0.2602</td>
</tr>
<tr>
<td>Time*Lymph node status</td>
<td>0.7170</td>
<td>0.8485</td>
</tr>
<tr>
<td>Time*Age</td>
<td>0.2201</td>
<td>0.8518</td>
</tr>
<tr>
<td>Time*Sex</td>
<td>0.8005</td>
<td>0.1566</td>
</tr>
<tr>
<td>Time*Type of surgery</td>
<td>0.0649</td>
<td>0.1051</td>
</tr>
</tbody>
</table>
Appendix 5 – Actual study power with $\alpha = 0.05$

<table>
<thead>
<tr>
<th>Hazard ratio</th>
<th>T2 N = 232, $\pi_1 = 76%$, $\pi_2 =$ 24%</th>
<th>T3 N = 138, $\pi_1 = 63%$, $\pi_2 =$ 37%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.40</td>
<td>99.9%</td>
<td>99.9%</td>
</tr>
<tr>
<td>0.50</td>
<td>99.5%</td>
<td>97.6%</td>
</tr>
<tr>
<td>0.60</td>
<td>91.3%</td>
<td>82.6%</td>
</tr>
<tr>
<td>0.70</td>
<td>64.0%</td>
<td>62.5%</td>
</tr>
<tr>
<td>0.78</td>
<td>36.5%</td>
<td>29.1%</td>
</tr>
</tbody>
</table>

Legend:
N = total sample size
$\pi_1$ = Proportion of cases receiving simple cholecystectomy
$\pi_2$ = Proportion of cases receiving extended surgery

Reference
Appendix 6 – Ethics Clearance

QUEEN’S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS
RESEARCH ETHICS BOARD (HSREB)
HSREB Renewal of Ethics Clearance

February 08, 2017

Dr. Senthuran Tharmalingam
Department of Public Health Sciences
Queen’s University

ROMEO/TRAQ #: 6014876
Department Code: EPID-502-15
Study Title: Gallbladder cancer: A population based analysis of surgical practice patterns and outcomes
Review Type: Delegated
Date Ethics Clearance Effective: February 20, 2017
Ethics Clearance Expiry Date: February 19, 2018

Dear Dr. Tharmalingam,

The Queen’s University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board (HSREB) has reviewed the application. This study, including all currently approved documentation has been granted ethical clearance until the expiry date noted above.

Prior to the expiration of your ethics clearance, you will be reminded to submit your renewal report through ROMEO. Any lapses in ethical clearance will be documented below.

Yours sincerely,

Chair, Health Sciences Research Ethics Board

The HSREB operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2), the International Conference on Harmonization Good Clinical Practice Consolidated Guideline (ICH GCP), Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations; Canadian General Standards Board, and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is qualified through the CTO REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP). Federallywide Assurance Number: FWA#: 00004134, IRB#: 000001173

HSREB members involved in the research project do not participate in the review, discussion, or decision.