# Diagnosis and treatment of gallbladder cancer

## an overview of contemporary practice

Elise A.J. de Savornin Lohman

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# DIAGNOSIS AND TREATMENT OF GALLBLADDER CANCER

## AN OVERVIEW OF CONTEMPORARY PRACTICE

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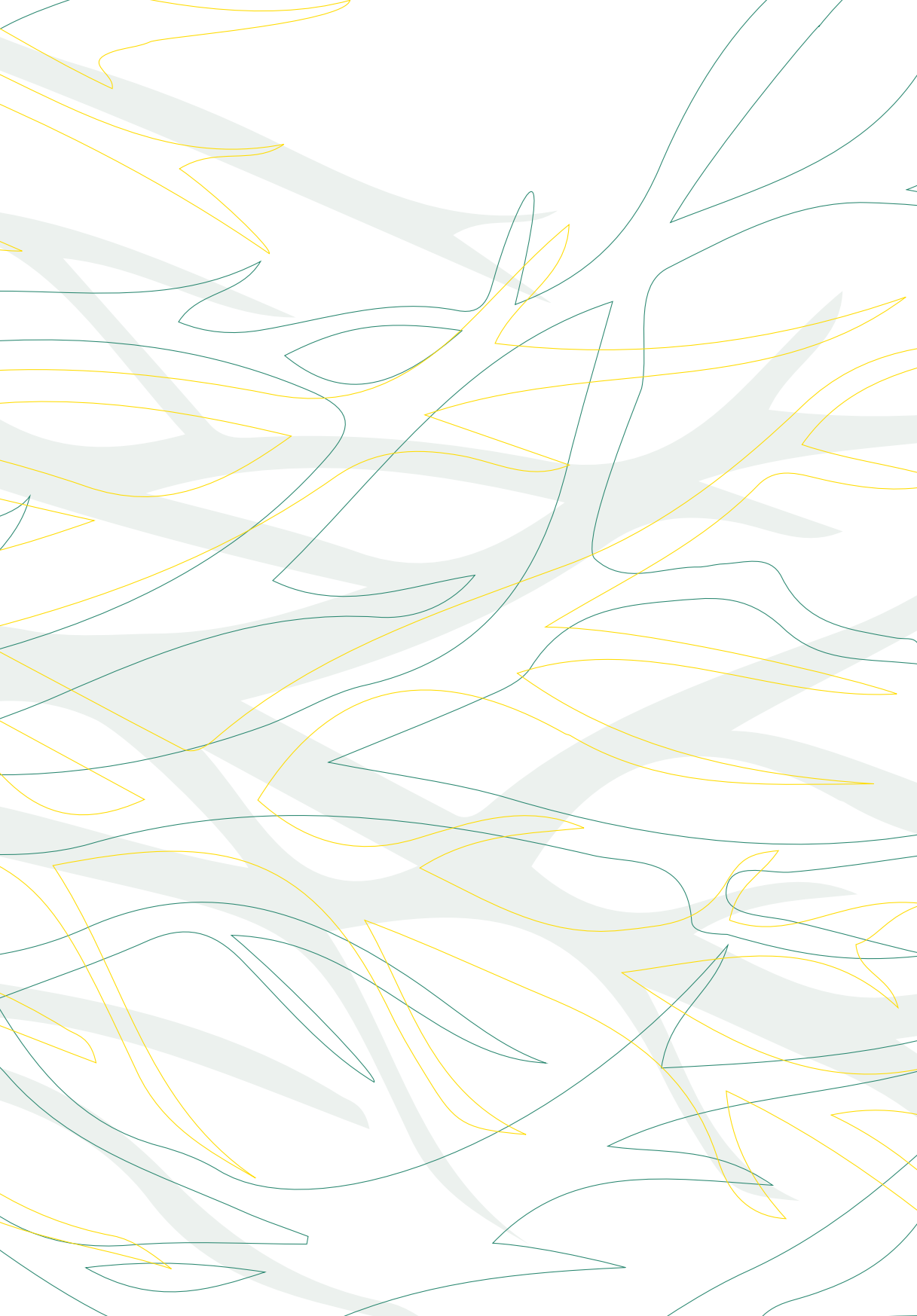

CHAPTER 1

# General introduction

# INTRODUCTION

The gallbladder is a small, sac-shaped organ situated in the gallbladder fossa on the visceral surface of the liver. It is composed of multiple layers; a simple, columnar epithelial layer, followed by the lamina propria, muscle layer, perimuscular fibrous layer and serosa. The gallbladder lies in direct relation to the visceral side of the liver and is entirely surrounded by peritoneum. The hepatic side of the gallbladder is attached to the liver by connective tissue of the fibrous capsule of the liver.

The primary purpose of the gallbladder is the storage and concentration of bile, which is produced in and supplied by the liver. The cystic duct connects the gallbladder neck to the common hepatic duct. When food enters the small intestine, the gallbladder contracts and subsequently bile is released into the duodenum via the bile duct. Bile aids in digestion by breaking up dietary fats and will also drain waste products from the liver into the duodenum. The formation of gallstones, caused by the oxidization of saturated bile, is the most prevalent pathology of the gallbladder. Gallstones are found in approximately 10-15% of the adult Western population and may lead to a variety of problems, such as acute abdominal pain due to passage of stones through the cystic duct or cholecystitis due to blocking of bile outflow. [1]

## GALLBLADDER CANCER

Although benign gallbladder disease is common, gallbladder cancer (GBC) is extremely rare. It is characterized by late and often nonspecific symptomatology such as upper abdominal pain and weight loss. Although GBC accounts for 80% of biliary tract cancers, it remains poorly understood, difficult to treat and highly lethal. [2, 3]

The primary mechanic driving the development of GBC is chronic inflammation and consequent disruption of normal cell homeostasis. Gallstones are present in 80% of patients with GBC and cause chronic inflammation of the gallbladder wall. This inflammatory process may cause DNA damage which can result in malignant transformation of the epithelial cells. Other major etiological factors include age, gender, obesity, genetics, environmental factors and certain hepatobiliary comorbidities such as primary sclerosing cholangitis or an anomalous pancreatobiliary duct junction. [4]

## INCIDENCE, MORTALITY AND SURVIVAL

In 2018, GBC was diagnosed in 219.000 people across the globe and 165.000 died from its consequences. [5, 6] It is one of the few cancers that boasts a higher mortality percentage than incidence, accounting for 1.2% of all cancers and 1.7% of all cancer mortality respectively. [2, 7, 8] Even though the global incidence of GBC has been steadily diminishing, mortality has not declined and even risen in some countries since 2000; 5-year survival of GBC, across all stages, is still only 10%. [5, 9, 10]

Incidence of gallbladder cancer shows remarkable geographic variation; it ranges from 1/100.000 women in Western countries to 26/100.000 in Chilean women. Differences in genetics, lifestyle and environmental factors all likely contribute to this remarkable range in incidence rates. [7]

Survival of GBC is poor, primarily because it is critically dependent on timely diagnosis and subsequent radical surgery. [4] Unfortunately, around half of patients presents with local or distant metastases and another 30% of patients already have locally advanced, non resectable disease. Median overall survival (OS) in these patients is only around 6 months. [11, 12] Only 20% of patients is resectable at diagnosis. [13] Even then, median survival in patients who received a radical resection is merely 24 months.

## CURRENT TREATMENT STRATEGIES FOR NON-METASTATIC PATIENTS

Gallbladder cancer is treated by radical surgical resection, of which the extent is primarily determined by tumor stage. The majority of patients that are diagnosed in an early stage (i.e. the tumor is confined to the gallbladder wall) are diagnosed incidentally after laparoscopic cholecystectomy for a benign indication; so called incidental GBC (iGBC). [14] Additional surgery is often warranted in order to achieve tumor-free margins and prolong survival.[13] Surgery for advanced GBC only positively affects survival if it is technically feasible to obtain tumor-free resection margins, which frequently requires extended resections of the liver or other organs. These major resections are associated with major morbidity and mortality. [4]

Even after radical resection over 50% of patients will suffer from either local recurrence or distant metastatic disease. [15] Adjuvant chemotherapy (aCT) has recently received increasing attention as a method to complement surgery and increase local and distant control. Trials investigating the efficacy of adjuvant chemotherapy (aCT) in GBC are sparse and included not just patients with GBC, but rather all patients with

biliary tract cancer. In recent years, four large trials, all investigating different treatment regimens, have been completed. Only the BILCAP trial, published in 2019, reported a potential survival benefit from adjuvant capecitabine. [16] This trial studied whether adjuvant capecitabine provides superior survival compared to surgery alone in patients with resected biliary tract cancer. Although the primary, intention-to-treat analysis did not show a statistically significant difference in survival, the per-protocol analysis showed that median overall survival was 53 months in patients treated with adjuvant capecitabine versus 36 months in patients treated with observation alone (P=0.028). As a result of the BILCAP trial, adjuvant capecitabine is now considered standard of care by the American Society of Clinical Oncology for all patients with resected biliary tract cancer. [17] It is important to note that the trial mostly included patients with good performance status and no subgroup analysis for patients with gallbladder cancer was conducted. Therefore, it is still uncertain whether capecitabine is a viable adjuvant treatment agent for patients with gallbladder cancer specifically.

## HISTOPATHOLOGY AND PROGNOSTIC FACTORS

Tumor stage, resection margin and lymph node status are major prognostic factors, but these factors alone are insufficient to predict survival after resection with reasonable precision. [18] In other cancers, not only pathological factors such as histology and differentiation grade are used to predict survival. Instead, assessment of histopathological features is combined with analysis of the specific molecular landscape of the tumor to establish prognosis and identify high-risk patients as candidates for additional treatment like adjuvant chemotherapy or other forms of personalized therapy. Primary examples are the presence of microsatellite instability and response to 5-FU therapy in colon cancer and BRCA-gene mutations and sensitivity to platinum-based chemotherapy in breast cancer. [19, 20] This knowledge has facilitated a more personalized approach to treatment and has greatly improved the prognosis of many patients with various forms of cancer. Histopathology, molecular profile and the correlation to response to chemotherapy and prognosis in GBC have barely been investigated due to financial and logistic constraints. The available literature states that the majority (80%) of GBC are adenocarcinomas and that other subtypes of GBC include papillary and squamous cell carcinomas. [21] Some state that squamous cell tumors have a worse prognosis than adenocarcinomas, whilst others find the opposite. [21] In addition to histological type, perineural invasion may be another histopathological feature predictive of survival and of response to chemotherapy, although randomized evidence is lacking. [22]

## CURRENT STATE OF AFFAIRS

Cancer survival has increased significantly in the past decades; it is estimated that half of patients diagnosed with cancer will survive beyond five years. [23, 24] In contrast, GBC survival has not improved, and median overall survival remains an abysmal six months. [10, 25, 26] Improved survival in other cancers is primarily attributed to personalized treatment; resection strategies and adjuvant therapies are increasingly tailored to specific patient and tumor characteristics.

Historically, research into treatment and prognostic factors for GBC has been limited. The lack of available evidence is primarily caused by the low incidence of GBC, which makes the logistics of performing an RCT very challenging. Most research stems from single-institute cohorts in high-incidence countries. Although evidence derived from single cohorts may sometimes be useful, results are prone to bias and are often not applicable to outside populations. [27]

In recent years, those involved in research of rare cancers are attempting to overcome these limitations through joint efforts. [28] In other cancers, such as pancreatic adenocarcinoma, multi-center consortia, multidisciplinary study groups and nation-wide databases have proven to be excellent means to perform high-quality research, including large prospective cohorts and RCT's. [29] Although this centralization is not as prominent in GBC, collaboration is rising and a number of multicenter initiatives have published several high-quality studies. [16, 30]

## OUTLINE OF THIS THESIS

The central aim of this thesis is to increase the knowledge on development and diagnostics, treatment and prognosis of GBC in order to facilitate a more personalized treatment approach for GBC patients in the Netherlands. To this end, in **Part 1** risk factors and imaging strategies for timely diagnosis are discussed. **Part 2** is focused on the optimization of treatment for patients with resectable GBC. In **Part 3,** the relationship between histopathology and prognosis is investigated and a prediction model for survival of GBC patients is proposed.

## PART I: ETIOLOGY AND DIAGNOSIS

It is estimated that around 20% of the global cancer burden can be attributed to infectious diseases.[31] *Salmonella* is a bacteria which harbors oncogenic potential and infection with the *Salmonella Typhi* serovar is a known risk factor for the development of GBC.[32] Other types of *Salmonella* infection may also be associated with increased risk of gallbladder (or other biliary) cancer but this hypothesis has never been studied. In **Chapter 2**, it is investigated whether infection with non-typhoid *Salmonella* is a risk factor for biliary tract cancer in the Netherlands.

Early detection of GBC is challenging because many symptoms are nonspecific and mimic those of benign gallbladder disease such as cholecystitis. Routine imaging with computed tomography (CT) is only sensitive in 50% of cases which frequently results in delayed diagnosis and erroneous staging; both dismal to prognosis. [33] Appropriate staging is especially important when attempting to identify candidates for radical resection, as lymph node metastases prove to be such a poor prognostic factor that surgery is virtually futile. [4] Imaging techniques like Magnetic Resonance Imaging (MRI) appear to have a higher sensitivity and specificity for important diagnostic and prognostic factors such as liver invasion, but their exact role remains unclear. [34] In **Chapter 3** a meta-analysis investigating the accuracy of CT compared to the accuracy of MRI for the diagnosis of lymph node metastases is presented.

## PART II: SURGICAL AND SYSTEMIC TREATMENT

Median OS of patients with resected GBC improved slightly in the past decade. [35] This improvement has coincided with optimization of surgical and chemotherapeutic treatment. Because most data on GBC treatment and survival is derived from non-Western, high-volume single center cohort studies it is unknown whether these trends in therapy and survival also translate to everyday clinical practice in the Netherlands. In **Chapter 4** we used data from the Netherlands Cancer Registry to identify general trends in survival and corresponding trends in treatment in a nation-wide setting.

In patients with advanced disease, extended hepatic resections are often required to achieve tumor-free margins. It is unclear whether the potential survival benefit of radical resection outweighs the significant surgical morbidity and mortality associated with such aggressive surgery. [36] In **Chapter 5**, postoperative morbidity, mortality and survival of patients that underwent hepatectomy or pancreatoduodenectomy for advanced GBC are analyzed to assess the value of extended surgery. Pre-operative obstructive jaundice is indicative of advanced stage disease and is consequently regarded as a relative contra-indication to surgery. To investigate the value of resection for patients with pre-operative jaundice, we compared the outcomes of jaundiced versus non-jaundiced patients after resection in **Chapter 6**.

Although adjuvant chemotherapy may improve survival in biliary tract cancer, its role remains controversial as it is likely only beneficial in patients with poor prognostic characteristics. In **Chapter 7**, we attempt to analyze the value of adjuvant chemotherapy and delineate characteristics associated with favorable response by analyzing data from the Surveillance, Epidemiology and End Results (SEER) program and linking this data to Medicare insurance claims.

## PART III: HISTOPATHOLOGY AND PROGNOSIS

In incidentally diagnosed GBC, it is known that residual disease after re-resection is the primary indicator of prognosis. [37] In patients in whom no residual disease is found, it is questionable whether re-resection actually improves prognosis or whether it merely acts as a staging procedure. To assess the value of re-resection and improve candidate selection, in **Chapter 8** we describe the outcomes of patients with- and without residual disease after re-resection and identify risk factors for the presence of residual disease. Finally, we sought to quantify the role of potential prognostic factors for patients with resected gallbladder cancer. Accurate estimation of prognosis is extremely important in order to inform patients about their prognosis and guide clinical decision making. Several predictive models have been proposed to fulfill those needs. [22, 38, 39] Unfortunately, these models are either based on very limited data or are derived from high-volume, single-center series and do not provide an accurate reflection of everyday clinical practice. We used gallbladder resection samples of a nation-wide database of over 400 patients to correlate histopathological findings to prognosis. This data is summarized in **Chapter 9**, where a novel prediction model is proposed to estimate survival in patients with resected GBC.

## SUMMARY OF THE RESEARCH QUESTIONS ADRESSED AND THE METHODOLOGY USED IN THIS THESIS

| Chapter | Research Question | Study Design |
|---|---|---|
| 2 | Is non-typhoid *Salmonella* or *Campylobacter* infection a risk factor for biliary tract cancer? | Retrospective, nation-wide registry study |
| 3 | How does CT compare to MRI in terms of performance and diagnostic accuracy for the detection of lymph node metastases in gallbladder cancer? | Systematic review & meta-analysis |
| 4 | What are the trends in incidence and treatment for gallbladder cancer in the Netherlands and how do these trends correspond with survival? | Retrospective, nation-wide registry study |
| 5 | Is extended resection (i.e. hepatectomy or pancreatoduodenectomy) beneficial or harmful in patients in patients with advanced gallbladder cancer, especially in light of potential morbidity and mortality? | Retrospective, multi-institute cohort study |
| 6 | Does jaundice preclude resection in patients with gallbladder cancer? | Retrospective, multi-institute cohort study |
| 7 | Does adjuvant treatment benefit patients with resected biliary tract cancer and if so, which subgroup of patients may benefit most? | Retrospective, nation-wide registry study |
| 8 | What factors are predictive for the presence of residual disease in patients with incidental gallbladder cancer after re-resection, and how does the presence of residual disease affect survival? | Retrospective, nation-wide registry study |
| 9 | What patient and tumor characteristics are predictive for survival in GBC and can they be used to establish a reliable prognostic model? | Retrospective, internarional multi-institute cohort study |

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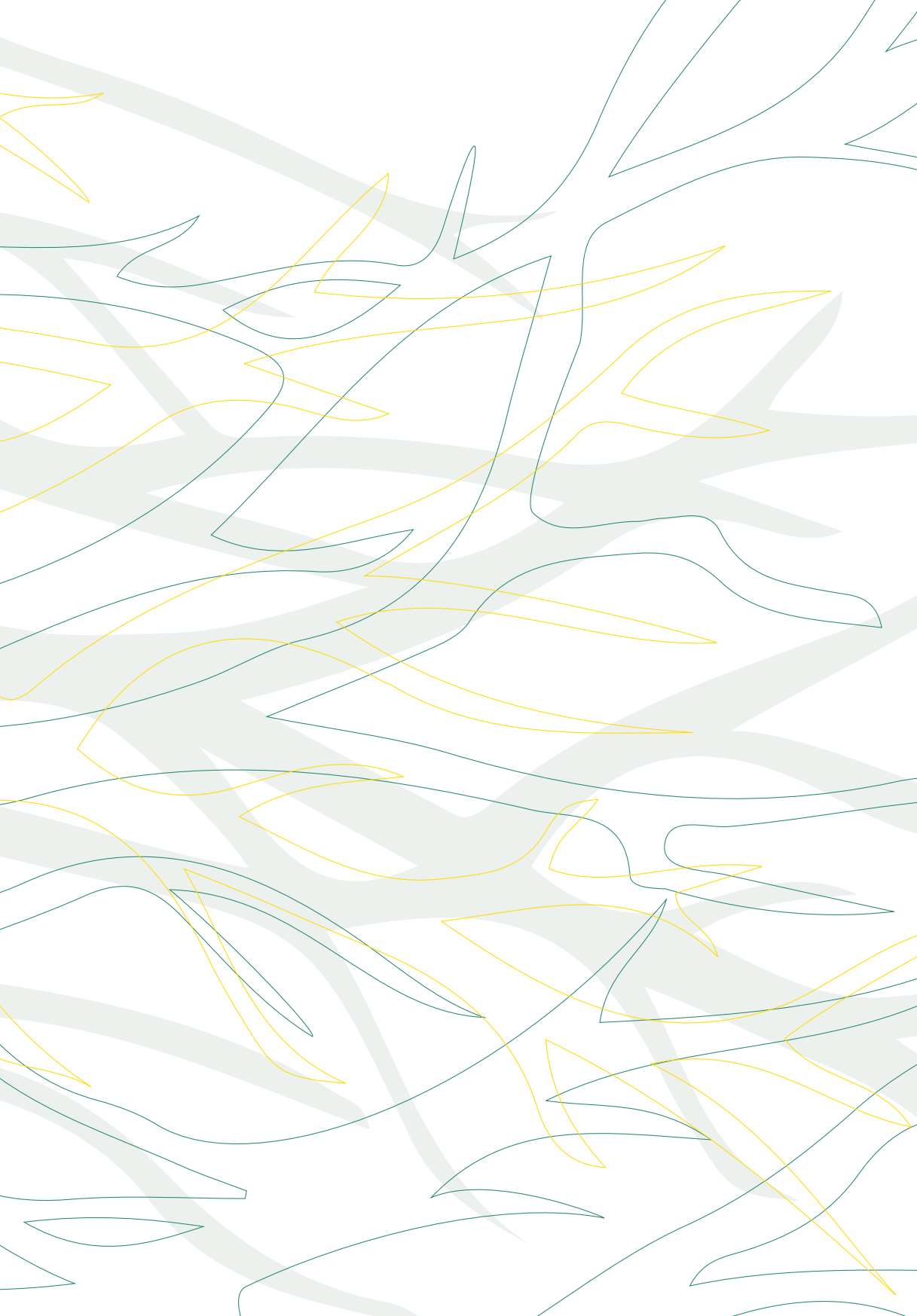
# 1

# PART 1

## ETIOLOGY AND DIAGNOSIS



CHAPTER 2

# Severe *Salmonella* spp. or *Campylobacter* spp. infection and the risk of biliary tract cancer: a population - based Study

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*Cancers 2020*

# ABSTRACT

*Salmonella* spp. infection has shown to have oncogenic transformative effects and thereby increases the risk of certain cancers. For *Campylobacter* spp., similar effects have been demonstrated. Risk factor identification may allow for timely diagnosis and preventive treatment. To substantiate the oncogenic potential of *Salmonella* and *Campylobacter* spp., this study compared the incidence of extrahepatic biliary tract cancer (BTC) in patients with diagnosed *Salmonella* or *Campylobacter* spp. infection with BTC incidence in the Netherlands. National infectious diseases surveillance records of patients diagnosed with a laboratory-confirmed *Salmonella* or *Campylobacter* spp. infection during 1999–2016 were linked to the Netherlands Cancer Registry. Incidence of BTC in *Salmonella* and *Campylobacter* spp. patients was compared to the incidence of BTC in the general population using Standardized Incidence Ratios (SIRs). In total, 16,252 patients were diagnosed with *Salmonella* spp. and 27,668 with *Campylobacter* spp. infection. Nine patients developed BTC at a median of 47 months (13–67) after *Salmonella* spp. infection and seven at a median of 61 months (18–138) after *Campylobacter* spp. infection. SIR of BTC in salmonellosis patients was 1.53 (95% CI 0.70–2.91). In patients aged <60 years, the SIR was 1.74 (95% CI 0.36–5.04). For campylobacteriosis patients, the SIR was 0.97 (95% CI 0.39–2.00). Even though *Salmonella* or *Campylobacter* spp. infection was not significantly associated with increased BTC risk in this cohort, it remains extremely important to study potential risk factors for cancer to facilitate screening and ultimately improve prognosis of cancer patients.

# INTRODUCTION



Biliary tract cancers (BTC) are rare malignancies of the distal and proximal bile ducts, the gallbladder and the cystic duct. Despite significant improvement in the overall survival of cancer patients, 5-year survival of patients with extrahepatic biliary tract cancer (i.e., gallbladder cancer, proximal and distal cholangiocarcinoma) is still only 10%. [1-3] Currently, radical surgery is the only curative treatment available. Unfortunately, surgery is not an option in the majority of patients, because BTC frequently goes undetected until the disease has progressed to an advanced, unresectable stage. [4 5, 6]

Geography appears to be the primary risk factor for the development of non-intrahepatic BTC, and as a result, incidence rates vary significantly per region. For example, gallbladder cancer (GBC) incidence ranges from 0.9/100,000 women in the Netherlands to 35/100,000 women in Chile. [6, 7] Other risk factors for BTC include age, parasitic infections, congenital malformations of the biliary tract, primary sclerosing cholangitis, and sex. [8] However, most patients with BTC do not have any of the known risk factors apart from age. [9] Screening for and detection of risk factors in addition to geography and age could lead to significantly faster detection of BTC and a subsequent improvement in survival.

An estimated 20% of the global cancer burden can be attributed to infectious diseases. [10] The association between viral infections, such as human papilloma virus, hepatitis B and C and certain forms of cancer, has been well-established. [11, 12] This knowledge has led to the implementation of successful targeted treatment and screening programs that can facilitate prevention and early detection of these cancers and improve survival, such as the Dutch national program for cervical cancer. [13] Although less studied, bacteria also have oncogenic potential and thereby increase the risk of cancer. [14] The primary example is Helicobacter pylori infection, which increases the risk of gastric cancer through the secretion of toxins that mediate cell signaling, as well as chronic inflammation. [15] Similarly, *Salmonella* spp. enforce bacterial uptake by manipulating host cell signaling pathways. Specifically, host AKT and ERK pathways are activated. Both pathways are active in many cancers and are an essential step in the malignant transformation of pre-transformed cells. [16] *Salmonella* spp. infection is common and represents a known risk factor for gallbladder and colon cancers, with the former pertaining specifically to *Salmonella* typhi, the agent of typhoid fever, and the latter to non-typhoidal Salmonellae. [16, 17] However, the role of non-typhoidal *Salmonella* has not yet been investigated for other biliary cancers. *Campylobacter* spp. is another

frequently-occurring gastrointestinal infection able to promote colon tumorigenesis by producing cytolethal distending toxins and is more frequently present in the microbiome of colorectal cancer patients, although a causal relationship between colorectal cancer and *Campylobacter* spp. infection has not been demonstrated. [18-21] *Salmonella* spp. is known to cause chronic inflammation of the bile ducts and to produce toxins with carcinogenic potential, which may lead to cancer of the extrahepatic biliary tract. [22] After an outbreak of *Salmonella* typhi in 1964, researchers found that the risk of biliary tract cancer was increased by 164 times in carriers compared to non-carriers. [23] Although non-typhoidal *Salmonella* has been associated with the development of colon cancer, its role has not been specifically investigated in biliary tract cancers other than gallbladder cancer. [17] *Campylobacter* spp. is found in abundance in the biliary microbiome of patients with BTC. [24] The potential association with non-typhoidal *Salmonella* or *Campylobacter* spp. and BTC has not been studied in large cohorts due to the rarity of BTC, especially in Western populations. In case an association is found, targeted screening for BTC in *Salmonella* spp. and *Campylobacter* spp. patients might be considered. To assess whether infection with non-typhoidal *Salmonella* or *Campylobacter* spp. is a risk factor for BTC, this study compares the incidence of BTC in patients with a registered non-typhoidal *Salmonella* or *Campylobacter* spp. infection in the past to the incidence of BTC in a Western–European population.

# MATERIALS AND METHODS

## DATA COLLECTION AND LINKAGE

Analyses were based on three linked health registries with national coverage. The first registry contains records from laboratory-confirmed human infections with *Salmonella* spp. (from 1999 onwards) and *Campylobacter* spp. (from 2002 onwards) based on the national laboratory surveillance system for gastrointestinal pathogens coordinated by the Dutch National Institute for Public Health and the Environment (RIVM). [25] The surveillance system has an estimated coverage of the resident Dutch population of 64% for *Salmonella* spp. and 52% for *Campylobacter* spp. infection. The second registry consisted of histopathological records provided by the automated pathological archive, the nation-wide network of histopathology and cytology in the Netherlands (PALGA). [26] The third registry was the Netherlands Cancer Registry (NCR), which contains data on all newly diagnosed malignancies since 1989, covering around 95% of the Dutch population. [27] The NCR is updated through PALGA and supplemented annually by information from hospital discharge records. Statistics Netherlands (CBS)

acted as a trusted third party to anonymize and link the data sets (www.cbs.nl). The CBS used the date of birth, gender and six digit postal code, which were available in all three registries, to generate a unique personal identifier (Record Identification Number (RIN)). After the RIN was generated, all personally identifying data was removed from the data sets. The researchers used the RIN to link all three data sets. Ethical Approvals were given by the CMO Arnhem-Nijmegen, code: 2017-3912 on the 18th of December 2017. A waiver of informed consent was provided.

## PATIENT SELECTION AND VARIABLE DEFINITIONS

All patients ≥20 years of age diagnosed with non-typhoidal *Salmonella* infection from the 1st of January 1999, and diagnosed with *Campylobacter* spp. infection from the 1st of January 2002, until the 31st of December 2016 were identified in the RIVM database. Additionally, all patients with non-intrahepatic biliary tract cancer (ICD-O-3 location codes C239, C240, C242, C243, C244, C248, C249) were identified in the NCR database. Patients who were diagnosed with intrahepatic BTC, BTC before or within 1 year of salmonellosis/campylobacteriosis diagnosis or had less than 1 year of follow-up were excluded. In case the patient had multiple recorded *Salmonella* spp./*Campylobacter* spp. infections, only the first diagnosis was considered. Both databases were cleared from duplicates. Time at risk was defined as the number of days between 1 year after salmonellosis or campylobacteriosis diagnosis and development of BTC, death, or end of the study period (31st of December 2017), whichever occurred first.

## OUTCOMES

The primary outcome of the study was the incidence of BTC among individuals with a registered non-typhoidal *Salmonella* or *Campylobacter* spp. infection in the past as compared to the incidence of BTC in the general Dutch population. Subgroup analyses were conducted to investigate the risk of BTC in patients ≤60 years of age (at the time of infection) and by gender.

## STATISTICAL ANALYSIS

Standardized incidence ratios (SIR) were calculated for salmonellosis and campylobacteriosis patients separately to compare the difference in incidence of BTC in patients with *Salmonella* spp. or *Campylobacter* spp. infection to an age-, gender- and calendar year-matched cohort of the general Dutch population. To this end, the observed number of BTC cases in the salmonellosis and campylobacteriosis patients was divided by the expected number of BTC cases in the matched cohort provided by the NCR. 95% confidence intervals (95% CI) for the SIRs were calculated assuming

a Poisson distribution. In all analyses, p-values < 0.05 were considered statistically significant. Statistical analysis was performed using STATA version 14 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

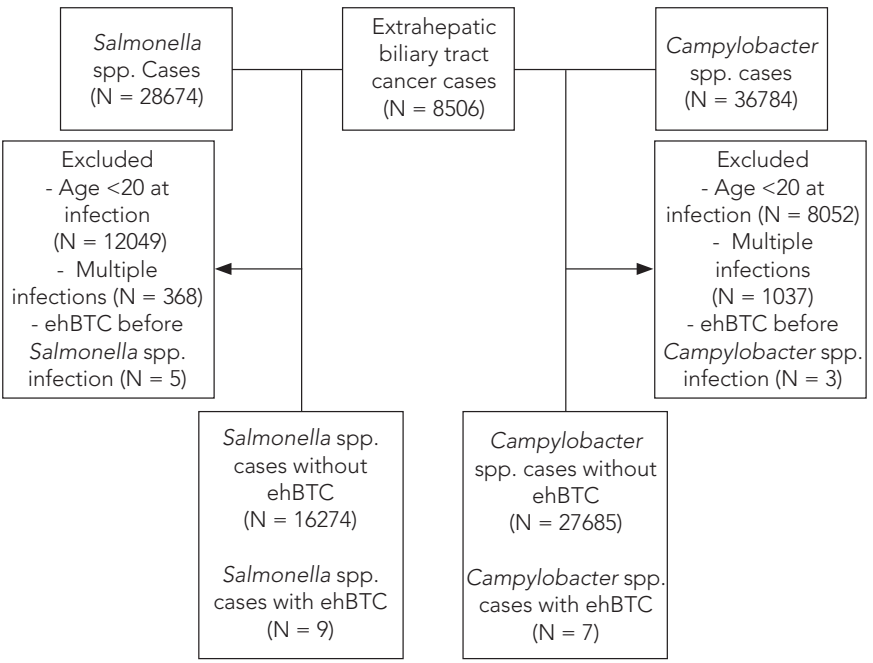
# RESULTS

## COHORT CHARACTERISTICS

The final cohort consisted of 16,283 *Salmonella* spp. patients (reported between 1999–2016), 27,692 *Campylobacter* spp. patients (reported between 2002–2016) and 8506 patients with BTC (Figure 1). After linkage, nine *Salmonella* spp. patients and seven *Campylobacter* spp. patients were diagnosed with BTC ≥1 year after infection.

Baseline characteristics of the cohorts are provided in Table 1 (Salmonellosis and Campylobacteriosis) and Table 2 (BTC). Median age at infection was 48.9 years (IQR:

**Figure 1.** Cohort selection. ehBTC = extrahepatic biliary tract cancer.



30.0–66.0) for salmonellosis patients (66.5%, <60 years) and 48.5 years (IQR: 31.3–62.4) for Campylobacterosis patients (70.8%, <60 years). Median follow-up after infection was 7 years (IQR 3–12) in salmonellosis patients and 5 years (IQR 3–9) in Campylobacterosis patients. Median age at diagnosis was 73 years (IQR 64–80) in BTC patients. Median follow-up time from diagnosis to death or end of study in in BTC patients was 55 months.

**Table 1.** Baseline characteristics of the Salmonellosis and Campylobacterosis patients.

| Characteristic | *Salmonella* N (%) | *Campylobacter* N (%) |
|---|---|---|
| **Age** | | |
| <40 | 6 167 (38%) | 10 125 (37%) |
| 40 – 49 | 2 190 (13%) | 4 499 (16%) |
| 50 – 59 | 2 445 (15%) | 4 985 (18%) |
| 60 – 69 | 2 273 (14%) | 4 153 (15%) |
| 70 - 79 | 2 003 (12%) | 2 703 (10%) |
| 80+ | 1 172 (7%) | 1 227 (4%) |
| **Sex** | | |
| Male | 7 640 (47%) | 14 293 (52%) |
| Female | 8 612 (53%) | 13 399 (48%) |
| **Serotype/species** | | |
| *S.* Typhymurium/monophasic | 4 487 (28%) | N.A. |
| *S.* Enteritidis | 5 544 (34%) | N.A. |
| *S.* (Para) tyhpi | 318 (2%) | N.A. |
| Other *Salmonella* serotypes | 5 903 (36%) | N.A. |
| *C.* jejuni | N.A. | 23 647 (85%) |
| *C.* coli | N.A. | 1 910 (7%) |
| Other *Campylobacter* species | N.A. | 2 135 (8%) |
| **Type of infection** | | |
| Septemic | 884 (5%) | [1] |
| Enteric | 13 864 (88%) | [1] |
| Other | 1.066 (7%) | [1] |

[1] Not registered for Campylobacterosis cases.

**Table 2.** Baseline characteristics of patients with BTC in the Netherlands (2000–2017).

| Characteristic | N (%) |
|---|---|
| **Age** | |
| <40 | 99 (1%) |
| 40 – 49 | 326 (4%) |
| 50 – 59 | 993 (12%) |
| 60 – 69 | 2 048 (24%) |
| 70 - 79 | 2 851 (33%) |
| 80+ | 2 189 (26%) |
| **Sex** | |
| Male | 3 822 (45%) |
| Female | 4 684 (55%) |
| **Tumor location** | |
| Gallbladder | 2 586 (30%) |
| Bile ducts, NOS | 2 421 (28%) |
| Proximal bile ducts | 1 846 (22%) |
| Distal bile ducts | 1 490 (18%) |
| Other[1] | 163 (2%) |
| **Clinical stage** | |
| Non-metastatic | 4 078 (48%) |
| Metastatic | 4 428 (52%) |
| **Treatment** | |
| Resection | 2 479 (29%) |
| Chemotherapy | 647 (8%) |
| Survival (months)[2] | 5.8 (5.5-6.0) |

[1] Includes cystic duct and mixed types. [2] Displayed as median and 95% CI.

## PATIENTS WITH SALMONELLA SPP. INFECTION AND BTC

Nine salmonellosis patients were diagnosed with BTC ≥1 year after salmonellosis diagnosis (Table 3). Mean time to BTC diagnosis was 47 months (range 13–81). Three of nine (33%) salmonellosis patients were ≤50 years old at time of BTC diagnosis, as opposed to the general BTC population, in which only 5.0% of patients were ≤50 years old at time of BTC diagnosis ($p < 0.001$). Four cases were diagnosed with S. enteritidis,

three with S. typhimurium, and two with other *Salmonella* serovars. Eight patients had an enteric infection, one had an invasive (bloodstream) infection. Two patients had a distal cholangiocarcinoma, one patient had a proximal cholangiocarcinoma, one patient had gallbladder cancer, and five had BTC NOS (not otherwise specified).

## PATIENTS WITH CAMPYLOBACTER SPP. INFECTION AND BTC

Seven campylobacteriosis patients were diagnosed with BTC ≥1 year after diagnosis (Table 3). Mean time to BTC diagnosis was 60.6 months (range 18–138). All patients were >50 years of age at time of BTC diagnosis. Five patients had a proximal cholangiocarcinoma and two patients had gallbladder cancer.

**Table 3.** Baseline characteristics of patients with *Salmonella* spp. or *Campylobacter* spp. infection and extrahepatic biliary tract cancer.

| Characteristic | Salmonella N (%) | Campylobacter (N%) |
|---|---|---|
| **Sex (male)** | 5 (56%) | 3 (43%) |
| Age | | |
| <60 | 3 (33%) | 2 (29%) |
| ≥60 | 6 (66%) | 5 (71%) |
| **Serotype** | | |
| Enteritidis | 4 (45%) | N.A. |
| Typhimurium/monophasic | 2 (22%) | N.A. |
| Other | 3 (33%) | N.A. |
| **Interval** | | |
| <60 months | 7 (78%) | 3 (43%) |
| ≥60 months | 2 (22%) | 4 (57%) |
| **Tumor location** | | |
| Gallbladder/proximal bile ducts | 2 (22%) | [1] |
| Distal bile ducts | 2 (22%) | [1] |
| Extrahepatic bile ducts, NOS | 5 (56%) | 0 (0%) |
| **Base of diagnosis** | | |
| Cytology/Imaging | 6 (67%) | 2 (29%) |
| Histology | 3 (33%) | 5 (71%) |

[1] Numbers cannot be provided due to risk of subject identification.

**Table 4.** Incidence of biliary tract cancer in patients ≥1 year after laboratory-confirmed infection with *Salmonella* spp. or *Campylobacter* spp., stratified by age at infection and gender.

| Type | Observed Incidence | Expected Incidence | SIR | 95% CI | *p*-value |
|---|---|---|---|---|---|
| ***Salmonella* spp.** | | | | | |
| All patients | 9 | 5.875 | 1.53 | 0.70–2.91 | 0.280 |
| 20–60 | 3 | 1.740 | 1.72 | 0.36–5.04 | 0.507 |
| Male | 5 | 2.665 | 1.88 | 0.61–4.38 | 0.264 |
| Female | 4 | 3.289 | 1.22 | 0.33–3.11 | 0.835 |
| ***Campylobacter* spp.** | | | | | |
| All patients | 7 | 7.221 | 0.97 | 0.39–2.00 | 0.868 |
| 20–60 | 2 | 2.126 | 0.94 | 0.11–3.40 | 0.715 |
| Male | 3 | 4.025 | 0.75 | 0.15–2.18 | 0.857 |
| Female | 4 | 3.233 | 1.24 | 0.34–3.17 | 0.810 |

### RISK OF BTC AFTER SALMONELLA SPP. OR CAMPYLOBACTER SPP. INFECTION

The SIR of BTC among the salmonellosis patients (compared to the general population) was 1.53 (95% CI 0.70–2.91, Table 4) and the absolute risk was 0.05%. Subgroup analysis in patients <60 years of age demonstrated that the SIR in this group was 1.72 (CI 0.36–5.04). Subgroup analysis according to gender revealed similar findings. In campylobacteriosis patients, the SIR was 0.97 (95% CI 0.39–2.00, Table 4) and the absolute risk was 0.03%. Subgroup analyses stratified according to gender and age revealed similar results.

## DISCUSSION

This study assessed whether *Salmonella* spp. or *Campylobacter* spp. infection represents a significant risk factor for BTC by comparing the incidence of BTC in patients with a history of *Salmonella* spp. or *Campylobacter* spp. infection to the (age-, gender- and calendar year-matched) incidence of BTC in the general Dutch population. Additionally, age and gender effects on the association between *Salmonella* spp. or *Campylobacter* spp. infection and BTC were investigated. No significant increase

in BTC occurrence in patients who had experienced a severe *Salmonella* spp. or *Campylobacter* spp. infection was observed.

The relatively low number of *Salmonella* spp. (and *Campylobacter* spp.) infections linked to the (already rare) BTC patients found in this study was the main limitation for statistical significance, as considerable uncertainty was introduced in the estimates by such low number of outcome events. The upper limit of the SIR for BTC in salmonellosis patients was 2.7, which implies that a clinically significant effect may be present, but the study is simply insufficiently powered to detect its presence. This issue is, however, not unique to this study alone, but rather affects all studies investigating rare diseases. Experts increasingly recognize that some evidence, although maybe imprecise, may be better than no evidence at all. [28]

In countries where typhoid fever is still endemic, such as the Indian subcontinent and some parts of South America, multiple epidemiological studies have shown an increased risk for the development of BTC and especially gallbladder cancer. Besides chronic infection, an increased risk of gallstones in these populations, a higher incidence of obesity, and potential environmental pollution have been mentioned as potentially contributing to this phenomenon. [22] However, none of these factors (apart from gallstones and gallbladder cancer, which is not unique to these countries) show an extremely high correlation with the incidence of BTC. On the other hand, researchers have demonstrated a clear association with chronic S. typhi infection and the development of gallbladder cancer in these countries. [29] In contrast, a Chinese study investigating the correlation between chronic infection with S. typhi and biliary tract cancer failed to find a significant association due to a very low occurrence of such infection. [30] One may argue that association does not equal causation and that in areas with endemic typhoid fever and high rates of gallbladder cancer, other factors might be at play as well. However, even in Western countries with typically extremely low incidence of S. typhi infection (as typhoid fever has been eradicated in most Western countries thanks to modern sanitation), after large outbreaks of typhoid fever, an increase in number of BTC diagnoses is observed. [23]

This paper focusses primarily on the incidence of BTC in non-typhoidal *Salmonella*. We hypothesized that, similar to gallbladder cancer, the increased incidence of BTC after typhoidal *Salmonella* infection would translate to increased BTC risk in non-typhoidal *Salmonella*. [31] The lack of significant correlation in non-typhoid *Salmonella* infection may be attributed to the fact that non-typhoid *Salmonella* strains are less likely to

cause chronic infection and thus have lower oncogenic potential compared to their typhoid counterparts. [32]

Remarkably, one third of the patients with both *Salmonella* spp. infection and BTC were under 50 years of age at time of BTC diagnosis. This proportion was significantly higher than in the general BTC population, in which only 5% is aged 50 years or younger. [33] Because the risk of BTC increases exponentially with age, we performed a subgroup analysis in all patients aged <60. Although this subgroup analysis also failed to reach significance due to the even lower numbers, the relatively high proportion of young patients suggests that *Salmonella* spp. infection at a young age might contribute to the risk of developing BTC later in life. Possibly, patients who acquire a *Salmonella* spp. infection at the age of 70 or older may die from other diseases before they develop BTC and are thus less well-represented. The median time between *Salmonella* spp. infection and BTC diagnosis was 4 years. This finding implies that the potential oncogenic effect of *Salmonella* spp. results in malignant transformation of epithelial cells in a relatively short timeframe and is concurrent with other studies. [17] Another explanation may be that patients with inflammatory bowel disease (IBD) are at a higher risk for developing a serious *Salmonella* spp. infection. Since IBD often has an onset in early adulthood and is also a potential independent risk factor for the development of BTC, it is possible that this difference in age can be explained by the fact that the patients with *Salmonella* spp. infection also had IBD and therefore were at greater risk for developing BTC at a younger age. [34]

No tendency towards increased BTC incidence after *Campylobacter* spp. infection was seen in this study. *Campylobacter* spp. and *Salmonella* spp. bacteria both release the genotoxic protein cytolethal distending toxin (CDT). However, whereas *Salmonella* spp. is linked to the development of BTC by overexpression of C-myc in tissue samples, *Campylobacter* spp. is not. [16] Differences in bacterial mechanisms, specifically concerning the alteration of host cell signaling pathways during invasion, may account for differences in oncogenic potential between the two species.

Molecular characterization of cancers and subsequent personalization of therapy is a prime topic in current oncological research. Although the genomic landscape of BTC is incredibly diverse, multiple preclinical and clinical models show that BTC development may be associated with the alteration of several actionable genes. A particular example is the overexpression of cyclophilin-A in patients with liver-fluke-associated cholangiocarcinoma. [35, 36] Identification of inflammation-associated driving

mutations is an important topic as it has implications for both risk profiling and potential personalized treatment. Although molecular profiling of patients with salmonellosis and BTC was outside of the scope of this study, a study in gallbladder cancer has managed to identify the signaling pathway associated with S. typhi development and gallbladder cancer. [16] Further research investigating molecular alterations in infected cancer patients is paramount to increase our understanding of tumor cell transformation and cancer development.

The primary limitation of this study is the low number of *Salmonella* spp. and *Campylobacter* spp. infected patients that also developed BTC, leading to a high risk of type-2 error. Typically, patients with *Salmonella* spp. infection in the Netherlands who require medical attention, laboratory diagnosis and reporting to health authorities are severely ill. As most patients with *Salmonella* spp. infection only show mild symptoms, the actual number of *Salmonella* spp. cases in the Netherlands is much higher than reported. It is estimated that close to 1 million inhabitants developed a symptomatic *Salmonella* spp. infection in the Netherlands between 1999–2015, which is 35 times the number of cases included in this study. Campylobacteriosis cases are estimated around 81,000 in the Netherlands annually. [37] As a result, a number of patients with mild and therefore unreported *Salmonella* spp. or *Campylobacter* spp. infection, but with a BTC diagnosis, may have been misclassified and included in the group of BTC patients without (reported) *Salmonella* spp. or *Campylobacter* spp. infection. Since the contribution of these mild infections to the risk of developing BTC is implicitly included in the baseline risk, our results may be considered as very conservative estimates of their true contribution to BTC risk. Moreover, although chronic infections are those mostly implicated in BTC formation, they could not be studied as such in this study because this information (i.e., differentiation between acute and chronic infection) is simply not available in the RIVM data set. [25] Yet, we included all reported infections, and because these infections represent the most severe ones (in terms of magnitude and duration of symptoms) occurring in the population, our analysis implicitly focused on a selection of salmonellosis and campylobacteriosis patients that showed extreme clinical manifestations. Finally, the RIVM registry only contains data on *Salmonella* spp. and *Campylobacter* spp. infection from 1999 onwards and consequently we only had a median follow-up period of 7 years. If, like in pancreatic cancer, the interval between first mutation and cancer development is over 10 years, the study period may have been insufficient to detect a correlation between infection and BTC development. [38]

A major strength of this study is the cohort size and nation-wide design. Indeed, it should be acknowledged that the low number of BTC events in our cohort—despite the large surveillance data sets used—reflects mainly the rare occurrence of these tumors. The cohort analyzed in this paper is large and comprehensive, being nation-wide and covering all available years of systematic data collection. Previous studies investigating the role of bacterial infections in the development of BTC have typically drawn from case-control cohorts or small case series. Additionally, to our knowledge, this paper describes the first Western cohort of patients with *Salmonella* spp. or *Campylobacter* spp. infection and BTC. [29]

# CONCLUSIONS

There is accumulating evidence that pathogenic bacteria like *Salmonella* spp. play a role in cancer development, including cancers of the digestive system. However, we could not demonstrate a significantly increased occurrence of BTC among reported salmonellosis or campylobacteriosis patients as compared to the general population. Potentially, the study was either underpowered due to the low number of BTC events or *Salmonella* and *Campylobacter* spp. infections are not associated with the development of BTC in Western countries. Additional research is needed to unravel the biological mechanisms behind bacterial infections as a cause of cancer and identify potential infections that may warrant early screening and therefore facilitate early cancer detection, especially in third-world countries with high rates of (hyper) endemic bacterial infections.

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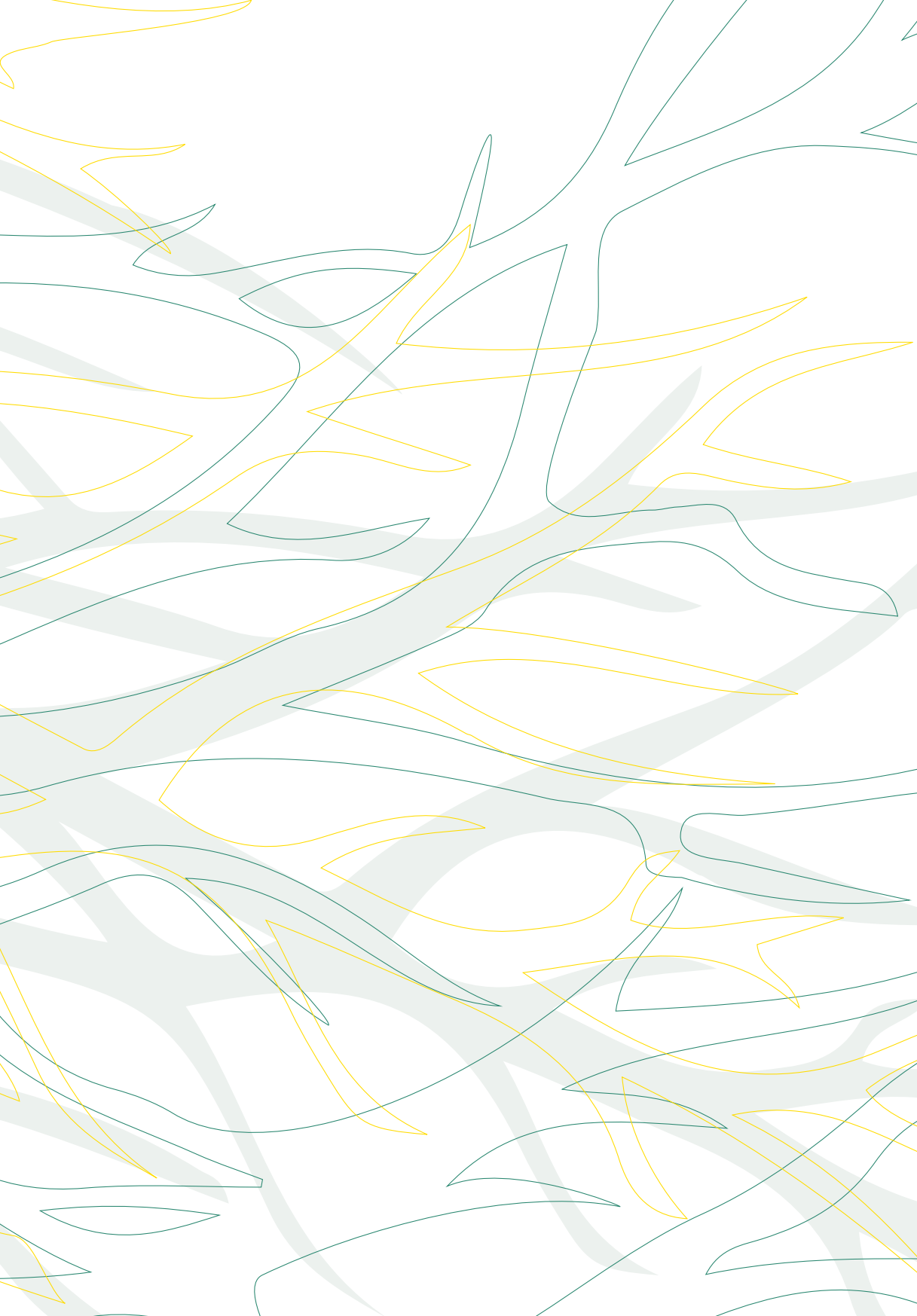

CHAPTER 3

# The diagnostic accuracy of CT and MRI for the detection of lymph node metastases in gallbladder cancer; a systematic review and meta - analysis

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# ABSTRACT


## BACKGROUND

Lymph node metastases (LNM) are an ominous prognostic factor in gallbladder cancer (GBC) and, when present, should preclude surgery. However, uncertainty remains regarding the optimal imaging modality for pre-operative detection of LNM and international guidelines vary in their recommendations. The purpose of this study was to systematically review the diagnostic accuracy of computed tomography (CT) versus magnetic resonance imaging (MRI) in the detection of LNM of GBC.

## METHODS

A literature search of studies published until November 2017 concerning the diagnostic accuracy of CT or MRI regarding the detection of LNM in GBC was performed. Data extraction and risk of bias assessment was performed independently by two reviewers. The sensitivity of CT and MRI in the detection of LNM was reviewed. Additionally, estimated summary sensitivity, specificity and diagnostic accuracy of MRI were calculated in a patient based meta-analysis.

## RESULTS

Nine studies including 292 patients were included for narrative synthesis and 5 studies including 158 patients were selected for meta-analysis. Sensitivity of CT ranged from 0.25 to 0.93. Estimated summary diagnostic accuracy parameters of MRI were as follows: sensitivity 0.75 (95% CI 0.60 – 0.85), specificity 0.83 (95% CI 0.74 – 0.90), LR+ 4.52 (95% CI 2.55 – 6.48) and LR- 0.3 (95% CI 0.15 – 0.45). Small (<10mm) LNM were most frequently undetected on pre-operative imaging. Due to a lack of data, no subgroup analysis comparing the diagnostic accuracy of CT versus MRI could be performed.

## CONCLUSION

The value of current imaging strategies for the pre-operative assessment of nodal status in GBC remains unclear, especially regarding the detection of small LNM. Additional research is warranted in order to establish uniformity in international guidelines, improve pre-operative nodal staging and to prevent futile surgery.

# INTRODUCTION



Gallbladder cancer (GBC) is the fifth most prevalent malignancy of the gastrointestinal tract worldwide. [1,2] Due to an asymptomatic course in early stages and a propensity for aggressive local growth, most gallbladder cancers are only diagnosed in an advanced stage. Outcomes are poor with reported 5-year survival rates ranging from 10-20%. [3] Radical excision currently remains the only curative treatment option. However, only 20-30 % of pre-operatively diagnosed patients are candidates for resection. [4]

Prognosis after surgery is primarily determined by tumour- and lymph node stage; one-year survival rate after radical resection in T3 tumours drops from 50% to 2% when distant lymph nodes (outside of the hepatoduodenal ligament) are involved. [5] Once the tumour has metastasised to the periaortic, pericaval, superior mesenteric and celiac lymph nodes, resection does not appear to increase survival and surgery is deemed futile. [6] Adequate pre-operative detection of lymph node metastases (LNM) is therefore of vital importance to adequately select surgical candidates and to prevent surgery-related morbidity and mortality.

Agarwal et al. [7] analysed 60 patients with irresectable gallbladder cancer and found that 4 (7%) patients were irresectable due to pre-operatively undetected distant LNM. Other studies show that up to 50% of locally advanced gallbladder tumours appear to be irresectable during exploratory laparotomy due to undetected lymph node or peritoneal metastases. [8] Recently, staging laparoscopy has been incorporated into clinical practice in order to determine the resectability of gallbladder cancer before committing to exploratory laparotomy. However, a study found that among 314 patients who were deemed resectable after pre-operative CT imaging and staging laparoscopy, 47 (15%) ultimately were irresectable due to nonlocoregional LNM. [8] Clearly, better pre-operative radiological detection of nonlocoregional lymph nodes in addition to staging laparoscopy is paramount in order to prevent redundant surgery in gallbladder cancer patients. However, it is unclear which is the optimal imaging modality with the highest diagnostic accuracy.

Currently, computed tomography (CT) is the most widely used imaging modality for pre-operative staging. [5] However, the reported sensitivity of CT for the detection of LNM is only around 24%. [9] Evidence suggests that magnetic resonance imaging (MRI) might outperform CT with a sensitivity of up to 80% for nodal metastases and 100% for liver invasion. [10] Additionally, opposed to CT, MRI does not rely on the use of ionizing

radiation, using magnetic stimulation of hydrogen atoms to depict the targeted tissue. MRI is especially useful for creating highly detailed images of soft tissues since these contain a high amount of hydrogen atoms. Although availability of MRI is less compared to CT, it is increasingly being used in clinical practice due to a better safety profile, suspected superior diagnostic performance and increasing availability.

The aim of the current systematic review is to determine the diagnostic accuracy of CT and MRI in the detection of LNM in order to define the optimal pre-operative imaging strategy in patients with gallbladder carcinoma.

# METHODS

## STUDY SELECTION

All prospective and retrospective cross-sectional studies analysing the diagnostic accuracy of CT and MRI in the detection of LNM of gallbladder cancer were considered eligible. Studies were included for narrative review if the following additional criteria were met: (a) all patients were > 18 years of age, (b) histopathological analysis or follow-up imaging was available as a reference standard, (c) sufficient data was reported in order to extract the number of true positive and false negative results of CT and/or MRI in the detection of lymph node metastases, regardless of level of reporting.

For the meta-analysis, studies reporting anything other than the patient as unit of analysis were excluded since data from varying levels of reporting (for example patient versus lymph node versus regional) cannot be pooled. Only studies reporting patient-level data using true positives, true negatives, false positives and false negatives were included for meta-analysis. Case-control studies were excluded due to a high risk of bias. There were no restrictions based on publication status. Studies reporting results in any language but English, Dutch or German were excluded. When cohort overlap was suspected, the study with the smallest number of participants was excluded.

## SEARCH STRATEGY

A systematic literature search was conducted in the databases of MEDLINE (8$^{th}$ of November, 2017) and EMBASE (10$^{th}$ of November, 2017). The search was performed including terms for ''gallbladder cancer'', ''Magnetic Resonance Imaging'' and ''Computed Tomography'' (full search strategy is provided in Appendix A, Table A1&A2). Additionally, the references of all included studies and major meta-analyses

were searched for additional studies not included in the results of electronic searches. Online clinical trial registries such as ISRCTN (www.isrctn.com) and ClinicalTrials.gov (www.clinicaltrials.gov) were searched as an additional source for related studies. This study was carried out in accordance with the protocol as registered in PROSPERO (record ID 83752).



## DATA EXTRACTION

One reviewer (E.S.L.) screened titles and abstracts of records retrieved by the electronic searches for eligibility. A second reviewer (T.B.) assessed the accuracy of decision making on a random sample of 20%. Full text of the studies possibly meeting inclusion criteria was obtained. Two independent reviewers (E.S.L. and T.B.) applied the inclusion criteria to the full records. Any disagreement in study selection was resolved by discussion or arbitration by a third reviewer (P.R.). When not enough information was provided in order to assess eligibility of the study for inclusion, the study authors were contacted with a request for additional information.

Data was extracted into Review Manager version 5.3 *(*Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) by two independent reviewers (E.S.L and T.B.) using a standardized data extraction form. The following data was extracted for all studies included for review: year of publication, country of publication, study design (e.g. retrospective cohort study, prospective cohort study, randomised controlled trial), full text publication or abstract, inclusion and exclusion criteria, number of participants, participant age and gender, years of experience and expertise of assessors, MRI/CT characteristics, reference standard characteristics and sensitivity for the detection of LNM.

Additionally, for studies included for meta-analyses the following additional data was extracted: number of true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN) and diagnostic criteria and cut-off values / version of TNM staging used.

When raw data on diagnostic accuracy was not available, study authors were contacted in order to obtain additional data. Any differences were resolved by discussion or by input from a third reviewer (P.R.).

## ASSESSMENT OF METHODOLOGICAL QUALITY

Using a modified version of the QUADAS-2 assessment-tool, study design characteristics were extracted and analysed by two independent reviewers (E.S.L. and T.B.) to assess methodological quality. [11] Any discrepancies were resolved by means of discussion and consensus. When discrepancies persisted, a third author (P.R.) was requested for additional input in order to reach consensus. Appendix B provides the criteria used to classify responses (yes, no or unclear) to each of the QUADAS-2 checklist items.

## STATISTICAL ANALYSIS AND DATA SYNTHESIS

For each study included in the meta-analysis, data was extracted to generate 2x2 contingency tables displaying true-positives, true-negatives, false positives and false negatives. Patients with N0 nodal status were regarded as disease negative and patients with either N1 or N2 nodal status were disease positive. True positives were defined as cases in which patients were categorised as having disease by both the index- and reference test. False positives were defined as patients categorised as having disease by the index test but categorised as not having disease by the reference standard. True negatives were patients categorised as not having disease by the index- and reference test. False negatives were defined as patients categorised as not having disease by the index test and having disease by the reference standard. Forest plots were constructed for all included studies displaying sensitivity, specificity and the corresponding 95% confidence interval for both index tests. Summary sensitivity and specificity of MRI were also plotted on a ROC curve. Since a common implicit cut-off value for test positivity is to be expected, estimates of pooled sensitivity and specificity were calculated by fitting a bivariate random effects model. A P-value of $<0.05$ was considered statistically significant. All analyses were conducted using Review Manager 5.3 and R 3.6 statistical package (R Core Team (2016). R, R Foundation for Statistical Computing, Vienna, Austria)

## ASSESSMENT OF REPORTING BIAS

To date, no formal tool for the assessment of reporting bias in diagnostic accuracy studies exists. However, we highlighted possible sources of detection, selection and reporting bias and consequently excised caution in the interpretation of results.

## HETEROGENEITY EXPLORATION

Due to the nature of diagnostic accuracy studies, heterogeneity is expected to be present. [12] We planned to conduct a sensitivity analysis exploring the influence of the following characteristics on sensitivity and specificity; year of publication, experience

of assessor (defined as ≤ 5 or > 5 years of experience), type of MRI/CT (single-slice vs. multi-slice, 1.5 vs. 3T), study design (prospective vs. retrospective), full text publications versus abstracts, age of participants, type of reference standard, version of TNM-staging used and use of contrast agents. However, due to the small sample of included studies, we did not conduct any sensitivity analyses.



# RESULTS

## SEARCH STRATEGY AND STUDY SELECTION

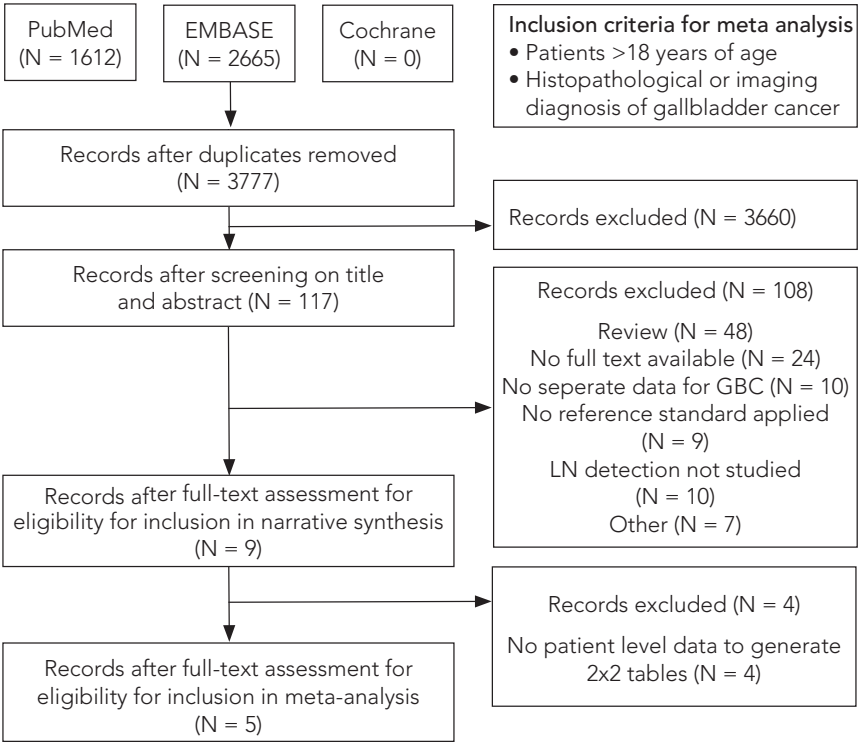
A flowchart of the selection process is provided in Figure 1. Our search strategy identified 1612 records in MEDLINE, 2665 in EMBASE and 0 in the Cochrane Library of Diagnostic Test Accuracy Studies. A hand-search of references from these studies and reviews did not yield any additional records. After removal of duplicates, the titles and abstracts of 3777 records were screened for relevance and 117 studies were selected for full text evaluation. A total of 108 studies were excluded after full-text assessment for the following reasons: narrative review/editorial/comment (N = 48); no full text available (N = 24); not addressing the research question (N = 10); not reporting data in such a way that sensitivity for LNM detection can be calculated (i.e. not all patients received verification by a reference test or not reporting data for GBC separately) (N = 19) or other reasons (N = 7). Five studies reported patient-level data and were included for meta-analysis. An additional four studies provided sensitivity data for CT or MRI, and were included for narrative synthesis.

The studies that met our inclusion criteria for meta-analysis or narrative review are described in Table 1.

## STUDIES INCLUDED FOR META-ANALYSIS.

Five studies were included for meta analysis. Four investigated the diagnostic accuracy of MRI in 138 participants and one (Engels et al.) investigated the diagnostic accuracy of CT in 20 participants. [13-17] All except one were of retrospective design. [15] None of the studies directly compared the diagnostic accuracy of CT with MRI. All except one (Engels et al) only included patients in which either curative or palliative surgery was performed, arguing the need for histopathological analysis of the final resection specimen as a reference standard. The authors of the study by Engels et al. chose to include irresectable patients, using a combination of percutaneous biopsy and

**Figure 1.** Study selection flowchart.




autopsy results as an alternative reference standard. Another MRI study (Kim et al. 2015) excluded patients in which CT imaging was considered to be diagnostic for tumour stage. Criteria for lymph node positivity were reported in 3 out of 5 studies. The prevalence of node-positive disease ranged from 33% to 72% with a median of 54% (IQR 43 - 68%).

## ADDITIONAL STUDIES INCLUDED FOR NARRATIVE REVIEW

Four studies could not be included for meta-analysis but did meet our eligibility criteria for narrative review. [18-21] Three studies investigated CT in 116 participants and one study (Kim et al. 2002) investigated MRI in 18 participants for the detection of LNM. All studies except one were retrospective in nature. Three studies chose to exclude patients in whom no surgical and/or histopathological analysis of a resection specimen was available as a reference standard. [18, 20, 21] One study did not exclude irresectable

Table 1. Characteristics of included studies.

| Basic study and patient characteristics | | | | | | |
|---|---|---|---|---|---|---|
| Authors | Year of publication | Number of participans | Age, mean | Study design | Reference standard | Modality |
| Engels et al. | 1989 | 20 | Unknown | Retrospective cohort | Surgical findings, autopsy, FNA biopsy results | CT |
| Kalra et al. | 2006 | 20 | 50 | Prospective cohort | Surgical findings, histopathology | CT |
| Kaza et al. | 2006 | 15 | 52 | Prospective cohort | Surgical findings, histopathology | MRI |
| Kim et al. | 2002 | 18 | 57 | Retrospective cohort | Surgical findings, histopathology | MRI |
| Kim et al. | 2015 | 86 | 65 | Retrospective cohort | Surgical findings, histopathology | MRI |
| Ohtani et al. | 1996 | 59 | 65 | Retrospective cohort | Surgical findings, histopathology | CT |
| Oikarinen et al. | 1993 | 37 | 69 | Retrospective cohort | Histopathology, FNA biopsy results, follow-up imaging results | CT |
| Schwartz et al. | 2002 | 19 | 68 | Retrospective cohort | Surgical findings, histopathology | MRI |
| Tseng et al. | 2002 | 18 | Unknown | Retrospective cohort | Surgical findings | MRI |



patients, but chose to compare pre-operative imaging findings to a combination of follow-up imaging and fine-needle biopsy results. [19] None of the studies excluded patients based on age, gender or tumour stage. Three studies used a cut-off value of a diameter >10mm as criterion for lymph-node positivity. [18-20] One study did not describe diagnostic criteria. [21]

## METHODOLOGICAL QUALITY

An overview of the methodological quality of included studies as assessed by QUADAS-2 is provided in Figure 2. Additionally, scores on each individual QUADAS-2 item for all included studies are displayed in Appendix C. As illustrated, there is a substantial amount of underreporting in the included studies, resulting in many ''unclear" judgements and consequently diminishing the quality of the data.

# FINDINGS

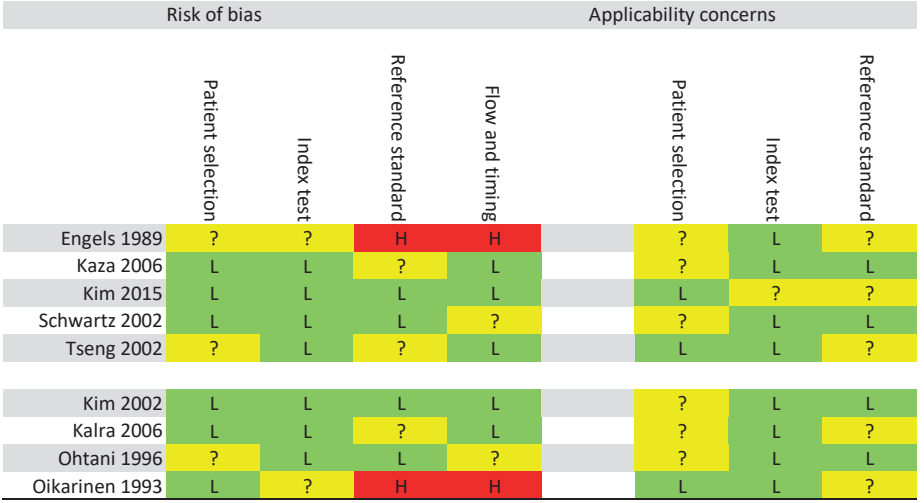
## SENSITIVITY OF CT AND MRI IN THE DETECTION OF LNM

Five studies investigated the diagnostic accuracy of MRI in 156 participants and four studies investigated the diagnostic accuracy of CT in 136 participants. In Figure 3, an overview of the sensitivity of CT and MRI in the detection of lymph node metastases is displayed. Results from all studies are shown, including studies not reporting patient level data. As demonstrated, sensitivity of both modalities varied greatly, ranging from 0.25 to 0.93. Of note, all studies in which the size of the false negative lymph nodes was reported stated that all missed LNM were <10mm in size. [15-17, 20]

## ACCURACY OF MRI AND CT FOR THE DETECTION OF LNM IN GALLBLADDER CANCER

A Forest Plot of sensitivity and specificity of MRI and CT for nodal staging from individual studies is displayed in Figure 4. Our search identified only one study which investigated the diagnostic accuracy of CT and reported data on patient level. [17] In this study, 20 patients with gallbladder cancer were included and the respective sensitivity and specificity of CT were 0.93 (95% CI 0.66 - 1.00) and 1.00 (95% CI 0.54 - 1.00). The negative likelihood ratio including 95% confidence interval was 0.07 (0.01 - 0.47). No false positives were reported in this study; therefore, the positive likelihood ratio could not be calculated.

**Figure 2.** Risk of bias assessment according to QUADAS2. Abbreviations: L = low, H = high, ? = unknown.



The sensitivity of MRI ranged from 60% to 92%. Specificity ranged from 83% to 100%. The estimated summary sensitivity and specificity values (Figure 5) including 95% confidence intervals were 0.75 (0.60 - 0.85) and 0.83 (0.74 - 0.90), respectively. The pooled positive likelihood ratio was 4.52 (95% CI 2.55 to 6.48), and the pooled negative likelihood ratio was 0.30 (95% CI 0.15 to 0.45).



The assessors in the study by Kim et al. (2015) did not perform consensus readings. [17] Instead, diagnostic accuracy data was reported from the separate readings of both assessors. We chose to incorporate the readings of both assessors into our model as separate studies. We also attempted to conduct two sensitivity analyses, incorporating only the data from either one of the assessors. However, not enough data was available and a summary ROC point could not be estimated. Figure 5 displays a summary ROC point for the diagnostic accuracy of MRI as well as individual accuracy estimates of all included studies.

Figure 3. Forest plot of reported sensitivity of all included studies.

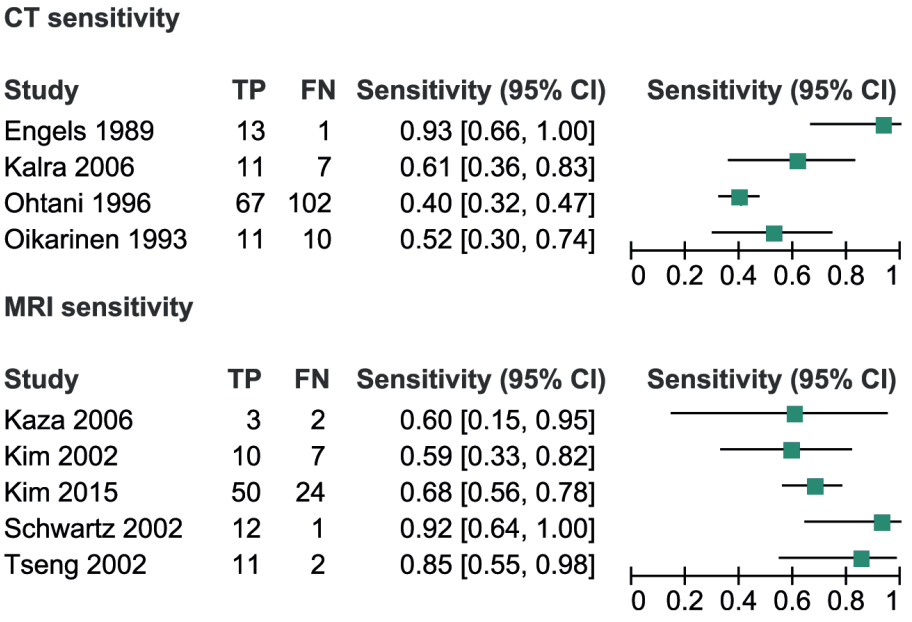


Figure 4. Forest plot of reported sensitivity and specificity of studies included in the meta-analysis.

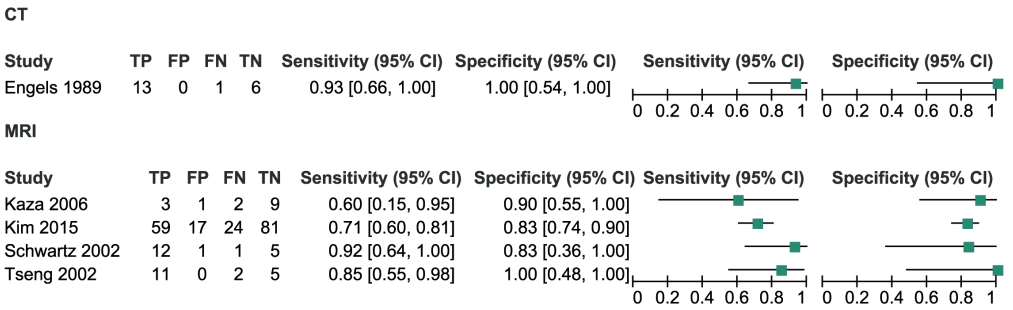
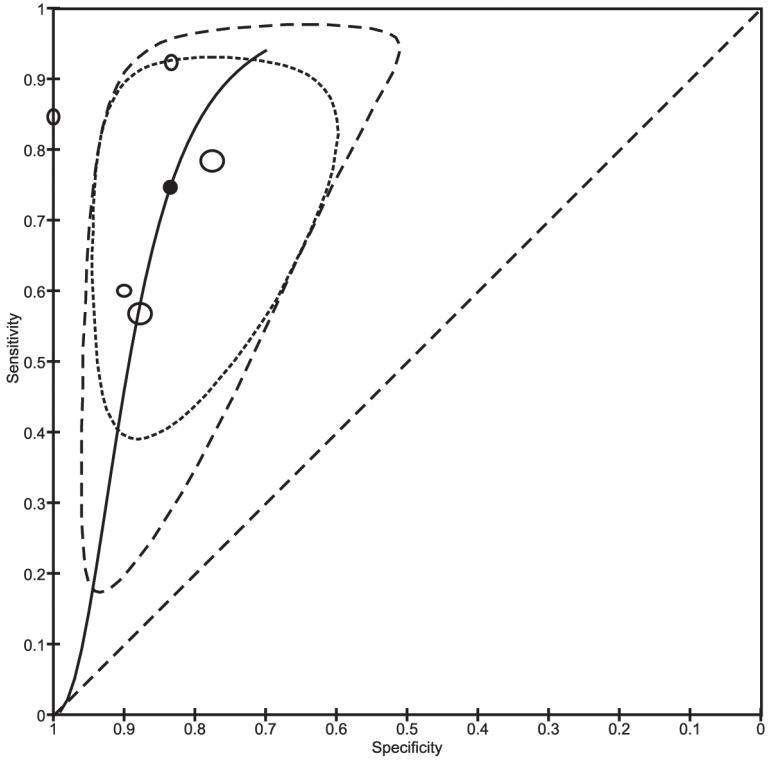



Figure 5. Summary receiver operating characteristic (SROC) curve of the diagnostic accuracy of MRI. The black spot represents the summary ROC point for the diagnostic accuracy of MRI.



# DISCUSSION



Pre-operative staging of gallbladder cancer still presents a significant clinical challenge. The presence of LNM, especially in non locoregional sites (i.e. outside of the hepatoduodenal ligament) is associated with a poor prognosis. [22] Pre-operative detection of LNM in gallbladder cancer is essential in order to determine the treatment approach, prevent unnecessary surgery and establish prognosis.

Five studies investigating the diagnostic accuracy of CT or MRI met our inclusion criteria for meta-analysis. An additional 4 studies were included for narrative review. Based on these studies, the estimated summary sensitivity of MRI for detection of LNM was 0.75. Sensitivity of CT could not be pooled as only one study was included, but the sensitivity of CT ranged from 0.25 - 0.93 in the studies included for narrative review.

Currently, substantial controversy exists regarding the optimal pre-operative imaging strategy for gallbladder cancer. The guideline from the European Society of Medical Oncology (ESMO) states that all patients should receive pre-operative MRI since within the literature superior sensitivity of MRI compared to CT has been reported for the staging of various tumours, but other guidelines state that not enough evidence is available to support this approach. [5, 23-26] Our findings confirm this notion and no difference between CT and MRI could be demonstrated. Significant heterogeneity was found in reported sensitivity and specificity, patient population characteristics, and reference standards.

Almost all studies (with the exception of two) were retrospective case series in which the criteria for additional MRI were not clearly outlined. In current clinical practice virtually all patients receive CT-imaging; additional MRI is only conducted when deemed necessary by the treating physician; for example when liver involvement cannot be clearly outlined on CT imaging. This may result in selection bias as only those patients with ambiguous CT results will receive an additional pre-operative MRI. Furthermore, a variety of reference standards was used for the verification of imaging results. Most studies only included resectable patients and chose to use histopathological analysis of the resection specimen as the reference standard. However, some studies also included inoperable patients and used follow-up imaging or biopsy results to verify imaging results. Evidently, arguments supporting the validity of both strategies can be made. On the one hand, surgical exploration and histopathological analysis remain the gold standard for the verification of imaging results. On the other hand, this is

obviously not possible in patients not undergoing surgery and excluding irresectable patients might result in significant selection bias, as more patients with irresectable e.g. locally advanced tumours are more likely have positive distant LNM. [27] Thus, the use of an alternate reference standard like adequate follow-up imaging can provide valuable additional information.

The materials and scanning protocols used in the included studies differed significantly. Notably, slice thickness of the CT and MRI scanners varied considerably. As nodal size is the most important characteristic used for LNM detection which is obviously influenced by slice thickness, up-to-date imaging devices with smaller slice thickness may detect LNM more accurately. Furthermore, most studies stated that metastatic lymph nodes missed on pre-operative imaging were usually smaller than 10mm in size. Newer imaging devices with a higher resolution or techniques like diffusion-weighted MRI or MRI using nano-sized contrast particles have demonstrated promising results in other hepatobiliary malignancies and could more accurately detect LNM. [28-30]

This systematic review and meta-analysis has several limitations that need to be considered. First and foremost, the number of included studies was limited, resulting in a paucity of data available for meta-analysis. Diagnostic accuracy data for CT could not be pooled since only one study was included and MRI and CT could not be compared. Although we planned to conduct sensitivity analyses in order to identify sources of heterogeneity, the limited quantity of data made this impossible. Second, there was considerable heterogeneity regarding patient characteristics, test characteristics and reference standards used. Some of the studies were published before 2000 and might have been conducted using out-of-date imaging techniques. Finally, the quality of the studies was rated as unclear on many aspects due to serious underreporting regarding methodology and patient selection. This is an important cause of concern and should be taken into consideration when interpreting the results.

# CONCLUSIONS

Our systematic review and meta-analysis show significant uncertainty regarding the optimal imaging strategy for the pre-operative detection of LNM in GBC. Current clinical practice involves standard pre-operative imaging by CT and additional MRI only in case of inconclusive CT results regarding pre-operative staging. Although a potential superior diagnostic accuracy of MRI has been reported, this is not supported by our results. Both CT and MRI demonstrate varying sensitivity and seem to be unreliable for the detection of LNM <10mm in size as demonstrated by the finding that in the studies included in this review all false negative lymph nodes were <10mm in size. More advanced pre-operative imaging techniques and better knowledge of metastatic lymph node imaging characteristics are needed to improve the pre-operative detection of LNM in gallbladder cancer and prevent unnecessary surgery-related morbidity and mortality.

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# APPENDIX

Table A.1: MEDLINE search strategy.

| | |
|---|---|
| 1 | Gallbladder Neoplasms[Mesh] |
| 2 | (gall bladder[tw] OR gallbladder[tw]) AND (cancer[tw] OR carcinoma[tw] OR neoplasm*[tw] OR tumor*[tw] OR tumour*[tw]) |
| 3 | (biliary cancer[tiab] OR billiary cancer[tiab] OR biliary carcinom*[tiab] OR billiary carcinom*[tiab]) |
| 4 | #1 OR #2 OR #3 |
| 5 | "Magnetic Resonance Imaging"[Mesh] |
| 6 | NMR Imag*[tw] OR MR Tomograph*[tw] OR NMR Tomograph*[tw] OR NMRI[tw] OR MRI[tw] OR MRIs[tw] OR magnetic resonance imag*[tw] OR diffusion weighted imag*[tw] OR cholepancreatograph*[tw] OR T2-weighted imag*[tw] |
| 7 | ((magnetic resonance[tw] OR MR[tw] OR NMR[tw]) AND (imag*[tw] OR tomograph*[tw] or scan*[tw])) |
| 8 | #5 OR #6 OR #7 |
| 9 | #4 AND #8 |
| 10 | "Tomography, X-Ray Computed"[Mesh] |
| 11 | (CT [tw] AND (Scan[tw] OR scans[tw] OR imag*[tw] OR x-ray*[tw])) OR X-Ray Computed Tomography[tw] OR Computed X Ray Tomography[tw] OR X-Ray Computer Assisted Tomography[tw] OR X Ray Computer Assisted Tomography[tw] OR X-Ray Computerized Tomography[tw] OR CT X Ray[tw] OR CT X Rays[tw] OR Computed X-Ray Tomography[tw] OR Xray Computed Tomography[tw] OR X-Ray CAT Scan[tw] OR X-Ray CAT Scans[tw] OR Transmission Computed Tomography[tw] OR X Ray Computerized Tomography[tw] OR Electron Beam Computed Tomography[tw] OR X-Ray Computerized Axial Tomography[tw] OR X Ray Computerized Axial Tomography[tw] OR MDCT[tw] OR Multidetector Computed tomogr*[tw] OR Mutidetector CT[tw] |
| 12 | #10 OR #11 |
| 13 | #4 AND #12 |
| 14 | #4 AND (#8 OR #12) |

Table A.2: EMBASE search strategy.

| | |
|---|---|
| 1 | exp biliary tract tumor/ or ((gall bladder or gallbladder) adj5 (cancer or carcinoma or neoplasm* or tumor* or tumour*)).mp. or biliary cancer.ti,ab,kw. or billiary cancer.ti,ab,kw. or biliary carcinom*.ti,ab,kw. or billiary carcinom*.ti,ab,kw. |
| 2 | exp nuclear magnetic resonance imaging/ or NMR Imag*.mp. or MR Tomograph*.mp. or NMR Tomograph*.mp. or NMRI.mp. or MRI.mp. or MRIs.mp. or magnetic resonance imag*.mp. or diffusion weighted imag*.mp. or cholepancreatograph*.mp. or T2-weighted imag*.mp. |
| 3 | ((magnetic resonance or MR or NMR) and (imag* or tomograph* or scan*)).mp. |
| 4 | 2 or 3 |
| 5 | ((CT and (Scan or scans or imag* or x-ray*)) or X-Ray Computed Tomography or Computed X Ray Tomography or X-Ray Computer Assisted Tomography or X Ray Computer Assisted Tomography or X-Ray Computerized Tomography or CT X Ray or CT X Rays or Computed X-Ray Tomography or Xray Computed Tomography or X-Ray CAT Scan or X-Ray CAT Scans or Transmission Computed Tomography or X Ray Computerized Tomography or Electron Beam Computed Tomography or X-Ray Computerized Axial Tomography or X Ray Computerized Axial Tomography or MDCT or Multidetector Computed tomogr* or Mutidetector CT).mp. |
| 6 | exp computer assisted tomography/ or exp emission tomography/ or exp whole body tomography/ or exp x-ray tomography/ |
| 7 | 5 or 6 |
| 8 | 4 and 7 |
| 9 | 1 and 8 |
| 10 | limit 9 to conference abstract |
| 11 | 9 not 10 |

**Appendix B:** QUADAS-2 checklist operational definitions.

| Risk of bias | | |
|---|---|---|
| **Domain** | **Scoring** | **Summary judgement** |
| **Patient selection** | | |
| Was a consecutive or random sample of participants enrolled? | *''Yes" if participants were enrolled in a random or consecutive manner*<br>*''No" if participants were not enrolled in a random or consecutive manner*<br>*''Unclear" if the method of participant enrollment is not clearly outlined* | Could the selection of participants have introduced bias?<br><br>*''Low" if all three items above are answered yes*<br><br>*''High" if one or more items above are answered no*<br><br>*''Unclear" for the remainder of combinations* |
| Was a case-control design avoided? | *''Yes"*<br>*''No"*<br>*''Unclear" if insufficient information regarding study design was provided* | |
| Did the study avoid inappropriate exclusions? | *''Yes" if included patients were representative of a typical sample in clinical practise.*<br>*''No" if the patient sample was not representative of clinical practise. Examples of inappropriate exclusions are exlusions based on age, prior therapy, TNM stage, ECOG-status or gender.*<br>*''Unclear" if no information regarding inclusion criteria was provided.* | |

**Appendix B:** QUADAS-2 checklist operational definitions.

| Index test | | |
|---|---|---|
| Were the index test results interpreted without knowledge of the results of the reference standard? | *''Yes" if assessors were either blinded for the outcome of histopathological analysis or if MRI/CT images were analysed before obtaining the results of histopathological anlysis.*<br>*''No" if assessors were not blinded for the outcome of histopathological analysis and CT/MRI were analysed after obtaining the results of histopathological analysis.*<br><br>*"Unclear" if no or insufficient information is provided regarding timing of imaging assessment and blinding of assessors.* | Could the conduct or interpretation of the index test have introduced bias?<br><br>*''High" if concerns are present regarding the conduct or interpretation of index tests. Examples include the index test being performed with out-of-date material, concerns regarding the quality of the scanning protocol or a high rate of inter- or intra-observer variability.*<br><br>*''Low" if no concerns are present.*<br><br>*''Unclear" if no or insufficient information is provided regarding the conduct or interpretation of index tests.* |
| If a threshold was used, was it specified? | *''Yes" if clear criteria are outlined for lymph node positivity.*<br>*''No" if criteria for lymph node positivity are not clearly outlined.* | |

| Reference standard | | |
|---|---|---|
| Is the reference standard likely to correctly classify the target condition? | *''Yes" if the reference standard is was sampling of the lymph nodes with pathological confirmation or follow-up imaging over a period of >6 months in case surgery was not performed*<br>*''No" if alternative reference standards were used*<br>*''Unclear" if no information was providing regarding the conduct of the reference standard* | Could the reference standard, its conduct, or its interpretation have introduced bias?<br><br>*A detailed assessment of risk of bias based on information provided regarding the reference standard, its conduct and its interpretation will be made with special regard to the version of the TNM staging system used. Based on this assessment studies will be categorised as having a low, high or unclear risk of bias due to the conduct and interpretation of the reference test used in included studies.* |
| Were the reference standard results interpreted without knowledge of the results of the index test? | *''Yes" if the pathologist investigating the histopathological specimen was blinded to CT/MRI results*<br>*''No" if the pathologist investigating the histopathological specimen was not blinded to CT/MRI results*<br>*''Unclear" if no or insufficient information regarding blinding of the pathologist was provided* | |

| Flow and timing | | |
|---|---|---|
| Was there an appropriate interval between index test(s) and reference standard? | *''Yes" if the interval between pre-operative CT/MRI and histopathological analysis or follow-up imaging was less than six weeks*<br><br>*''No" if the interval between pre-operative CT/MRI and histopathological analysis or follow-up imaging was longer than six weeks*<br>*''Unclear" if the interval between pre-operative CT/MRI and histopathological analysis or follow-up imaging was not reported* | Could the patient flow have introduced bias?<br><br>*'Low" if all four items above are answered yes*<br><br>*''High" if one or more of four items above are answered no*<br><br>*''Unclear" for the remainder of combinations* |
| Did all patients receive a reference standard? | *''Yes" if all patients received either histopathological validation or follow-up imaging*<br>*''No" if a subset of patients did not receive either histopathological validation or follow-up imaging*<br>*''Unclear" if the proportion of patients receiving the reference standard was not clearly reported* | |
| Did patients receive the same reference standard? | *''Yes" if all resectable patients received histopathological analysis as the reference standard and if all unresectable patients received the same alternative reference standard.*<br>*''No" if a subgroup of resectable patients did not receive histopathological analysis or if unresectable patients received a different alternative reference standard*<br>*''Unclear" if details outlining the delivery of the reference standard are not clearly stated* | |
| Were all patients included in the analysis? | *''Yes" if all patients recruited into the study were included in the analysis*<br>*''No" if not all patients recruited into the study were included in the analysis*<br>*''Unclear" if no or insufficient information regarding the final number of patients included in the analysis is available.* | |

| Appliccability |
| --- |
| Patients |
| Are there concerns that the included participants and setting do not match the review question?<br><br>*A detailed assessment of applicability will be made based on a combination of resemblance of the study population to the target population and study characteristics and setting. Based on this assessment studies will be categorised as having a high, low or unclear risk of bias due to concerns regarding applicability of the patient population used in the study to the general population.* |
| Index test |
| Are there concerns that the index test, its conduct or interpretation differ from the review question?<br><br>*A detailed assessment of applicability will be made based on assessment of the information regarding the conduct and interpretation of the index test with special regard to version of TNM staging used and quality and applicability of imaging protocols. Based on this assessment studies will be categorised as having a low, high or unclear risk of bias due to concerns regarding applicability of the conduct and interpretation of the index test(s) used in included studies.* |
| Reference standard |
| Is there concern that the target condition as defined by the reference standard does not match the review question?<br><br>*This item is not applicable since only studies in which the target condition defined by the reference standard matches the target condition of the review question will be included.* |

**Appendix C:** Risk of bias assessment according QUADAS 2, individual scores of each included study

| Engels 1989 | | |
|---|---|---|
| Item | Authors' judgement | Applicability concerns |
| **Domain 1: Patient selection** | | |
| Was a consecutive or random sample of participants enrolled? | Yes | |
| Was a case-control design avoided? | Yes | |
| Did the study avoid inappropriate exclusions? | Yes | |
| Could the selection of participants have introduced bias? | **Low** | |
| Are there concerns that the included participants and setting do not match the review question? | | **Low** |
| **Domain 2: Index test** | | |
| Were the index test results interpreted without knowledge of the results of the reference standard? | Unclear | |
| If a threshold was used, was it specified? | Yes | |
| Could the conduct or interpretation of the index test have introduced bias? | **Unclear** | |
| Are there concerns that the index test, its conduct or interpretation differ from the review question? | | **Unclear** |
| **Domain 3: Reference standard** | | |
| Is the reference standard likely to correctly classify the target condition? | No | |
| Were the reference standard results interpreted without knowledge of the results of the index test? | Unclear | |
| Could the reference standard, its conduct, or its interpretation have introduced bias? | **Unclear** | |
| Is there concern that the target condition as defined by the reference standard does not match the review question? | | **Unclear** |
| **Domain 4: Flow and timing** | | |
| Was there an appropriate interval between index test(s) and reference standard? | Unclear | |
| Did all patients receive a reference standard? | Yes | |
| Did patients receive the same reference standard? | No | |
| Were all patients included in the analysis? | Yes | |
| Could the patient flow have introduced bias? | **Unclear** | |

| Kalra 2006 | | |
|---|---|---|
| Item | Authors' judgement | Applicability concerns |
| **Domain 1: Patient selection** | | |
| Was a consecutive or random sample of participants enrolled? | Yes | |
| Was a case-control design avoided? | Yes | |
| Did the study avoid inappropriate exclusions? | Yes | |
| Could the selection of participants have introduced bias? | **Low** | |
| Are there concerns that the included participants and setting do not match the review question? | | **Unclear** |
| **Domain 2: Index test** | | |
| Were the index test results interpreted without knowledge of the results of the reference standard? | Yes | |
| If a threshold was used, was it specified? | Yes | |
| Could the conduct or interpretation of the index test have introduced bias? | **Low** | |
| Are there concerns that the index test, its conduct or interpretation differ from the review question? | | **Low** |
| **Domain 3: Reference standard** | | |
| Is the reference standard likely to correctly classify the target condition? | Unclear | |
| Were the reference standard results interpreted without knowledge of the results of the index test? | No | |
| Could the reference standard, its conduct, or its interpretation have introduced bias? | **Unclear** | |
| Is there concern that the target condition as defined by the reference standard does not match the review question? | | **Unclear** |
| **Domain 4: Flow and timing** | | |
| Was there an appropriate interval between index test(s) and reference standard? | Yes | |
| Did all patients receive a reference standard? | Yes | |
| Did patients receive the same reference standard? | Yes | |
| Were all patients included in the analysis? | Yes | |
| Could the patient flow have introduced bias? | **Low** | |

| Kaza 2006 | | |
|---|---|---|
| Item | Authors' judgement | Applicability concerns |
| **Domain 1: Patient selection** | | |
| Was a consecutive or random sample of participants enrolled? | Yes | |
| Was a case-control design avoided? | Yes | |
| Did the study avoid inappropriate exclusions? | Yes | |
| Could the selection of participants have introduced bias? | **Low** | |
| Are there concerns that the included participants and setting do not match the review question? | | **Unclear** |
| **Domain 2: Index test** | | |
| Were the index test results interpreted without knowledge of the results of the reference standard? | Unclear | |
| If a threshold was used, was it specified? | No | |
| Could the conduct or interpretation of the index test have introduced bias? | **Low** | |
| Are there concerns that the index test, its conduct or interpretation differ from the review question? | | **Low** |
| **Domain 3: Reference standard** | | |
| Is the reference standard likely to correctly classify the target condition? | Unclear | |
| Were the reference standard results interpreted without knowledge of the results of the index test? | Unclear | |
| Could the reference standard, its conduct, or its interpretation have introduced bias? | **Unclear** | |
| Is there concern that the target condition as defined by the reference standard does not match the review question? | | **Unclear** |
| **Domain 4: Flow and timing** | | |
| Was there an appropriate interval between index test(s) and reference standard? | Yes | |
| Did all patients receive a reference standard? | Yes | |
| Did patients receive the same reference standard? | Yes | |
| Were all patients included in the analysis? | Yes | |
| Could the patient flow have introduced bias? | **Low** | |

| Kim 2002 | | |
|---|---|---|
| Item | Authors' judgement | Applicability concerns |
| **Domain 1: Patient selection** | | |
| Was a consecutive or random sample of participants enrolled? | Yes | |
| Was a case-control design avoided? | Yes | |
| Did the study avoid inappropriate exclusions? | Yes | |
| Could the selection of participants have introduced bias? | **Low** | |
| Are there concerns that the included participants and setting do not match the review question? | | **Unclear** |
| **Domain 2: Index test** | | |
| Were the index test results interpreted without knowledge of the results of the reference standard? | Yes | |
| If a threshold was used, was it specified? | No | |
| Could the conduct or interpretation of the index test have introduced bias? | **Low** | |
| Are there concerns that the index test, its conduct or interpretation differ from the review question? | | **Low** |
| **Domain 3: Reference standard** | | |
| Is the reference standard likely to correctly classify the target condition? | Yes | |
| Were the reference standard results interpreted without knowledge of the results of the index test? | Unclear | |
| Could the reference standard, its conduct, or its interpretation have introduced bias? | **Low** | |
| Is there concern that the target condition as defined by the reference standard does not match the review question? | | **Low** |
| **Domain 4: Flow and timing** | | |
| Was there an appropriate interval between index test(s) and reference standard? | Yes | |
| Did all patients receive a reference standard? | Yes | |
| Did patients receive the same reference standard? | Yes | |
| Were all patients included in the analysis? | Yes | |
| Could the patient flow have introduced bias? | **Low** | |

| Kim 2015 | | |
|---|---|---|
| Item | Authors' judgement | Applicability concerns |
| **Domain 1: Patient selection** | | |
| Was a consecutive or random sample of participants enrolled? | Yes | |
| Was a case-control design avoided? | Yes | |
| Did the study avoid inappropriate exclusions? | Yes | |
| Could the selection of participants have introduced bias? | **Low** | |
| Are there concerns that the included participants and setting do not match the review question? | | **Unclear** |
| **Domain 2: Index test** | | |
| Were the index test results interpreted without knowledge of the results of the reference standard? | Yes | |
| If a threshold was used, was it specified? | Yes | |
| Could the conduct or interpretation of the index test have introduced bias? | **Low** | |
| Are there concerns that the index test, its conduct or interpretation differ from the review question? | | **Low** |
| **Domain 3: Reference standard** | | |
| Is the reference standard likely to correctly classify the target condition? | Yes | |
| Were the reference standard results interpreted without knowledge of the results of the index test? | Unclear | |
| Could the reference standard, its conduct, or its interpretation have introduced bias? | **Low** | |
| Is there concern that the target condition as defined by the reference standard does not match the review question? | | **Low** |
| **Domain 4: Flow and timing** | | |
| Was there an appropriate interval between index test(s) and reference standard? | Yes | |
| Did all patients receive a reference standard? | Yes | |
| Did patients receive the same reference standard? | Yes | |
| Were all patients included in the analysis? | Yes | |
| Could the patient flow have introduced bias? | **Low** | |

| Ohtani 1996 | | |
|---|---|---|
| Item | Authors' judgement | Applicability concerns |
| **Domain 1: Patient selection** | | |
| Was a consecutive or random sample of participants enrolled? | Unclear | |
| Was a case-control design avoided? | Yes | |
| Did the study avoid inappropriate exclusions? | Yes | |
| Could the selection of participants have introduced bias? | **Unclear** | |
| Are there concerns that the included participants and setting do not match the review question? | | **Unclear** |
| **Domain 2: Index test** | | |
| Were the index test results interpreted without knowledge of the results of the reference standard? | Yes | |
| If a threshold was used, was it specified? | Yes | |
| Could the conduct or interpretation of the index test have introduced bias? | **Low** | |
| Are there concerns that the index test, its conduct or interpretation differ from the review question? | | **Low** |
| **Domain 3: Reference standard** | | |
| Is the reference standard likely to correctly classify the target condition? | Yes | |
| Were the reference standard results interpreted without knowledge of the results of the index test? | Unclear | |
| Could the reference standard, its conduct, or its interpretation have introduced bias? | **Low** | |
| Is there concern that the target condition as defined by the reference standard does not match the review question? | | **Low** |
| **Domain 4: Flow and timing** | | |
| Was there an appropriate interval between index test(s) and reference standard? | Unclear | |
| Did all patients receive a reference standard? | Yes | |
| Did patients receive the same reference standard? | Yes | |
| Were all patients included in the analysis? | Yes | |
| Could the patient flow have introduced bias? | **Unclear** | |

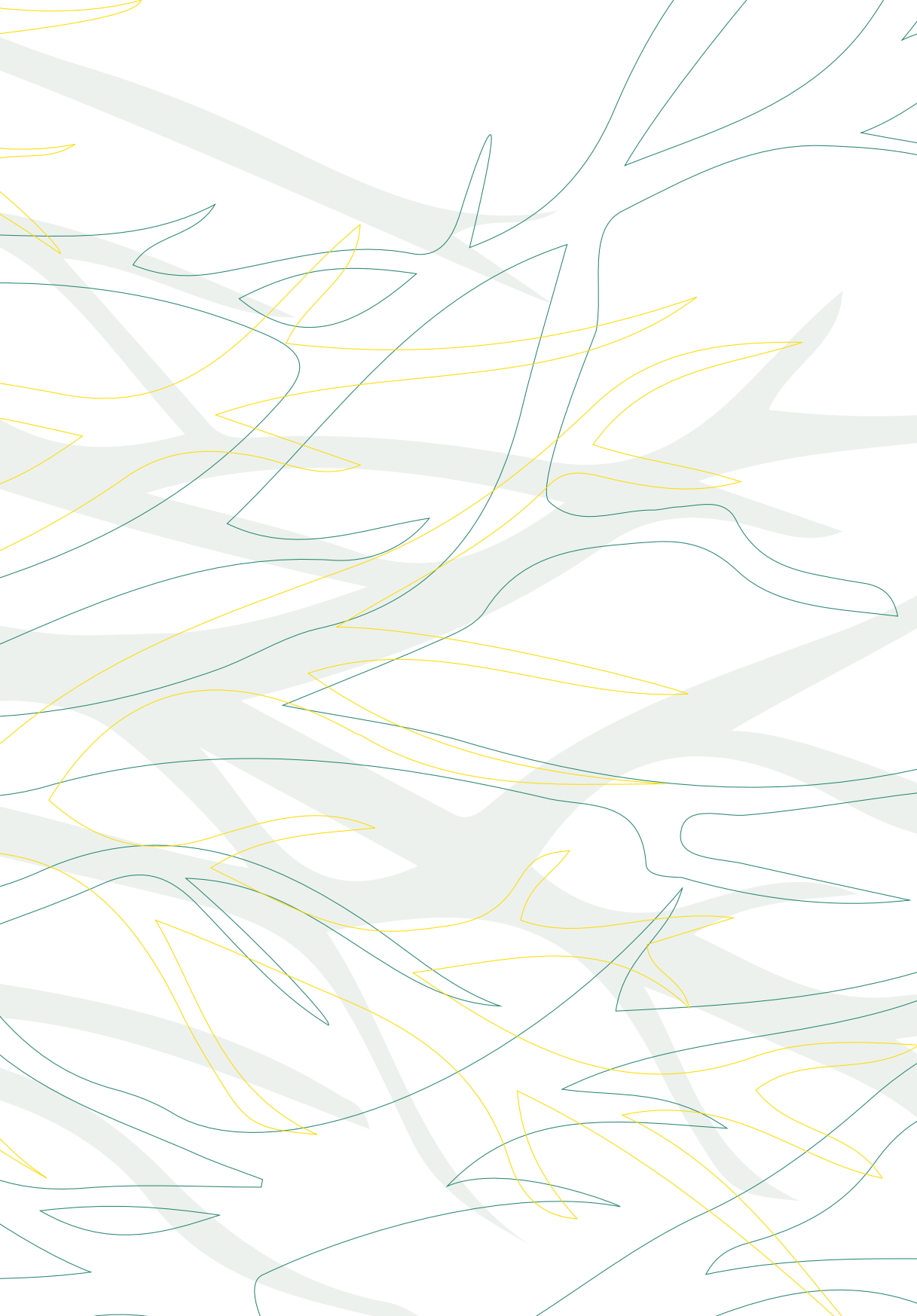
| Oikainen 1993 | | |
|---|---|---|
| Item | Authors' judgement | Applicability concerns |
| **Domain 1: Patient selection** | | |
| Was a consecutive or random sample of participants enrolled? | Yes | |
| Was a case-control design avoided? | Yes | |
| Did the study avoid inappropriate exclusions? | Yes | |
| Could the selection of participants have introduced bias? | **Low** | |
| Are there concerns that the included participants and setting do not match the review question? | | **Low** |
| **Domain 2: Index test** | | |
| Were the index test results interpreted without knowledge of the results of the reference standard? | Unclear | |
| If a threshold was used, was it specified? | Yes | |
| Could the conduct or interpretation of the index test have introduced bias? | **Unclear** | |
| Are there concerns that the index test, its conduct or interpretation differ from the review question? | | **Low** |
| **Domain 3: Reference standard** | | |
| Is the reference standard likely to correctly classify the target condition? | Unclear | |
| Were the reference standard results interpreted without knowledge of the results of the index test? | Unclear | |
| Could the reference standard, its conduct, or its interpretation have introduced bias? | **High** | |
| Is there concern that the target condition as defined by the reference standard does not match the review question? | | **Unclear** |
| **Domain 4: Flow and timing** | | |
| Was there an appropriate interval between index test(s) and reference standard? | Unclear | |
| Did all patients receive a reference standard? | Yes | |
| Did patients receive the same reference standard? | No | |
| Were all patients included in the analysis? | Yes | |
| Could the patient flow have introduced bias? | **High** | |

| Schwartz 2002 | | |
|---|---|---|
| Item | Authors' judgement | Applicability concerns |
| **Domain 1: Patient selection** | | |
| Was a consecutive or random sample of participants enrolled? | Yes | |
| Was a case-control design avoided? | Yes | |
| Did the study avoid inappropriate exclusions? | Yes | |
| Could the selection of participants have introduced bias? | **Low** | |
| Are there concerns that the included participants and setting do not match the review question? | | **Unclear** |
| **Domain 2: Index test** | | |
| Were the index test results interpreted without knowledge of the results of the reference standard? | Yes | |
| If a threshold was used, was it specified? | No | |
| Could the conduct or interpretation of the index test have introduced bias? | **Low** | |
| Are there concerns that the index test, its conduct or interpretation differ from the review question? | | **Low** |
| **Domain 3: Reference standard** | | |
| Is the reference standard likely to correctly classify the target condition? | Yes | |
| Were the reference standard results interpreted without knowledge of the results of the index test? | Unclear | |
| Could the reference standard, its conduct, or its interpretation have introduced bias? | **Low** | |
| Is there concern that the target condition as defined by the reference standard does not match the review question? | | **Low** |
| **Domain 4: Flow and timing** | | |
| Was there an appropriate interval between index test(s) and reference standard? | Unclear | |
| Did all patients receive a reference standard? | Yes | |
| Did patients receive the same reference standard? | Yes | |
| Were all patients included in the analysis? | No | |
| Could the patient flow have introduced bias? | **Unclear** | |

| Tseng 2002 | | |
|---|---|---|
| Item | Authors' judgement | Applicability concerns |
| **Domain 1: Patient selection** | | |
| Was a consecutive or random sample of participants enrolled? | Unclear | |
| Was a case-control design avoided? | Yes | |
| Did the study avoid inappropriate exclusions? | Yes | |
| Could the selection of participants have introduced bias? | **Unclear** | |
| Are there concerns that the included participants and setting do not match the review question? | | **Low** |
| **Domain 2: Index test** | | |
| Were the index test results interpreted without knowledge of the results of the reference standard? | Unclear | |
| If a threshold was used, was it specified? | Yes | |
| Could the conduct or interpretation of the index test have introduced bias? | **Low** | |
| Are there concerns that the index test, its conduct or interpretation differ from the review question? | | **Low** |
| **Domain 3: Reference standard** | | |
| Is the reference standard likely to correctly classify the target condition? | Unclear | |
| Were the reference standard results interpreted without knowledge of the results of the index test? | Unclear | |
| Could the reference standard, its conduct, or its interpretation have introduced bias? | **Unclear** | |
| Is there concern that the target condition as defined by the reference standard does not match the review question? | | **Unclear** |
| **Domain 4: Flow and timing** | | |
| Was there an appropriate interval between index test(s) and reference standard? | Yes | |
| Did all patients receive a reference standard? | Yes | |
| Did patients receive the same reference standard? | Yes | |
| Were all patients included in the analysis? | Yes | |
| Could the patient flow have introduced bias? | **Low** | |

# PART 2

## SURGICAL AND SYSTEMIC TREATMENT



CHAPTER 4

# Trends in treatment and survival of gallbladder cancer in the Netherlands; identifying gaps and opportunities from a nation - wide cohort

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*Cancers 2020*

# ABSTRACT

Gallbladder cancer (GBC) is rare in Western populations and data about treatment and outcomes are scarce. This study aims to analyze survival and identify opportunities for improvement using population-based data from a low-incidence country. GBC patients diagnosed between 2005–2016 with GBC were identified from the Netherlands Cancer Registry. Patients were grouped according to time period (2005-2009/2010-2016) and disease stage. Trends in treatment and overall survival (OS) were analyzed. In total 1834 patients were included: 661(36%) patients with resected, 278 (15%) with non-resected non-metastatic and 895(49%) with metastatic GBC. Use of radical versus simple cholecystectomy (12% vs. 26%,P<0.001) in early (pT1b/T2) GBC increased. More patients with metastatic GBC received chemotherapy (11% vs. 29%,P<0.001). OS improved from 4.8 months (2005-2009) to 6.1 months (2010-2016)(P=0.012). Median OS increased over time (2005-2009 vs. 2010-2016) in resected (19.4 to 26.8 months, P=0.038) and metastatic (2.3 vs. 3.4 months, P=0.001) GBC but not in unresected, non-metastatic GBC. In early GBC, patients with radical cholecystectomy had a median OS of 76.7 compared to 18.4 months for simple cholecystectomy (P<0.001). Palliative chemotherapy showed superior (P<0.001) survival in metastatic (7.3 versus 2.1 months) and non-resected non-metastatic (7.7 versus 3.5 months) GBC. In conclusion, survival of GBC remains poor. Radical surgery and palliative chemotherapy appear to improve prognosis but remain under-utilized.

# INTRODUCTION

Gallbladder cancer (GBC) is a rare and highly lethal neoplasm of the biliary tract. GBC demonstrates marked geographic, age-, gender- and ethnicity-related differences in incidence, implying (epi)genetics or environmental factors may play an important role in the development of GBC. [1-6] Other possible risk factors include cholelithiasis, obesity, gallbladder polyps, chronic infections and an abnormal pancreaticobiliary duct junction. [1, 7, 8] Treatment of GBC remains challenging. Diagnosis, unless incidentally after cholecy-stectomy for benign gallbladder disease, is often made in an advanced stage and survival is extremely poor due to the limited efficacy of systemic therapy options. [3] The only treatment with curative intent is surgical resection. However, due to late detection and a tendency towards invasive local growth only 10 to 25% of tumors are candidates for potential curative intent surgery at presentation. [9, 10] Even after resection 5-year survival rates are poor, ranging from 12 to 30% in non-incidental tumors. [3, 11, 12] Long-term survival is only observed in patients with early (T1/T2) GBC, which is mainly diagnosed incidentally. However, even for these patients additional radical surgery with resection of the gallbladder bed and lymph node dissection of the hepatoduodenal ligament is recommended because it is thought to considerably increase survival. [13, 14]



The limited benefit of systemic therapy in GBC has been shown in prospective trials; in 2010, the ABC-02 trial reported a median overall survival (OS) of 11.7 months vs. 8.1 months in unresectable biliary tract cancer treated with gemcitabine and cisplatin versus gemcitabine alone. [15] This has since been adopted as the standard regimen in the treatment of unresectable GBC. Although several randomized clinical trials have investigated the value of adjuvant chemotherapy for biliary tract cancers, none have found a survival benefit in the intention-to-treat analysis and no adequately powered subgroup analyses for GBC have been conducted. [16, 17]

Guidelines for the treatment of localized GBC are mainly based on retrospective evidence and expert opinion due to the minimal availability of randomized evidence. Previous studies investigating GBC have typically been conducted in high-volume, non Western centers and included patients with various biliary tract cancers. [15, 18, 19] Due to presumed different etiologies, results in GBC may differ from those in other biliary tract tumors. [20]

Our objective was to investigate trends in treatment, establish prognostic factors associated with survival and identify opportunities for improvement in treatment stratified for disease stage.

# METHODS

This is a cohort study using data from the nationwide population-based Netherlands Cancer Registry (NCR), containing information on all newly diagnosed malignancies. The NCR receives notifications from the automated pathological archive (PALGA), the nation-wide network and registry of histo- and cytopathology in the Netherlands, and is supplemented by alerts from the National Archive of Hospital Discharge Diagnosis. [21] Completeness of the registry is estimated to be at least 95%.[22] Since all data was anonymized a waiver for ethical approval was provided. The STROBE guidelines for reporting of observational studies have been followed. [23] This study was approved by the NCR ethical review board and a waiver for ethical approval was provided by the Medical Ethics Review Committee of the region Arnhem-Nijmegen (CMO A-N, nr. 2017-3912) on 27/12/2017. The study was conducted according to the Declaration of Helsinki.

## PATIENT SELECTION AND VARIABLE DEFINITIONS

Clinicopathological data on all adult patients diagnosed between 2005-2016 with invasive gallbladder neoplasms were extracted. The following variables were provided: age, gender, year of diagnosis, socioeconomic status (social deprivation scores based on a mean number of 4 000 inhabitants per 4-digit postal codes), histopathological or clinical diagnosis, tumor histology (based on the ICD-O3 classification, morphological codes are provided in Appendix A), clinical and pathological TNM stage (AJCC staging system, version 6 for patients diagnosed from 2005 – 2009 and version 7 from 2010 – 2016[24, 25]), presence and location of metastatic disease, occurrence of syn- or metachronous primary tumors, type of resection performed, resection margin (R0: microscopically free of tumor, R1 microscopically positive for tumor, R2: macroscopically positive for tumor), systemic therapy (yes/no), radiation therapy (yes/no) and duration of follow-up in days from date of diagnosis. Missing data occurred in 4 out of 9 baseline variables (2-29%) and was not imputed because it was determined not to be missing at random.

Primary radical/extended cholecystectomy was defined as cholecystectomy with en-bloc excision of the gallbladder bed and dissection of the hepatoduodenal lymph nodes as the first surgery received by the patient. Re-resection was defined as any surgery for GBC after initial cholecystectomy alone within 180 days of diagnosis. Radicality was classified into R0 (resection margin microscopically free of tumor) and R1/2 (resection margin micro- or macroscopically positive). Supportive therapy included

endoscopic procedures, biliary drainage and metastasectomy. 90-day mortality was defined as death within 90 days of diagnosis. Chemo- and radiotherapy were defined as administration of at least one dose. Information regarding type of systemic therapy received was not available. Follow-up data on vital status (complete until February 2018) were provided by linkage to the automated Municipal Personal Records Database.

## QUALITY CONTROL AND COMPLETENESS OF DATA ASSESSMENT

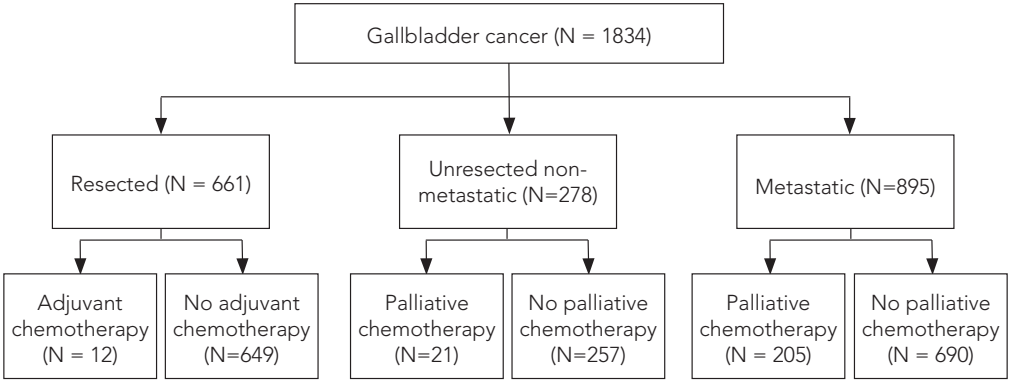
Accuracy of diagnosis and completeness of histopathological assessment was assessed by comparing data from the resected patients provided by the NCR with data extracted from the medical records available from four academic centers in the Netherlands: Radboudumc, Amsterdam University Medical Center (location AMC), Erasmus MC and Leiden University Medical Center.



## STATISTICAL ANALYSIS

Characteristics were described using counts and percentages for discrete variables and means and ranges for continuous variables. $\chi$ – square testing or Fisher's exact test, where appropriate, were used to assess differences in patient characteristics. Incidence rates were calculated per 100 000 person years and age-standardized using the European standard population. Trends in incidence were assessed by calculating the estimated annual percentage change (EAPC). Patients were grouped according to T-stage (T1/T2 vs. T3/T4), N – stage (N0 vs. N1/N2) and resection margin (R0 vs. R1/R2 vs. Rx). For survival analyses, patients were categorized as resected, non-metastatic non-resected (i.e. inoperable patients due to comorbidities and/or locally advanced disease) or metastatic at diagnosis. To assess trends in treatment over time, patients were grouped according to period of diagnosis (Period 1; 2005-2009 and Period 2; 2010-2016; these periods coincide with the introduction of gemcitabine-cisplatin chemotherapy as standard of care for unresected BTC). A subgroup analysis in patients with early (T1b/T2) disease was conducted to assess trends in surgical treatment. Kaplan-Meier curves were used to calculate median OS. OS was defined as time in days from date of diagnosis until date of death from any cause or the date of last follow-up (February 2018). Patients alive at the last date of follow-up were censored. Cox regression analysis was used to calculate hazard ratios for potential prognostic factors. Covariates were selected based on literature and entered in the multivariable model when statistically relevant ($p < 0.1$) on univariable analysis. P-values $< 0.05$ were considered statistically significant. All tests of significance were two-tailed. Statistical analyses were conducted using the SPSS 24.0 statistical package (SPSS, Inc., Chicago, IL).

**Figure 1.** Patient flow.




# RESULTS

## INCIDENCE AND PATIENT AND TUMOR CHARACTERISTICS

Patient and tumor characteristics are shown in Table 1. Between 2005 and 2016, 1 834 patients were diagnosed with GBC in the Netherlands (figure 1). Forty-nine percent of patients had metastatic disease at diagnosis (43% from 2005 – 2009 and 53% from 2010 – 2016, $P < 0.001$). The incidence of GBC did not change significantly (EAPC – 0.7%, $P = 0.32$) over time (Appendix B). Median age at diagnosis was 71 (IQR 64 - 80) years. Eighty-four percent of patients had histopathological confirmation of diagnosis.

## TREATMENT

Time trends in treatment in resected, non-resected non-metastatic and metastatic GBC are shown in figure 2. Among all patients with non-metastatic disease, primary resection rates increased; 64.7% in 2005 - 2009 to 74.8% in 2010-2016 ($P=0.001$). More extensive tumors (T3-T4) were resected between 2010 - 2016 compared to 2009 – 2015 (from 25.1% to 33.1%, $P<0.001$). In resected, non-metastatic patients 90-day mortality decreased from 12.0% to 5.6% ($P=0.003$) and the percentage of patients receiving R0 resection did not change significantly (from 70.3% to 74.7%, $P=0.294$). The number of patients receiving an extended cholecystectomy (with/without hepatoduodenal lymphadenectomy) opposed to simple cholecystectomy in early (T1b-T2) GBC increased significantly, from 12% to 26% ($P<0.001$). In the subgroup analysis conducted in patients with early GBC, 90-day mortality and the R0 resection rate did not change over time. Adjuvant chemotherapy was only administered to 12/661 (1.8%) patients.

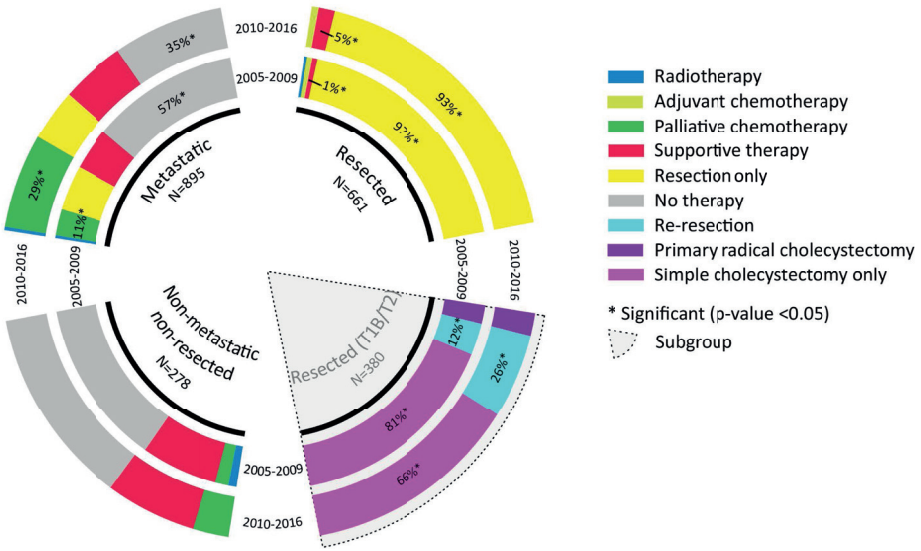
Table 1. Characteristics of patients with gallbladder cancer in the Netherlands (2005- 2016).

| | Total (n=1834) | Resected (n=661) | Non-resected non-metastatic (n=278) | Metastatic (n=895) |
|---|---|---|---|---|
| Patient and tumor characteristics | | | | |
| Age | 71.1 (22 – 97) | 69.2 (27 – 97) | 74.3 (32 – 95) | 71.2 (22 – 96) |
| Male sex | 545 (29.1%) | 206 (31.2%) | 82 (29.5%) | 250 (27.9%) |
| Socioeconomic Status | | | | |
| High | 501 (26.8%) | 183 (27.7%) | 82 (29.5%) | 229 (33.4%) |
| Medium | 741 (39.6%) | 253 (38.3%) | 110 (39.6%) | 367 (41.0%) |
| Low | 630 (33.7%) | 225 (34.0%) | 86 (30.9%) | 299 (33.4%) |
| Clinicopathologic T stage [1] | | | | |
| T1 | 202 (11.0%) | 147 (22.6%) | 1 (0.4%) | 54 (8.5%) |
| T2 | 325 (17.8%) | 303 (45.8%) | 1 (0.0%) | 22 (2.5%) |
| T3/T4 | 768 (41.9%) | 172 (26.2%) | 169 (60.8%) | 427 (47.7%) |
| TX | 353 (19.2%) | 38 (5.8%) | 13 (4.7%) | 302 (33.7%) |
| Unknown/missing | 185 (10.1%) | - | 95 (34.2%) | 90 (10.1%) |
| Clinicopathologic N stage [1] | | | | |
| N0 | 674 (36.0%) | 140 (21.2%) | 62 (22.3%) | 237 (26.5%) |
| N1 | 432 (23.1%) | 123 (18.6%) | 74 (26.6%) | 331 (37.0%) |
| NX | 559 (29.9%) | 387 (58.5%) | 47 (16.9%) | 237 (26.5%) |
| Unknown/missing | 207 (11.1%) | 11 (1.7%) | 95 (34.2%) | 90 (10.1%) |
| Location synchronous metastases | | | | |
| Liver | N/A | N/A | N/A | 350 (39.1%) |
| Peritoneal | N/A | N/A | N/A | 119 (13.3%) |
| Lymph node | N/A | N/A | N/A | 46 (5.1%) |
| Lung | N/A | N/A | N/A | 11 (1.2%) |
| Liver + peritoneum | N/A | N/A | N/A | 92 (10.3%) |
| Other | N/A | N/A | N/A | 22 (2.5%) |
| Multiple, other | N/A | N/A | N/A | 175 (19.6%) |
| Unknown/missing | N/A | N/A | N/A | 80 (8.9%) |
| Pathology confirmation of primary tumor(yes) | 1566 (83.7%) | 661 (100%) | 156 (56.1%) | 732 (81.8%) |
| Differentiation grade | | | | |
| Well | N/A | 102 (15.4%) | N/A | N/A |
| Moderate | N/A | 209 (31.6%) | N/A | N/A |
| Poor | N/A | 157 (23.7%) | N/A | N/A |
| Not determined | N/A | 193 (29.2%) | N/A | N/A |
| Radicality | | | | |
| R0 | N/A | 417 (63.1%) | N/A | N/A |
| R1 | N/A | 130 (19.7%) | N/A | N/A |
| R2 | N/A | 24 (3.6%) | N/A | N/A |
| Unclear | N/A | 90 (13.6%) | N/A | N/A |

[1] Clinical P- and N- for unresected patients and pathologic T- and N- stage for resected patients are provided.

Use of palliative chemotherapy did not increase in patients with unresected, non-metastatic GBC (15% vs. 15%, figure 2). The use of palliative chemotherapy in metastatic GBC increased from 11% to 29% ($P < 0.001$).

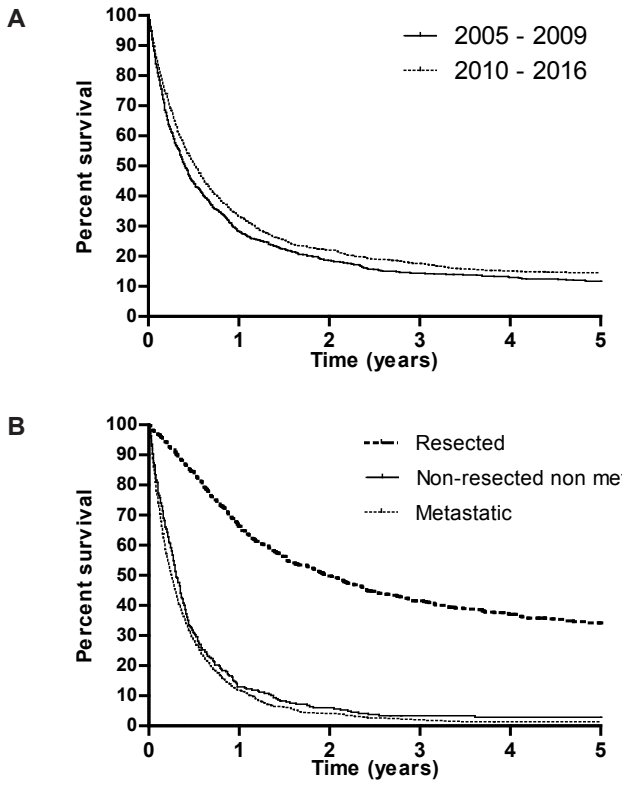
**Figure 2.** Trends in treatment in resected, non-resected non-metastatic and metastatic GBC. The grey area represents a subgroup analysis of resected patients with early (T1b/T2) gallbladder cancer. Percentages are only displayed when significant differences (P <0.05) between periods were found. Supportive treatment includes endoscopic procedures, biliary drainage and metastasectomy.




## SURVIVAL

Median OS of the entire cohort was 5.5 months (95% CI 5.0 – 6.0) and increased from 4.8 months (95% CI 4.2 – 5.4) in 2005 – 2009 to 6.1 months (95% CI 5.4 – 6.8) in 2010 – 2016 (p = 0.012, Figure 3A). Median OS differed significantly between resected and non-metastatic non-resected or metastatic disease: 23.7 (95% CI 19.6 – 27.8), 3.6 (95% CI 3.1 – 4.6) and 2.7 (95% CI 2.6 – 3.2) months respectively (P<0.001, Figure 3B). Resected patients showed improved survival over time; from 19.4 to 26.8 months (P=0.038, appendix C). Median OS in metastatic patients increased from 2.3 to 3.4 months (P<0.001, appendix C). In non-resected patients survival did not change significantly over time.

Figure 3. A: Survival according to time period. B: Survival according to disease stage.




| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Resected | N | 661 | 443 | 308 | 235 | 186 | 147 |
| Unresected M0 | N | 278 | 39 | 17 | 8 | 7 | 6 |
| Metastatic | N | 895 | 111 | 34 | 16 | 10 | 7 |

## THERAPY AND SURVIVAL

Survival in patient groups with resected, non-metastatic non-resected and metastatic GBC is shown in Table 2. The survival benefit of adjuvant chemotherapy could not be assessed since only 12 out of 661 patients received some form of adjuvant therapy. Radical surgery (either primary radical cholecystectomy or re-resection) in early GBC was associated with a significantly higher median OS compared to simple cholecystectomy, from 18.4 to 76.7 months (P<0.001). Palliative chemotherapy in non-resected non-metastatic and metastatic disease was associated with superior survival; from 3.5 to 7.7 (P=0.011) and 2.1 versus 7.3 (P<0.001) months respectively.

Table 2. Survival of patients with gallbladder cancer according to clinical stage and treatment strategy.

| | N | 5-year survival | Median OS, months (95% CI) | Log rank test P value |
|---|---|---|---|---|
| Total | 1834 | 13.2% | 5.5 (5.0 – 6.0) | |
| Resected non-metastatic | 661 | 34.2% | 23.7 (19.6 – 27.8) | |
| Adjuvant chemotherapy | 12 | 37.5% | 29.4 (21.4 – 37.5) | 0.521 |
| No adjuvant chemotherapy | 649 | 34.1% | 23.7 (19.4 – 27.6) | |
| *T1b/T2 tumor, no radical surgery* | *292* | *30.6%* | *18.4 (13.8 – 22.7)* | *<0.001* |
| *T1b/T2 tumor, radical surgery* | *88* | *52.7%* | *76.7 (43.0 – 110.3)* | |
| Non-resected non-metastatic | 278 | 2.9% | 3.6 (3.1 – 4.1) | |
| No palliative chemotherapy | 257 | 3.0% | 3.5 (2.9 – 4.0) | 0.011 |
| Palliative chemotherapy | 21 | - | 7.7 (4.5 – 10.8) | |
| Metastatic | 895 | 1.3% | 2.9 (2.6 – 3.2) | |
| No palliative chemotherapy | 690 | 0.6% | 2.1 (1.9 – 2.4) | <0.001 |
| Palliative chemotherapy | 205 | 3.7% | 7.3 (6.4 – 8.2) | |

## PROGNOSTIC FACTORS FOR SURVIVAL

Poor prognostic factors were increasing age, poor tumor differentiation, higher T-stage, presence of lymph node metastases and (in resected patients) non-radical resection (Table 3A+B).

Palliative surgery and chemotherapy were associated with a better prognosis in metastatic disease (HR 0.43 and 0.47 respectively, P<0.001).

## QUALITY CONTROL

In total, 108 patients (16% of resected patients) underwent a resection in one of the four academic hospitals. One patient (0.9%) turned out to have cholecystitis and was incorrectly registered by the NCR as having GBC.

Table 3a. Prognostic factors for patients with resected gallbladder cancer. N = 661.

| | Univariable cox regression | | | Multivariable cox regression | | |
|---|---|---|---|---|---|---|
| Characteristic | HR | 95% CI | P value | HR | 95% CI | P value |
| Grade | | | | | | |
| Well | 1 | | | 1 | | |
| Moderate | 1.41 | 1.02 – 1.95 | 0.036 | 1.17 | 0.84 – 1.61 | 0.354 |
| Poor | 2.67 | 1.93 – 3.70 | <0.001 | 2.07 | 1.49 – 2.86 | <0.001 |
| Unknown | 1.45 | 1.05 – 1.99 | 0.023 | 1.74 | 1.26 – 2.41 | 0.001 |
| Sex | | | | | | |
| Female | 1 | | | | | |
| Male | 0.88 | 0.71 – 1.08 | 0.214 | | | |
| Pathological T stage | | | | | | |
| T1 | 1 | | | 1 | | |
| T2 | 1.77 | 1.35 – 2.32 | <0.001 | 1.58 | 1.19 – 2.10 | 0.001 |
| T3/T4 | 3.59 | 2.69 – 4.78 | <0.001 | 2.61 | 1.89 – 3.61 | <0.001 |
| Tx | 3.23 | 2.01 – 5.18 | <0.001 | 2.16 | 1.34 – 3.50 | 0.002 |
| Pathological N stage | | | | | | |
| N0 | 1 | | | 1 | | |
| N1 | 2.96 | 2.13 – 4.12 | <0.001 | 1.95 | 1.39 – 2.74 | <0.001 |
| Nx | 2.48 | 1.86 – 3.31 | <0.001 | 1.86 | 1.46 – 2.66 | <0.001 |
| Radicality | | | | | | |
| R0 | 1 | | | 1 | | |
| R1/R2 | 3.78 | 3.03 – 4.71 | <0.001 | 2.69 | 2.11 – 3.43 | <0.001 |
| Unclear | 1.60 | 1.20 – 2.14 | 0.001 | 1.48 | 1.10 – 1.98 | 0.009 |
| Adjuvant chemotherapy (yes) | 0.67 | 0.33 – 1.36 | 0.268 | | | |
| Prior malignancy (yes) | 1.22 | 0.93 – 1.61 | 0.150 | | | |
| Increasing age (years) | 1.04 | 1.03 – 1.05 | <0.001 | 1.04 | 1.03 – 1.05 | <0.001 |

**Table 3b.** Prognostic factors for patients with metastatic gallbladder cancer. N = 895.

| | Univariable cox regression | | | Multivariable cox regression | | |
|---|---|---|---|---|---|---|
| Characteristic | HR | 95% CI | P value | HR | 95% CI | P value |
| Grade | | | | | | |
| Well | 1 | | | | | |
| Moderately | 1.02 | 0.61 – 1.71 | 0.931 | | | |
| Poor | 1.45 | 0.89 – 2.36 | 0.136 | | | |
| Unknown | 1.85 | 1.16 – 2.97 | 0.010 | | | |
| Sex | | | | | | |
| Female | 1 | | | | | |
| Male | 0.88 | 0.71 – 1.08 | 0.214 | | | |
| Clinical T stage | | | | | | |
| T1/T2 | 1 | | | 1 | | |
| T3/T4 | 2.01 | 1.57 – 2.58 | <0.001 | 1.33 | 1.02 – 1.73 | 0.036 |
| Tx | 1.82 | 1.41 – 2.35 | <0.001 | 1.33 | 1.02 – 1.74 | 0.035 |
| Unknown | 3.94 | 2.88 – 5.39 | <0.001 | 2.22 | 1.57 – 3.15 | <0.001 |
| Clinical N stage | | | | | | |
| N0 | 1 | | | 1 | | |
| N1 | 1.28 | 1.07 – 1.50 | 0.006 | 1.21 | 1.02 – 1.44 | 0.031 |
| Nx | 1.50 | 1.25 – 1.80 | <0.001 | 1.54 | 1.28 – 1.86 | <0.001 |
| Unknown | 2.70 | 2.11 – 3.47 | <0.001 | [1] | | |
| Supportive therapy (yes) | 1.07 | 0.90 – 1.27 | 0.443 | | | |
| Palliative surgery (yes) | 0.44 | 0.36 – 0.52 | <0.001 | 0.43 | 0.35 – 0.53 | <0.001 |
| Palliative chemotherapy (yes) | 0.46 | 0.39 – 0.54 | <0.001 | 0.47 | 0.39 – 0.55 | <0.001 |
| Prior malignancy (yes) | 0.93 | 0.80 – 1.08 | 0.358 | | | |
| Increasing age (year) | 1.03 | 1.03 – 1.04 | <0.001 | 1.02 | 1.01 – 1.03 | <0.001 |

[1] Removed due to collinearity.

# DISCUSSION

Between 2000 and 2016, no (clinically) significant changes in incidence and survival of GBC were seen. Although radical surgery in early GBC and palliative chemotherapy in unresectable and metastatic GBC significantly improved survival, these treatment modalities were only used in 26% (radical surgery) and 29% (palliative chemotherapy) of patients.

The survival rates as demonstrated in this study are comparable to those from a previously published Western cohorts, but inferior to survival rates from non-Western centers: 3-year survival was 73% for stage I (53% in stage II) in our study compared to 100% (80% in stage II) in a recently conducted Korean study including 142 patients. [26-28] Possibly, these differences are attributable to selection bias in high-volume expert centers in non-Western countries, different tumor biology or differences in the administration of adjuvant chemotherapy, which has not been standard practice in the Netherlands. [29]



In a subgroup analysis, improved survival over time was only seen in resected and metastatic GBC. The improved outcome of resected patients is likely the result of multiple factors. Although primary resection rates remained stable, larger tumors (T3/T4) were increasingly resected and 90-day mortality decreased significantly over time, suggesting an improvement in operative techniques or postoperative care. A sharp increase in re-resection rates for early GBC was seen after 2010, coinciding with a change in national guidelines advocating the use of additional gallbladder bed resection and regional lymphadenectomy in early (pT1b/T2) GBC, which is associated with significantly improved outcomes. [14, 30-33] Our results support this notion; patients with early GBC who received radical surgery had a median OS that was over three times larger (76.7 vs. 18.4 months) than the survival of patients who did not undergo radical resection.

Unfortunately our results suggest substantial undertreatment; even during the last study period only 26% of patients with early-stage GBC received the recommended radical surgery in addition to cholecystectomy alone. Probably, most early GBC patients are diagnosed incidentally after cholecystectomy for suspected benign gallbladder disease by a general gastrointestinal surgeon in a community hospital. We hypothesize that many clinicians still perceive advanced GBC as an untreatable disease and thus may be reluctant to refer patients to a specialized hepatobiliary center for additional surgery or chemotherapy. We believe that multidisciplinary, specialized care and better adherence to (inter-)national guidelines may improve prognosis of GBC patients.

Previous studies show conflicting results on the value of adjuvant chemotherapy. Most evidence is based on small, retrospective series and only one recently published phase-3 trial showed a survival benefit in the per-protocol analysis alone. [17] Currently recruiting large, prospective trials may show more positive results. [34] Unfortunately, the effect of adjuvant therapy after resection could not be assessed as adjuvant therapy is currently not standard of care in the Netherlands and was only administered to a small number of cases (most likely in a clinical trial setting).

In 2010, the ABC-02 trial demonstrated a survival benefit of gemcitabine and cisplatin in metastatic biliary tract cancer [15] resulting in an update of the national guidelines and palliative chemotherapy becoming standard of care. Although a subsequent rise from 11% to 29% in the use of palliative chemotherapy was seen after 2010, it was still infrequently administered. Since (subsidized) healthcare insurance is mandatory for all inhabitants of the Netherlands and travel distance to healthcare is generally small, the most likely explanation for this poor delivery rate is nihilism regarding the efficacy of chemotherapy. Evidently, chemotherapy in non-resectable GBC warrants further attention since the increase in use of palliative chemotherapy is a likely cause for the (minor) improvement in median OS in metastatic GBC.

The major limitation of this study pertains to the nature of registration data. Because of the retrospective nature of this study selection bias is present. Caution should be exercised when interpreting results, especially when analyzing treatment strategies and associated differences in survival. Also, possible incompleteness of data in the earlier years and changes in registry guidelines resulted in missing data on prognostic factors such as T- and N-stage (16%) in unresected patients and tumor grade (29%) in resected patients. Second, distinguishing GBC from perihilar cholangiocarcinoma (proximal extrahepatic cholangiocarcinoma, pCC) is challenging in locally advanced disease.[35] Diagnosis in unresected patients was based on imaging only and histopathological confirmation was available in 76% of patients. However, recent research highlights the importance of this distinction, as GBC and pCC show different molecular landscapes and consequently might benefit from different treatment options. [20, 36, 37] The results from this study reflect current clinical practice until more reliable diagnostic methods to differentiate between GBC and pCC become available.

A unique strength of this study is the nation-wide, population based design resulting in an accurate representation of treatment and survival patterns of gallbladder cancer in daily clinical practice in a low incidence population. In addition, we were able to

perform a quality control and demonstrated that the accuracy of the registration data is very high since only 1 out of 108 patients received an incorrect diagnosis.
In conclusion, survival of GBC is poor and minimal improvement has been made in the past decade in the Netherlands. Radical surgery in early GBC and palliative chemotherapy in unresectable and metastatic GBC are associated with increased OS. However, the use of these treatment modalities is still limited. A multidisciplinary approach in GBC involving radical surgery and systemic therapy may lead to improvement in the survival of GBC patients.

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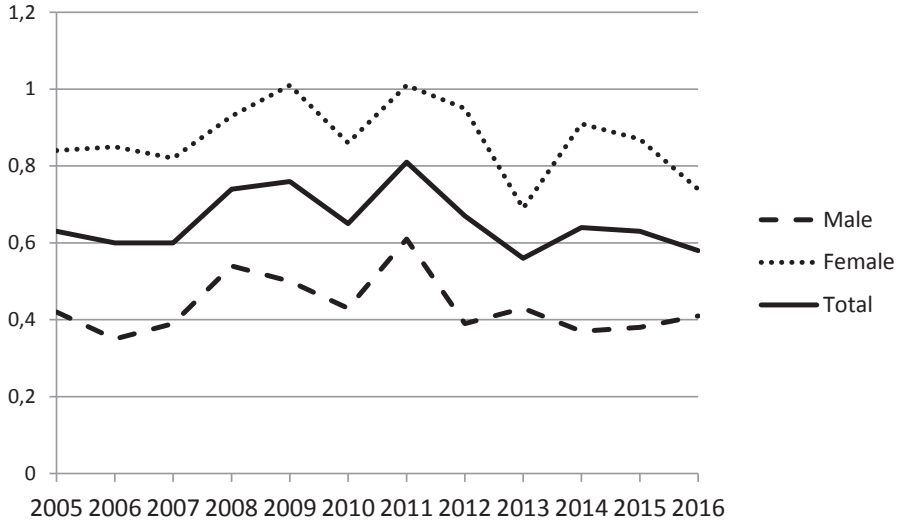
# APPENDIX

Appendix A.

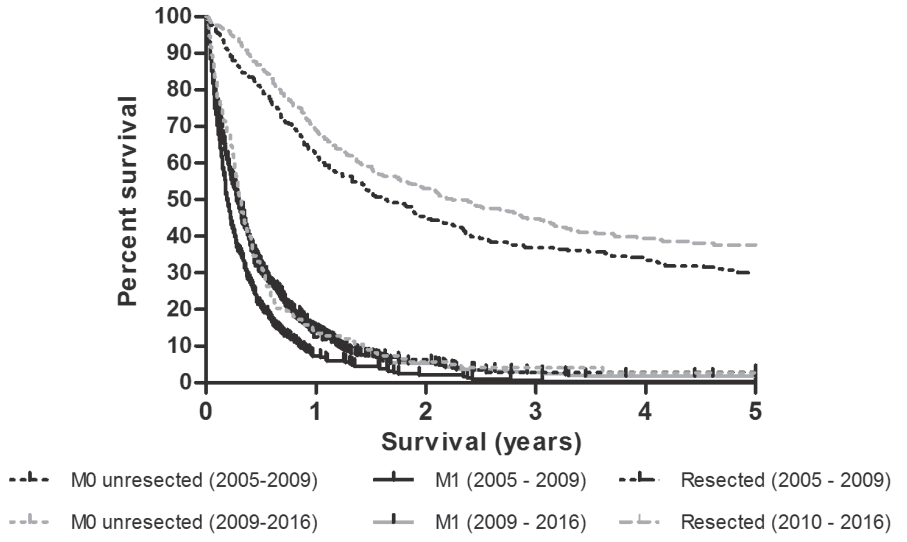
| ICD-03 code | | Frequency *(%)* |
|---|---|---|
| 8000 | Neoplasma | 310 *(16.6)* |
| 8001 | Tumor cells | 2 *(0.1)* |
| 8010 | Carcinoma, NOS | 50 *(2.7)* |
| 8012 | Large cell carcinoma NOS | 35 *(1.9)* |
| 8013 | Large cell neuroendocrine carcinoma | 6 *(0.3)* |
| 8020 | Carcinoma, undifferentiated, NOS | 5 *(0.3)* |
| 8030 | Giant cell and spindle cell carcinoma | 2 *(0.1)* |
| 8032 | Spindle cell carcinoma, NOS | 2 *(0.1)* |
| 8033 | Pseudosarcomatous carcinoma | 3 *(0.2)* |
| 8041 | Small cell carcinoma, NOS | 10 *(0.5)* |
| 8046 | Non-small cell carcinoma | 4 *(0.2)* |
| 8070 | Squamous cell carcinoma, NOS | 19 *(1.0)* |
| 8071 | Squamous cell carcinoma, keratinizing, NOS | 2 *(0.1)* |
| 8074 | Squamous cell carcinoma, spindle cell | 1 *(0.1)* |
| 8140 | Adenocarcinoma, NOS | 1171 *(62.6)* |
| 8144 | Adenocarcinoma, intestinal type | 21 *(1.1)* |
| 8160 | Cholangiocarcinoma | 6 *(0.3)* |
| 8163 | Pancreatobiliary-type carcinoma | 5 *(0.3)* |
| 8210 | Adenocarcinoma in adenomatous polyp | 7 *(0.4)* |
| 8211 | Tubular adenocarcinoma | 2 *(0.1)* |
| 8240 | Carcinoid tumor, NOS | 13 *(0.7)* |
| 8244 | Mixed adenoneuroendocrine carcinoma | 2 *(0.1)* |
| 8246 | Neuroendocrine carcinoma, NOS | 6 *(0.3)* |
| 8249 | Atypical carcinoid tumor | 2 *(0.1)* |
| 8260 | Papillary adenocarcinoma, NOS | 36 *(1.9)* |
| 8263 | Adenocarcinoma in tubolovillous adenoma | 3 *(0.2)* |
| 8310 | Clear cell adenocarcinoma, NOS | 3 *(0.2)* |
| 8312 | Renal cell carcinoma, NOS | 1 *(0.1)* |
| 8350 | Nonencapsulated sclerosing carcinoma | 1 *(0.1)* |

| | | |
|---|---|---|
| 8480 | Mucinous adenocarcinoma | 31 *(1.7)* |
| 8481 | Mucin-producing adenocarcinoma | 44 *(2.4)* |
| 8490 | Signet ring cell carcinoma | 19 *(1.0)* |
| 8500 | Infiltrating duct carcinoma, NOS | 2 *(0.1)* |
| 8503 | Intraductal papillary adenocarcinoma with invasion | 4 *(0.2)* |
| 8560 | Adenosquamous carcinoma | 26 *(1.4)* |
| 8570 | Adenocarcinoma with squamous metaplasia | 1 *(0.1)* |
| 8574 | Adenocarcinoma with neuroendocrine differentiation | 10 *(0.5)* |
| 8575 | Metaplastic carcinoma, NOS | 1 *(0.1)* |
| 8576 | Hepatoid adenocarcinoma | 1 *(0.1)* |
| 8980 | Carcinosarcoma, NOS | 3 *(0.2)* |

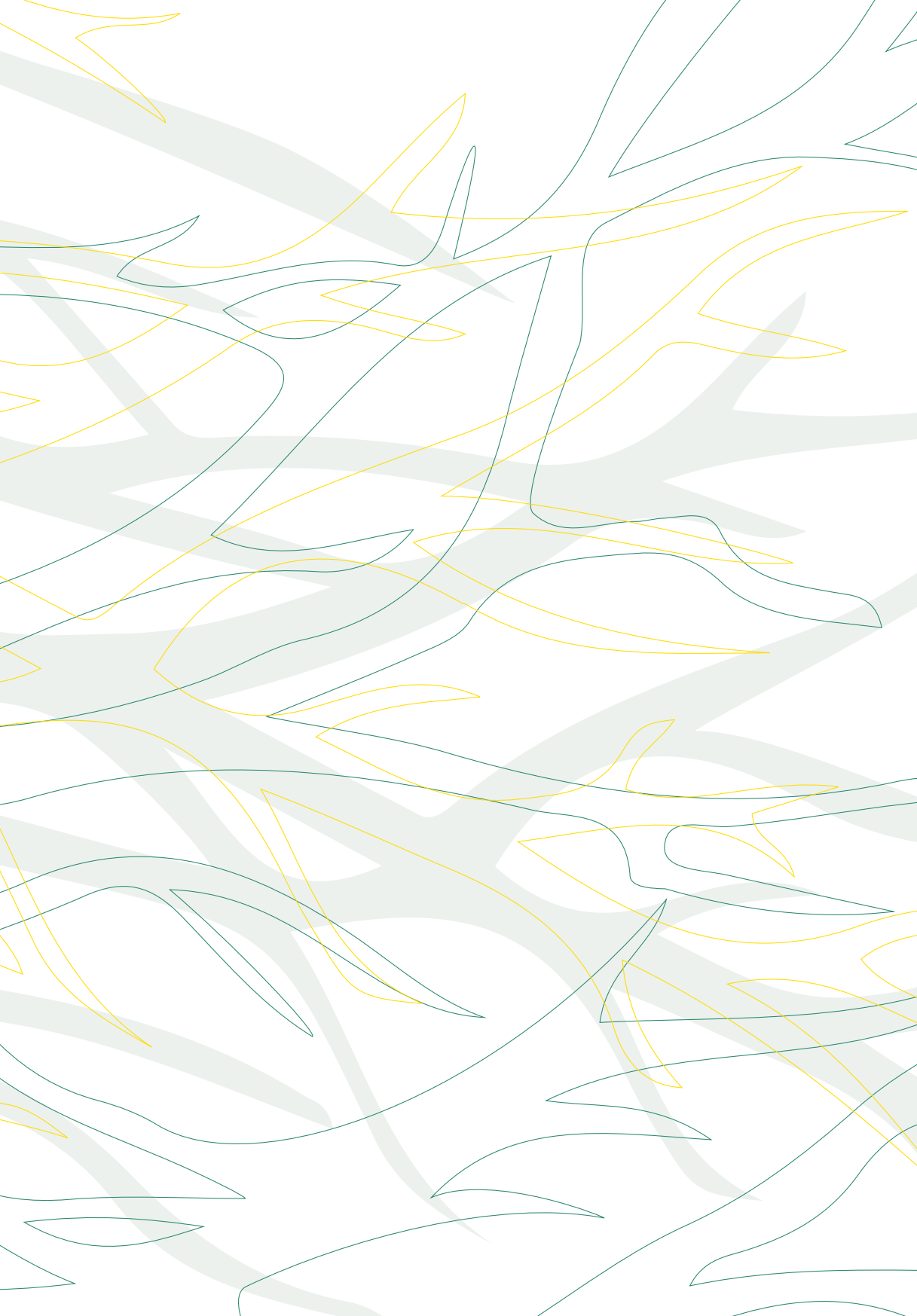
Appendix B




Appendix C



# 4



CHAPTER 5

# Extended resections for advanced gallbladder cancer: results from a nationwide cohort study

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# ABSTRACT

## BACKGROUND

Extended resections (i.e. major hepatectomy and/or pancreatoduodenectomy) are rarely performed for gallbladder cancer (GBC) as outcomes remain inconclusive. Little data regarding extended resections is available from Western centers. In this Dutch, multicenter cohort study outcomes of patients who underwent extended resections for locally advanced GBC are analyzed.

## METHODS

Patients with GBC who underwent extended resection with curative intent between January 2000 and September 2018 were identified from the Netherlands Cancer Registry (NCR). Extended resection was defined as a major hepatectomy (resection of ≥ 3 liver segments) and/or a pancreatoduodenectomy. Treatment and survival data were obtained. Post-operative morbidity, mortality, survival and characteristics of short- and long-term survivors were assessed.

## RESULTS

A total of 33 patients was included. R0-resection margins were achieved in 16 patients. Major post-operative complications (≥Clavien Dindo 3A) occurred in 19 patients and post-operative mortality <90 days in four. Recurrence occurred in 24 patients. Median overall survival (OS) was 12.8 months (95% CI 6.5 – 19.0). Two-year survival was achieved in 10 patients (30%) and 5-year survival in 5 patients (15%). Jaundice, common bile duct-, liver-, perineural- and perivascular invasion were associated with reduced survival. All three recurrence-free patients had R0 resection margins and no liver invasion.

## CONCLUSION

Median OS after extended resections for advanced GBC was 12.8 months in this cohort. Although post-operative morbidity and mortality were significant, long term survival (≥ 2 years) was achieved in a subset of patients. Therefore, GBC requiring major surgery does not preclude long-term survival and a subgroup of patients benefit from surgery.

# INTRODUCTION

Gallbladder cancer (GBC) is a rare tumor; worldwide incidence rates are less than 2 per 100 000 with significant geographic variation. [1,2] Nevertheless, it is the most common biliary tumor. [3,4] GBC is characterized by locally aggressive behavior and early spread to regional lymph nodes. [1] Timely diagnosis is difficult due to the late, non-specific symptoms and tendency for early metastatic spread. [5] As a result, GBC is diagnosed at an advanced stage in the majority of cases. [6-8]

Complete surgical resection is the only curative treatment. [9] The majority of long-term survivors is observed in patients with GBC diagnosed incidentally after cholecystectomy for presumed benign gallbladder disease. When diagnosed pre-operatively, only 10 – 20% of tumors are amenable to resection at presentation and prognosis after resection remains unfavorable. [10,11-14] In patients with T4 disease, even after radical resection median overall survival is only 11 months. [14]



To achieve resection with tumor-free margins, extended resections like major hepatectomy, pancreatoduodenectomy (PD) and even hepatopancreatoduodenectomy (HPD) have been performed in advanced tumors. [15-17] However, extended resections are associated with significant (>50%) postoperative morbidity and mortality whilst the benefit in terms of survival remains unclear [15]. Moreover, almost all studies were published are single center series from non-Western countries: only two Western studies published over one decade ago. [15,18] Nation-wide data are essential to shed light on actual, clinical outcomes of GBC patients.

The purpose of this study was to analyze the results of extended resections in patients with advanced GBC in a Dutch multicenter nationwide study and determine post-operative morbidity, mortality and survival as well as to identify factors associated with short- and long-term survival.

# METHODS

The study was approved by the Medical Ethics Committee of the region Arnhem-Nijmegen (METc number 2017-3912) and carried out according to the STROBE guidelines for observational cohort studies. [19] Patients were identified from the Netherlands Cancer Registry (NCR). The NCR contains data on all newly diagnosed

malignancies, including year of diagnosis, patient age and gender, tumor characteristics (TNM stage), patient identification number and treatment hospital. The data from the NCR is based on data from the automated pathological archive (PALGA), the nation-wide network and registry of histo- and cytopathology in the Netherlands and supplemented by data from the National Archive of Hospital Discharge Diagnosis. [20] Patients treated for GBC in any tertiary referral center in the Netherlands were included in a national, retrospective database.

## PATIENT SELECTION AND VARIABLE DEFINITIONS

Patients with histopathologically proven GBC who underwent extended resection were included for the present study. Extended resection was defined as a major hepatectomy (resection of ≥ 3 liver segments) and/or a pancreatoduodenectomy, with or without en-bloc resection of adjacent organs (duodenum, colon or stomach) with curative intent. Patients who underwent a simple cholecystectomy, a (re)resection of the gallbladder bed, a minor hepatectomy (< 3 segments) and/or a lymph node dissection along the hepatoduodenal ligament without associated major liver resection were excluded. Patients with incidentally diagnosed GBC (i.e. during or after cholecystectomy for presumed benign disease) were also excluded. Data on patient characteristics, pre-operative bilirubin and carbohydrate antigen 19-9 (CA19-9), operative characteristics, tumor characteristics, post-operative morbidity and mortality, recurrence, and overall survival were obtained from the medical records. Tumor staging was reported according to the American Joint Committee on Cancer (AJCC) staging system. [21] Resection margins were classified into R0 (distance margin to tumor ≥ 1mm) and R1 (micro- or macroscopically positive margin). Intra-operative hemorrhage was defined as intra-operative blood loss of ≥ 1000ml or requiring a blood transfusion intra-operatively. Data of post-operative complications were determined according to the Clavien-Dindo Classification System and included complications up to 90-days after surgery. [22] Major complications were defined as Clavien-Dindo grade ≥ 3A. Post-operative mortality was defined as death due to any cause <90 days post-operatively. Overall survival (OS) included deaths from any cause. Short-term survival was defined as survival ≤ 6 months, including post-operative mortality. Long-term survival was defined as survival ≥ 2 years. Disease-free survival (DFS) was defined as the number of months from extended resection to date of recurrence or date of last follow-up. Adjuvant therapy was only administered in clinical trial setting as it was not considered standard of care during the study period.

### STATISTICAL ANALYSIS

Continuous variables are presented as median (inter quartile range) and categorical data are presented as numbers (percentages). Survival was reported using Kaplan-Meier methods and differences in survival were analyzed using the Log-Rank test. P-values ≤ 0.05 were considered statistically significant. All statistical analysis were conducted using SPSS Statistics for Windows, version 23.0 ® (IBM Corporation, Armonk, NY, USA).

## RESULTS

### PATIENT CHARACTERISTICS

Between January 2000 and September 2018, 289 patients underwent a surgical resection for pre- or post-operatively diagnosed GBC with curative intent in the participating centers. During this period, 33 pre-or intra- operatively diagnosed patients (11%) underwent an extended resection and were included.



The cohort consisted of 13 men (39%) and 20 women (61%). Median age at time of diagnosis was 64 years (IQR 57.0 – 68.5) (Table 1). Presenting symptoms were jaundice (n=21), abdominal pain (n=16), nausea (n=8), weight loss (n=7), discolored defecation (n=3), fever (n=1), back pain (n=1) and liver enzyme disorders (n=1). CA19-9 at time of presentation was tested in 12 patients and had a median value of 542 kU/l (IQR 87 – 3500). Bilirubin levels at time of presentation were available in 19 patients, with a median value of 94 µmol/l (IQR 12 – 159). None of the patients were diagnosed with primary sclerosing cholangitis (PSC).

### PRE-OPERATIVE WORK-UP AND TREATMENT

Pre-operative work-up consisted of ultrasonography (US) (n=25), endoscopic US (n=5), computed tomography (CT) (n=33), magnetic resonance imaging (n=20) and positron emission tomography - CT (n=5). Adjacent organ invasion was seen on pre-operative imaging in 30 patients (91%); into the extrahepatic bile ducts (n=22; 67%), liver (n=16; 49%), pancreas (n=7; 21%) and duodenum (n=1; 3%). Pre-operative biliary drainage was performed in 20 patients; an endoscopic retrograde cholangiopancreatography was performed in all 20 patients (61%); in two patients (6%) an additional percutaneous transhepatic cholangiography was required. In eight patients (24%), a diagnostic laparoscopy was performed. A portal vein embolization was performed in five patients (15%). None of the patients received neo-adjuvant chemotherapy (NACT).

Table 1. Patient and operative characteristics of GBC patients that underwent extended resection.

| | Total n=33 | |
|---|---|---|
| Median age, in years (IQR) | 64 | (57-69) |
| Gender | | |
| Female | 20 | (61%) |
| Male | 13 | (39%) |
| ASA classification [a] | | |
| 1 | 5 | (20%) |
| 2 | 14 | (56%) |
| 3 | 6 | (24%) |
| Pre-operative biliary drainage (yes) | 20 | (61%) |
| PVE performed (yes) | 5 | (15%) |
| Type of surgery | | |
| Left hemihepatectomy | 1 | (3%) |
| Extended right hemihepatectomy | 7 | (21%) |
| Right hemihepatectomy | 11 | (33%) |
| Right hemihepatectomy + PD | 2 | (6%) |
| PD + wedge | 11 | (33%) |
| PD + segment 4,5 | 1 | (3%) |
| Portal vein reconstruction (yes) | 10 | (30%) |
| Post-operative complications ≥ CD 3 | 19 | (58%) |

ASA: American society of anesthesiologists; PVE: portal vein embolization;
PD: pancreatoduodenectomy; CD: Clavien-Dindo Classification System. Medians are presented as number (interquartile range [IQR]). Numbers are presented as n (percentage of group).
[a] eight missing values.
[a] one missing value; [b] two missing values; [c] eleven missing values.
Numbers are presented as n (percentage of group).

Indications for extended resection were a suspicion of GBC, cholangiocarcinoma (CCA) or pancreatic cancer with invasion of adjacent organs. Main indications for major hepatectomies were a suspicion of hilar CCA (n=4) or GBC with liver involvement (discovered on pre-operative imaging (n=11) or intra-operatively (n=4, of which in one patient a clear indication for a major hepatectomy instead of a minor hepatectomy could not be obsoleted retrospectively)). Main indications for a pancreatoduodenectomy (PD) were a suspicion of distal CCA (n=2), a suspicion of pancreatic cancer (n=2), GBC

with pancreatic involvement or suspicious lymph nodes around the pancreas pre-operatively (n=5) and GBC with involvement of the duodenum intra-operatively (n=3). Indication for hepatopancreatoduodenectomy was GBC with involvement of liver and extension in distal bile duct and portal vein on preoperative imaging (n=1) and GBC with involvement of liver and intra-operative suspected lymph node invasion around the pancreas requiring resection of the pancreatic head (n=1).

## OPERATIVE CHARACTERISTICS

The operative characteristics of the entire cohort are presented in Table 1. Surgical procedures consisted of a right hepatectomy (n=11), extended right hepatectomy (n=7), PD with wedge resection (n=11), PD with concurrent segment 4 and 5 resection (n=1) and left hepatectomy (n=1) and PD combined with right hepatectomy (n=2). Additionally, the colon was partially resected due to intra-operative involvement in two cases and the ovaries were resected in one case.



All patients underwent a lymph node dissection and resection of the common bile duct (CBD). The portal vein was reconstructed in 10 patients (30%). Intra-operative complications occurred in five patients (15%), consisting of hemorrhage in five and an additional systemic inflammatory response syndrome (SIRS) in one patient. All intra-operative complications occurred in patients who underwent a major hepatectomy.

## TUMOR CHARACTERISTICS

Histopathological analysis showed tumor-free margins in 16 patients (48%) (Table 2). Histology revealed adenocarcinoma (n=29), squamous-cell carcinoma (n=1), adenosquamous carcinoma (n=1). Histopathological subtype was not described in two patients. Tumor differentiation grade was reported in twenty-two patients and was well in six patients (27%), moderate in nine patients (41%) and poor in seven patients (32%). Perineural invasion was found in twenty-four patients (73%) and perivascular invasion in sixteen (48%).

## POST-OPERATIVE MORBIDITY AND MORTALITY

Major post-operative complications <90 days occurred in nineteen patients (58%) and are described in Table 3. Four post-operative deaths (12%) occurred; two due to sepsis (due to liver failure and due to anastomotic leakage), one due to liver failure after portal vein thrombosis and one due to aspiration and hypoxia.

Table 2. Pathological characteristics of GBC patients after extended resections.

| | Total n=33 | |
|---|---|---|
| pT [a] | | |
| T2 | 4 | (13%) |
| T3 | 18 | (55%) |
| T4 | 10 | (31%) |
| pN | | |
| N0 | 13 | (39%) |
| N1 | 11 | (33%) |
| N2 | 9 | (27%) |
| pM [b] | | |
| M0 | 22 | (71%) |
| M1 | 9 | (29%) |
| Radical resection margin (R0) | 16 | (48%) |
| Differentiation grade [c] | | |
| Good | 6 | (27%) |
| Moderate | 9 | (41%) |
| Poor | 7 | (32%) |
| Perineural invasion (yes) | 24 | (73%) |
| Vascular invasion (yes) | 16 | (48%) |
| Liver invasion (yes) | 21 | (64%) |
| Histology [b] | | |
| Adenocarcinoma | 29 | (94%) |
| Squamous-cell carcinoma | 1 | (3%) |
| Adenosquamous carcinoma | 1 | (3%) |

[a] one missing value; [b] two missing values; [c] eleven missing values.
Numbers are presented as n (percentage of group).

Table 3. Serious (≥CD3a) post-operative complications <90 days of extended resections in GBC patients.

| | Major Hepatectomy (N=19) | PD n=12 | Major hepatectomy + PD (N=2) |
|---|---|---|---|
| Post-operative complications ≥ CD3 | 11 (58%) | 7 (58%) | 1 (50%) |
| Intra-abdominal abscess | 1 | 3 | 0 |
| Ascites | 4 | 0 | 0 |
| Abdominal hemorrhage | 0 | 3 | 1 |
| Anastomotic leakage | 1 | 3 | 0 |
| Pancreatic fistula | 1 | 0 | 0 |
| Respiratory | 3 | 1 | 1 |
| Cardiac | 1 | 0 | 0 |
| Liver failure | 1 | 1 | 0 |
| Sepsis/SIRS | 4 | 3 | 0 |
| Other | 2 | 2 | 1 |
| Post-operative mortality | 3 (16%) | 1 (8%) | 0 (0%) |

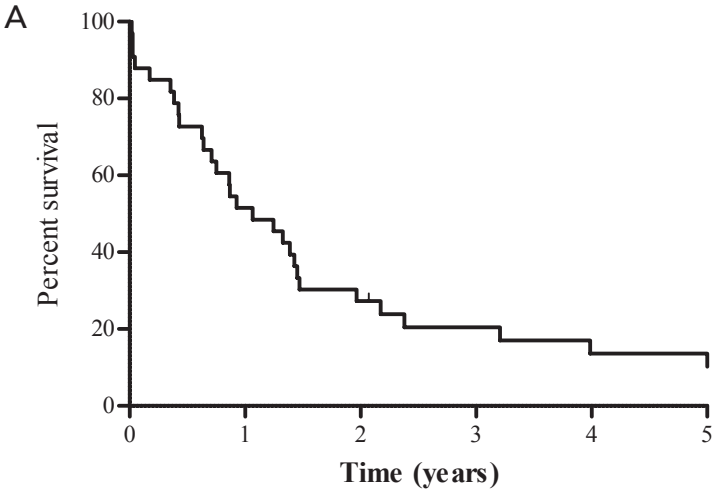
PD: pancreatoduodenectomy; CD: Clavien-Dindo Classification System; SIRS: systemic inflammatory response syndrome.
Numbers are presented as n (percentage of group).



## ADJUVANT TREATMENT, FOLLOW-UP AND SURVIVAL

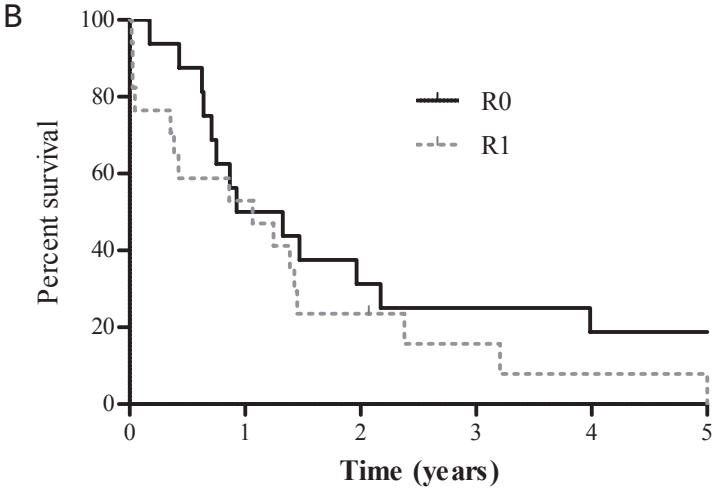
Two patients received adjuvant chemotherapy (gemcitabine and cisplatin in one and capecitabin in one) and five received chemotherapy with palliative intent at the time of recurrence. Recurrence occurred in twenty-four patients (73%). Imaging during follow-up showed recurrence locally (n=13), on the peritoneum (n=10), in the liver (n=9), in the lungs (n=1) and other locations (n=7). All mortality was disease-related (i.e. due to post-operative complications, progression or recurrence). Median OS was 12.8 months (95% CI 6.5 – 19.0) (Figure 1A). Median OS excluding post-operative mortality was 15.9 months (95% CI 9.1 – 22.7) and median DFS was 10.1 months (95% CI 4.5 – 15.8). No significant survival difference was found between patients with a R0 vs. R1 resection; median OS in R0 patients was 11.1 months versus 12.8 in patients with R1 resection (p=0.203, Figure 1B).

Figure 1. A: Survival in years. B: Survival according to resection margin.




No. at risk

| | | | | | | |
|---|---|---|---|---|---|---|
| OS | 33 | 17 | 10 | 7 | 5 | 5 |




No. at risk

| | | | | | | |
|---|---|---|---|---|---|---|
| R0 | 16 | 8 | 6 | 5 | 4 | 4 |
| R1 | 17 | 9 | 4 | 2 | 1 | 1 |

Three patients were still alive without signs of disease at the time this study was conducted (median follow-up 97 months). Characteristics of short-term survivors (≤6 months, n=9) and long-term survivors (≥ 2 year survival, n=10) are reported in Appendix table A and B, respectively. When excluding those patients who died due to post-operative complications, eight out of nine short term survivors were jaundiced at time of presentation and all patients showed perineural and perivascular invasion, invasion of the liver parenchyma and CBD upon histopathological analysis. All long-term survivors without recurrence (n=3) showed tumor free resection margins without signs of perivascular invasion or liver invasion after histopathological analysis. One long-term survivor without recurrence received adjuvant chemotherapy.

# DISCUSSION



This national, retrospective cohort study is the first Western study in the past decade on the outcomes of extended resections for GBC. This series shows that major hepatectomies and PD's are rarely performed for advanced GBC in the Netherlands. Despite a reported median DFS of 10.1 months and median OS of 12.8 months, 21% of patients survived beyond 3 years. Major post-operative complications occurred in 58% of patients and post-operative mortality in 12% of patients. R0 resection margins were achieved in 48% of patients. In a study from the Memorial Sloan Kettering Cancer Center, comparable mortality rates were described, with a post-operative mortality of 5/36 (14%) for major hepatectomies. [15] Recurrence occurred in 24 patients (73%), R0 resection margins in their series were achieved in 91% and 5-year survival was 27%. Nevertheless, in their study, some patients without evidence of inflow involvement underwent empiric major hepatectomy, whereas in our study all but one patient were suspected of tumor extension in other organs necessitating extended resection for tumor-free margins.

The value of extended surgery for advanced GBC remains questionable. Results from previous studies investigating patients undergoing (hepato)pancreaticoduodenectomy show virtually no survival beyond 2 years and a R0 resection rate of only 20%. [23,24] In our cohort, two-year survival was 30% and 5-year survival was 15%. Patients with an R0 resection, achieved in 48% of patients, had a 5-year survival of 19%. A previous study by Fong et al. showed that extent of liver resection did not influence survival in multivariable analysis. [25] Another study argues that wedge resection is to be reserved for patients with minimal liver invasion and extensive liver resections should

be performed in patients with advanced tumors with extensive liver invasion or hepatic-hilar type tumors. [26] Unfortunately, due to low numbers we could not study the association between extent of liver invasion, resection and survival. However, it is known from a population-based study that 1-year survival in patients with unresected, advanced GBC is less than 10%, opposed to a median OS of 12.8 months in our cohort including patients who died due to surgery-related complications. [27] Median OS of all patients with unresected GBC treated with palliative chemotherapy was 6.4 months in our nation-wide cohort (unpublished results). Moreover, in the ABC-02 trial (gemcitabine plus cisplatin vs. gemcitabine alone in unresected biliary tract cancer) no survivors beyond 3 years were reported, whilst in our cohort 3-year survival was 20%. [28] Even though no significant survival difference was seen between R0 and R1 patients, all three long-term survivors without recurrence clearly all had R0 resection margins. The lack of statistical significance therefore is most likely caused by our small sample size. Other explanations include possible per-center and over time differences in R0 or R1 resection margin criteria and differences in experience and quality of pathologists.

The identification of prognostic factors is vital for adequate patient selection. Our results show that all except one short-term survivors were jaundiced at presentation; a factor known to negatively influence survival. [29] Additionally, all short-term survivors had a pT3 or pT4 stage tumor and the majority had positive lymph nodes. In contrast, few long-term survivors had a high T stage and positive lymph nodes as well. Excluding patients who died due to post-operative complications, all short-term survivors had perivascular invasion as well as invasion of the liver parenchyma. These factors were also associated with poor survival in other studies. [24,30,31] In-depth pre-operative assessment using imaging techniques such as contrast-enhanced Magnetic Resonance Imaging may identify patients with smaller, localized tumors amenable to resection. [32] The use of PET (positron emission tomography) scans and PET-CT might be especially helpful in detecting unsuspected metastasis. [33-35]

Although our cohort likely exists of a highly selected subgroup of patients who are fit to undergo extensive surgery and have no suspicion of metastasis, our results do demonstrate that in these patients long-term survival after extended surgery is possible. However, the high morbidity and mortality rates associated with extensive liver surgery need to be weighed against the apparent survival benefit. Post-operative quality of life (QoL) must be taken into account when considering performing an extended resection. Unfortunately, due to the retrospective nature of this study, we were not able to assess QoL by using questionnaires.

Identifying the correct tumor type and location pre-operatively may be difficult in GBC. In our series 12 out of 33 cases were suspected of CCA or pancreas carcinoma instead of GBC pre-operatively. Infiltration by tumor or inflammation in surrounding tissues (i.e. pancreas, hilum or extrahepatic bile ducts) makes identification of primary tumor location on pre-operative imaging challenging. Moreover, differentiating malignant invasion in adjacent organs from inflammation is difficult. In a study from MSKCC a subgroup of patients required resection of adjacent organs due to tumor adhesion; definitive histopathology showed tumor invasion in only half of these patients. [15]

Results from the recently published BILCAP trial suggested that adjuvant capecitabine can improve OS in patients with resected biliary tract cancer. [36] Currently, (neo-) adjuvant chemotherapy ((N)ACT) is not considered standard of care in the Netherlands, as reflected by the small number of patients in our cohort receiving chemotherapy. [37] However, one out of three long-term survivors without recurrence received adjuvant capecitabine, providing additional support for the use of adjuvant chemotherapy.



Moreover, a recent article reported significantly lower recurrence rates and higher OS in patients receiving adjuvant chemoradiation therapy (compared to surgery alone) for resected GBC. Noteworthy, this benefit was only seen in patients with positive lymph node status and patients with N0 disease did not appear to benefit from adjuvant therapy. [38] Therefore, administration of adjuvant chemoradiation might be helpful in pN1/2 patients fit to undergo adjuvant therapy. A recent systematic review stated that although favorable tumor response and increased resectability rates have been reported after NACT, there is currently insufficient evidence to its support routine use. [39] However, the authors also concluded that future randomized trials should be conducted in order to investigate the role of NACT in advanced GBC. Since radical resection seems the only way to achieve long-term survival, NACT before resection of locally advanced gallbladder cancer patients (such as the patients in our cohort) may further improve outcomes.

Our study has several limitations. First, the low number of included patients makes it impossible to draw statistical conclusions on prognostic factors for prolonged survival after extended resections for GBC. Secondly, due to the retrospective nature of this study there was a large amount of missing data. Unfortunately, prospective research is logistically challenging due to the low incidence of this type of tumor. Future international collaborative studies should include a larger cohort of patients, preferably based on prospective data collection.

In conclusion, median overall survival after major resections for advanced GBC in this cohort was 12.8 months and 10 patients survived longer than 2 years. Jaundice at time of presentation, perineural and perivascular invasion, positive lymph nodes, invasion of the liver parenchyma and CBD demonstrated on histopathological examination were present in patients with poor survival. Although major post-operative complications were frequent and post-operative mortality occurred in 12% of patients, prognosis of these patients is extremely poor if no surgery is conducted. Therefore, extended resections for patients with locally advanced GBC should be considered if the morbidity and mortality is acceptable for the patient compared to the presumed benefit in survival and QoL.

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# 5

**Table A.** Characteristics of short-term survivors (≤ 6 months).

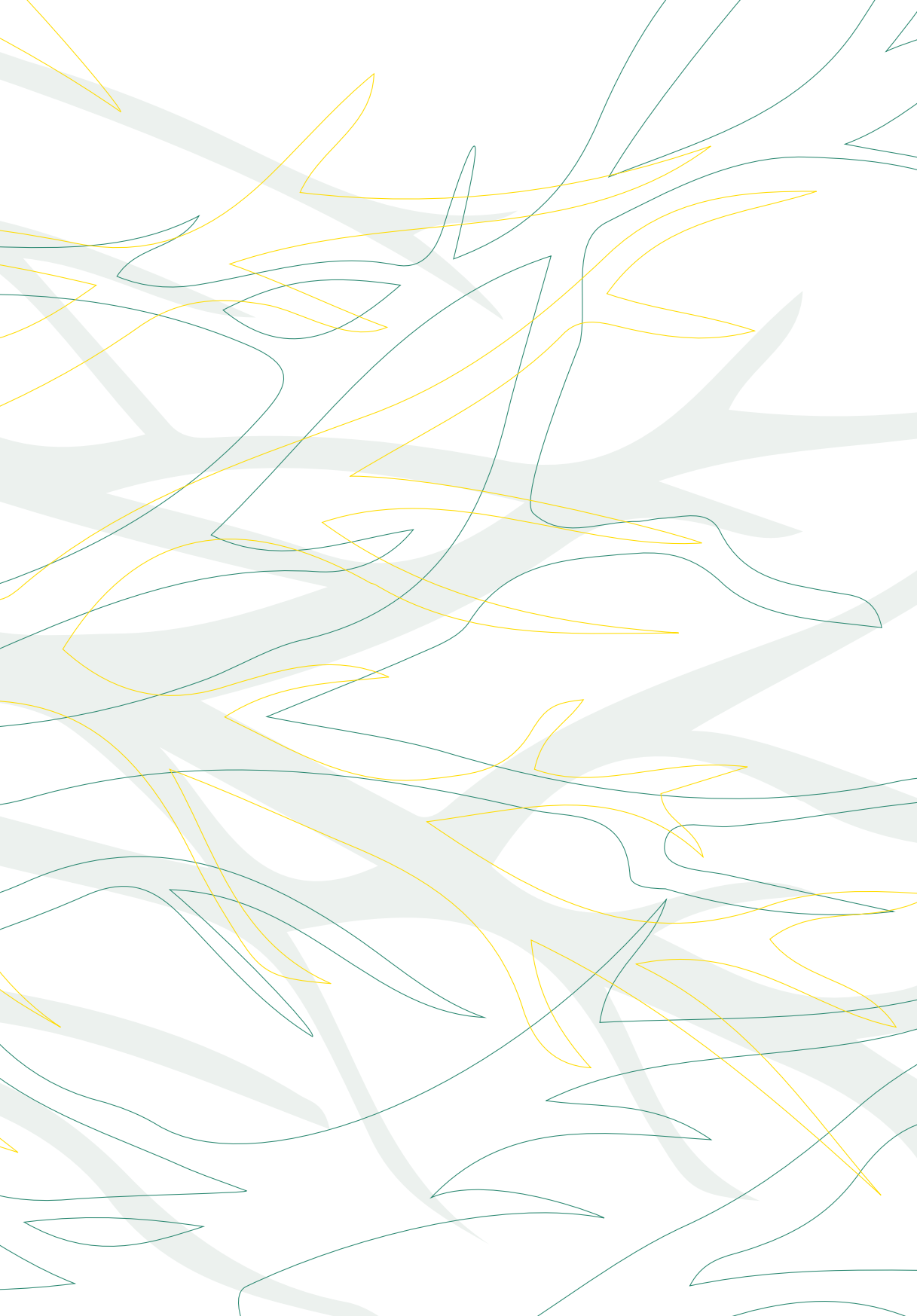
| Age[a], gender, ASA | Jaundice[a] | Procedure | pTNM R-status | Perineural invasion | Vascular invasion | Liver invasion | CBD invasion | Follow-up (days) | Cause of death |
|---|---|---|---|---|---|---|---|---|---|
| 66, M, ASA 3 | Yes | RH | T4N0M0 R0 | Yes | Yes | Yes | No | 6 | Post-op comp |
| 77, M, ASA - | Yes | RH | T3N1M0 R1 | Yes | Yes | Yes | Yes | 10 | Post-op comp |
| 73, M, ASA 2 | Yes | RH | T3N0Mx R1 | Yes | No | Yes | Yes | 10 | Post-op comp |
| 64, F, ASA - | Yes | PD | T4N1M1 R1 | Yes | Yes | Yes | Yes | 17 | Post-op comp |
| 64, F, ASA 2 | Yes | PD | T3N2M1 R0 | Yes | Yes | Yes | Yes | 63 | Progression |
| 70, F, ASA 3 | No | RH | T4N1M0 R1 | Yes | Yes | Yes | Yes | 130 | Progression |
| 58, F, ASA 3 | Yes | PD | T4N2M1 R1 | Yes | Yes | Yes | Yes | 141 | Progression |
| 64, M, ASA 2 | Yes | Ext RH | T3N1M0 R1 | Yes | Yes | Yes | Yes | 155 | Progression |
| 67, M, ASA - | Yes | Ext RH | T3N0M0 R0 | Yes | Yes | Yes | Yes | 157 | Recurrence |

M: male; F: female; ASA: American society of anesthesiologists; PD: pancreatoduodenectomy; RH: right hemihepatectomy; Ext RH: extended right hemihepatectomy; CBD: common bile duct; post-op comp: post-operative complications. [a] at time of presentation.

Table B. Characteristics of long-term survivors (≥ 24 months).

| Age[a], gender, ASA | Jaundice[a] | Procedure | pTNM R-status | Perineural invasion | Vascular invasion | Liver invasion | CBD invasion | Follow-up (months) | Status |
|---|---|---|---|---|---|---|---|---|---|
| 65, M, ASA 2 | Yes | PD | T3N0M0 R0 | No | No | Yes | No | 24 | Deceased, recurrence |
| 63, M, ASA 2 | Yes | RH | T2N0M0 R1 | Yes | Yes | No | No | 24 | Alive, with recurrence |
| 67, F, ASA 2 | No | RH | T4N1M0 R0 | Yes | No | No | No | 27 | Deceased, recurrence |
| 79, F, ASA 2 | No | PD | T2N2M0 R1 | No | Yes | No | No | 29 | Deceased, recurrence |
| 63, M, ASA 3 | Yes | PD | T4N1M0 R1 | Yes | No | No | No | 39 | Deceased, recurrence |
| 46, M, ASA 2 | Yes | RH + PD | T3N1M0 R0 | Yes | Yes | Yes | Yes | 49 | Deceased, recurrence |
| 69, F, ASA 2 | Yes | RH + PD | T4N2M1 R1 | Yes | No | No | No | 71 | Deceased, recurrence |
| 65, M, ASA 3 | No | RH | T3N0M0 R0 | Yes | No | No | Yes | 86 | Alive, no recurrence |
| 53, F, ASA 1 | No | RH | T2N2M0 R0 | No | No | No | No | 91 | Alive, no recurrence |
| 68, F, ASA 2 | No | PD | T3N0M0 R0 | No | No | No | No | 119 | Alive, no recurrence |

M: male; F: female; ASA: American society of anesthesiologists; PD: pancreatoduodenectomy; RH: right hemihepatectomy.
[a] at time of presentation.



CHAPTER 6

# Should jaundice preclude resection in patients with gallbladder cancer? Results from a nation - wide cohort study

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# ABSTRACT

## BACKGROUND

It is controversial whether patients with gallbladder cancer (GBC) presenting with jaundice benefit from resection. This study re-evaluates the impact of jaundice on resectability and survival.

## METHODS

Data was collected on surgically explored GBC patients in all Dutch academic hospitals from 2000–2018. Survival and prognostic factors were assessed.

## RESULTS

In total 202 patients underwent exploration and 148 were resected; 124 non-jaundiced patients (104 resected) and 75 jaundiced patients (44 resected). Jaundiced patients had significantly ($P<0.05$) more pT3/T4 tumors, extended (≥3 segments) liver- and organ resections, major post-operative complications and margin-positive resection. 90-day mortality was higher in jaundiced patients (14% vs. 0%, $P<0.001$). Median overall survival (OS) was 7.7 months in jaundiced patients (2-year survival 17%) vs. 26.1 months in non-jaundiced patients (2-year survival 39%, $P<0.001$). In multivariate analysis, jaundice (HR1.89) was a poor prognostic factor for OS in surgically explored but not in resected patients. Six jaundiced patients did not develop a recurrence; none had liver- or common bile duct (CBD) invasion on imaging.

## CONCLUSION

Jaundice is associated with poor survival. However, jaundice is not an independent adverse prognostic factor in resected patients. Surgery should be considered in patients with limited disease and no CBD invasion on imaging.

# INTRODUCTION

Gallbladder cancer (GBC) is an aggressive malignancy with a notoriously poor prognosis. [1] Five-year survival is less than 5%. [2, 3] The only curative treatment remains complete surgical resection. [1] Unfortunately, due to nonspecific symptoms and subsequent late detection, most patients present with advanced disease. GBC tends to grow rapidly and disseminates early to the surrounding hepatic parenchyma, lymph nodes, and peritoneum. [4] Extended liver resections are often necessary to achieve tumor-free margins. [5] Consequently, patients with GBC are frequently either unresectable or require extensive surgery with a high risk of morbidity and mortality to achieve potential cure.

Obstructive jaundice is one of the indicators for advanced disease since it implies infiltration of the hepatic hilum. [6] The first paper reporting on obstructive jaundice in GBC specifically consisted of a series of 107 jaundiced patients; only 6 (7%) patients were deemed resectable and no survival beyond two years was reported. [7] Based on these data the traditional consensus was that obstructive jaundice should preclude surgery. [8] However, recently published studies do report survival beyond 2 years in a small number of patients. Results from a recent meta-analysis show that surgery for GBC presenting with obstructive jaundice should be considered in medically fit patients where R0 resection appears feasible. [9] Nevertheless, most previous studies stem from single center series and generalizability may be limited. Moreover, none of the published studies focus on pre-operative factors associated with prolonged survival in jaundiced patients.

The aim of this study was therefore to analyze the prognostic impact of jaundice in GBC patients who underwent surgical exploration in a nation-wide setting.

# METHODS

## PATIENT INCLUSION AND DATA COLLECTION

Patients were identified from the Netherlands Cancer Registry (NCR). The NCR contains data on all newly diagnosed malignancies, including year of diagnosis, patient age and gender, tumor characteristics (histology and TNM stage), patient identification number and treatment hospital. The data from the NCR is based on data from the automated pathological archive (PALGA), the nation-wide network and registry of

histo- and cytopathology in the Netherlands, and supplemented by data from the National Archive of Hospital Discharge Diagnosis. [10] Patients treated for GBC in any of the expert, tertiary referral centers in the Netherlands were included in a retrospective database. Patients diagnosed in community hospitals were not included because in the Netherlands patients with gallbladder cancer will be referred to and treated in an expert center.

All patients with pre-operatively diagnosed GBC from January 2000 – September 2018 who underwent surgical exploration were included. Patients with GBC diagnosed during or after cholecystectomy for presumed benign disease (incidental GBC) were excluded from analysis. Data on patient medical history, pre-operative imaging and laboratory results, operative characteristics, histopathological characteristics, post-operative morbidity, mortality and recurrence were obtained from the medical records, which were available for all included patients. Data on follow-up was obtained through linkage with the automated Municipal Personal Records Database and was last accessed at 1/3/2019. The study was approved by the Medical Ethics Committee of the region Arnhem-Nijmegen (number 2017-3912).

## VARIABLE AND SUBGROUP DEFINITIONS

Patients were classified as jaundiced when yellow pigmentation of the skin or sclera was present at primary presentation or when serum bilirubin was ≥30 μmol/L. [11] Performance status was assessed using the ASA Physical Status Classification System. [12] All laboratory values were reported in μmol/L. Postoperative complications were classified according to the Clavien-Dindo Classification System and included complications up to 90-days after surgery. [13] Major complications were defined as Clavien-Dindo grade ≥ 3A. Post-operative mortality was defined as death due to any cause <90 days postoperatively. Radical (R0) resection was defined as distance margin to tumor ≥ 1mm. TNM staging was reported according to the American Joint Committee on Cancer (AJCC) staging system, 7$^{th}$ edition. [14] Conversion to the 8$^{th}$ edition was not possible since the location of the tumor since the location of the tumor (i.e. on the liver- or peritoneal side of the gallbladder) was frequently unknown. Early GBC was defined as AJCC stage ≤II and late stage GBC was defined as AJCC ≥IIIA. Overall survival (OS) was defined as number of days between date of surgical exploration and death from any cause. Long-term survival was defined as survival beyond two years.

### STATISTICAL ANALYSIS

Categorical variables were reported as counts with percentages and continuous variables as median values with corresponding Interquartile Ranges (IQR). Differences in baseline variables were assessed using the student's *t*-test, Mann-Whitney-U test, Chi-Squared test or Fisher's exact test. Overall survival was analyzed using the Kaplan-Meier method. Differences in OS between jaundiced and non-jaundiced patients were analyzed across the entire cohort and in the subgroup of resected patients. Differences in survival between subgroups (N0 vs. N1) were assessed using log-rank testing. Multivariable Cox regression analysis with backward elimination was used to identify prognostic factors. Included potential prognostic factors were those with p-values < 0.10 in univariable analysis or those known from literature. Missing values were presumed to be not missing at random (related to the value itself, potentially related to the outcome). Imputation was not conduced since it may result in biased estimates and/or overestimation of test statistics. [15] Results were reported as hazard ratio (HR) with 95% confidence interval (CI). All analyses were conducted using SPSS Statistics for Windows, version 25.0 (IBM Corporation, Armonk, NY, USA). P-values ≤ 0.05 were considered statistically significant.



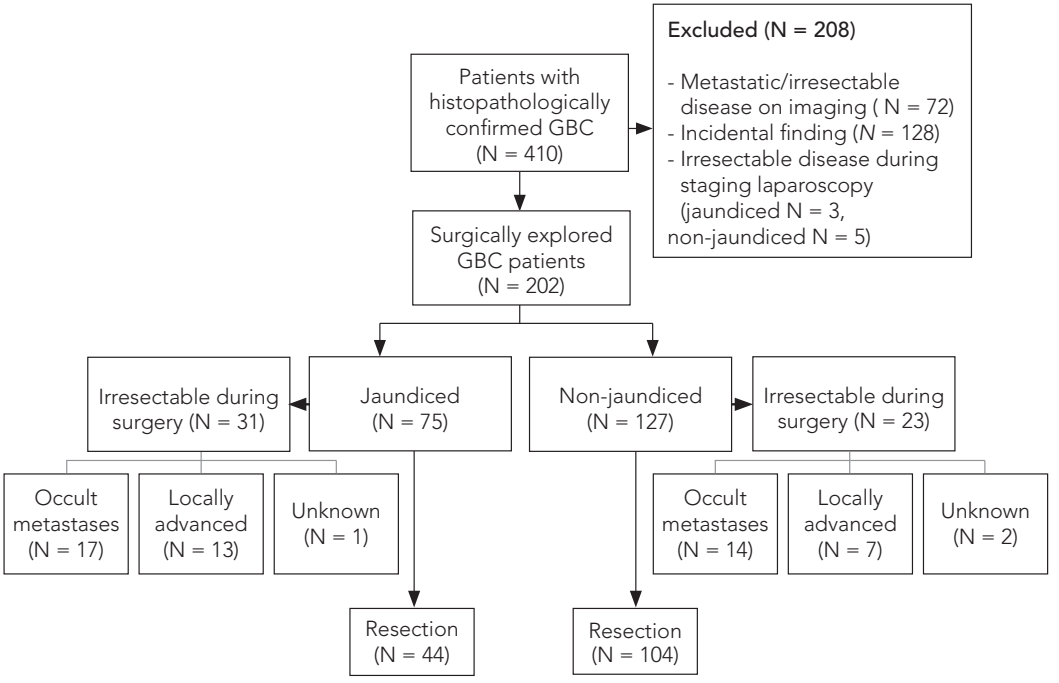
## RESULTS

### PATIENT INCLUSION AND BASELINE CHARACTERISTICS

A total of 410 patients with GBC was identified through the NCR. Of those, 208 patients were excluded; 128 patients had incidental GBC and 80 did not undergo surgical exploration (Figure 1).

The cohort consisted of 202 patients who underwent surgical exploration; 127 non-jaundiced patients (63%) and 75 (37%) jaundiced patients. Patient demographics and medical history were comparable in both groups (Table 1). Jaundiced patients presented more frequently with weight loss (36% vs. 14%, P<0.001). Median bilirubin at presentation was 9 (IQR 5-12) µmol/L in non-jaundiced patients and 170 (IQR 88-229) µmol/L in jaundiced patients. Tumor location on imaging was significantly different between groups; non-jaundiced patients more frequently presented with a tumor in the gallbladder fundus/corpus (38% vs. jaundiced 13%) whilst jaundiced patients more frequently had a tumor in neck of the gallbladder (16% vs. non-jaundiced 8%) (P=0.003).

**Figure 1.** Patient inclusion flow diagram.




## (PRE-)OPERATIVE TREATMENT

Pre-operative biliary drainage was performed in 76% of patients who presented with jaundice (Table 2). Of non-jaundiced patients at time of presentation, 13 (10%) underwent pre-operative biliary drainage due to; development of jaundice after presentation (N=6), cholangitis (N=2) and diagnostic purposes (N=5). In 54 (27%) patients unresectable or metastatic disease was discovered during surgical exploration and no resection was performed. Jaundiced patients had a higher rate of unresectable disease during surgical exploration (N = 31/75, 41%) compared to non-jaundiced patients (N= 23/124, 18%, P<0.001).

A curative-intent resection was performed in 44 (59%) jaundiced and 104 (82%) non-jaundiced patients. Jaundiced patients had a higher rate of extended (≥ 3 segments) liver resection (14/44 vs. 7/104, P<0.001), vascular reconstruction (8/44 vs. 3/104, P=0.003), common bile duct (CBD) resection (30/44 vs. 11/104, P<0.001) and peri-operative complications (6/44 vs. 3/104, P=0.043). Adjuvant chemotherapy (capecitabine) was administered to one non-jaundiced patient. None of the jaundiced patients received (neo)adjuvant therapy.

Table 1. Baseline patient- and tumor characteristics.

| Characteristic | Non-jaundiced (N = 127) | Jaundiced (N = 75) | P value |
|---|---|---|---|
| Gender (male) | 48 (38%) | 33 (40%) | 0.458 |
| Age (years) | 65 (57 - 74) | 66 (60 - 76) | 0.818 |
| Medical history | | | |
| ASA >2 | 34 (28%) | 15 (20%) | 0.240 |
| Previous malignancy | 31 (24%) | 10 (13%) | 0.071 |
| Gallbladder polyp | 8 (6%) | 3 (4%) | 0.750 |
| Cholecystitis | 8 (6%) | 2 (3%) | 0.328 |
| Cholelithiasis | 29 (23%) | 12 (16%) | 0.280 |
| Presenting symptoms | | | |
| Nausea / vomiting | 32 (25%) | 24 (32%) | 0.331 |
| Abdominal pain | 74 (58%) | 39 (52%) | 0.463 |
| **Weight loss** | **18 (14%)** | **27 (36%)** | **<0.001** |
| **Bilirubin [1]** | **9 (5 - 12)** | **170 (88 – 229)** | **<0.001** |
| CA 19.9 [2] | 57 (14 - 328) | 152 (59 - 1951) | 0.216 |
| T stage (imaging) | | | |
| <T3 | 25 (19%) | 8 (24%) | |
| T3/T4 | 31 (23%) | 25 (45%) | 0.070 |
| Missing | 71 (56%) | 42 (56%) | |
| Gallstones (imaging) | 8 (6%) | 6 (8%) | 0.764 |
| Liver invasion (imaging) | 35 (28%) | 27 (36%) | 0.262 |
| N1/2 stage (imaging) | 31 (24%) | 26 (35%) | 0.141 |
| M1 stage (imaging) | 8 (6%) | 4 (5%) | 0.326 |
| Tumor location (imaging) | | | |
| **Fundus / corpus** | **48 (38%)** | **10 (13%)** | |
| **Neck** | **10 (8%)** | **12 (16%)** | **0.003** |
| **Diffuse** | **20 (16%)** | **17 (23%)** | |
| Unreported | 49 (39%) | 36 (48%) | |

[1] Reported in 60/127 non-jaundiced patients and 60/75 jaundiced patients. [2] Reported in 36/127 non-jaundiced patients and 34/75 jaundiced patients.

Table 2. Surgical procedures and complications.

| All patients (N=202) | Non-jaundiced (N = 127) | Jaundiced (N = 75) | P-value |
|---|---|---|---|
| Pre-operative therapy | | | |
| No | 113 (89%) | 18 (24%) | <0.001 |
| ERCP | 12 (11%) | 51 (68%) | |
| PTC | 1 (1%) | 0 (0%) | |
| ERCP + PTC | 0 (0%) | 7 (9%) | |
| PVE | 1 (0%) | 5 (7%) | |
| Diagnostic laparoscopy (yes) | 19 (15%) | 14 (20%) | 0.437 |
| Non-resectable during surgery | 23 (18%) | 31 (41%) | <0.001 |
| **Resected patients (N=148)** | **Non-jaundiced (N=104)** | **Jaundiced (N = 44)** | |
| Hepatic resection | | | |
| Minor (≤2 segments) | 39 (38%) | 16 (36%) | <0.001 |
| Major (≥3 segments) | 7 (7%) | 14 (32%) | |
| Vascular reconstruction | 3 (3%) | 8 (18%) | 0.003 |
| CBD resection | 11 (11%) | 30 (68%) | <0.001 |
| Other organ resection [1] | 12 (11%) | 17 (39%) | 0.001 |
| Perioperative complications [2] | 3 (3%) | 6 (14%) | 0.043 |
| Postoperative complications CD≥3A | 16 (15%) | 18 (41%) | 0.001 |

PVE: Portal Vein Embolization. [1] Includes head of pancreas (jaundice = 11, no jaundice = 3), duodenum (jaundice = 10, no jaundice = 6), colon (jaundice = 2, no jaundice = 4) and other (jaundice = 5, no jaundice =4). [2] Includes bleeding (jaundice = 7, no jaundice = 2), bowel injury (jaundice = 0, no jaundice = 1) and SIRS reaction (jaundice = 1, no jaundice = 0).

## MORBIDITY AND MORTALITY IN PATIENTS WITH RESECTION WITH CURATIVE INTENT

In all patients who received resection with curative intent, jaundiced patients (N=44) had a higher rate of major (Clavien-Dindo grade ≥ 3A) complications after resection compared to non-jaundiced (104) patients (18/44 vs. 16/104, P<0.001). In patients who underwent extended surgery (defined as resection of ≥3 liver segments or resection of adjacent organs) no differences in major complications occurred when comparing jaundiced (13/27) to non-jaundiced patients (9/18) (P=0.852). Multivariable analysis including pre-operative T-stage, ASA classification, extent of resection, CBD resection (yes/no) and presence of jaundice showed that only resection of the CBD was an

independent predictor for major complications (HR 26.51, 95%CI 2.94-239.02, P=0.003). Six patients died within 90 days postoperatively due to surgical complications; two due to liver failure, two due to leakage of the hepatoduodenal anastomosis and subsequent sepsis and multi-organ failure, one due to post-operative hemorrhage and one due to aspiration pneumonia. All postoperative mortality occurred in jaundiced patients (6/44 vs. 0/104, P<0.001). In all deceased patients the CBD was resected. Two patients underwent a right hemihepatectomy, one received a radical cholecystectomy with CBD resection, one received a right hepatectomy with adjacent colon resection, one underwent a Whipple's procedure and one patient underwent a Whipple's procedure with portal vein reconstruction.

## HISTOPATHOLOGY OF RESECTED PATIENTS

Jaundice was associated with higher pT-stage (T3/T4 in 31/44 vs. 28/104, P<0.001) and a higher rate of R1/R2 resection (25/44 vs. 28/104, P=0.001) (Figure 2). Histopathological characteristics also differed significantly between jaundiced and non-jaundiced patients; jaundiced patients more frequently had a tumor located in the neck (9/44 vs. 12/104, P=0.001), a diffuse tumor (18/44 vs. 17/104, P=0.001), perineural invasion (24/44 vs. 30/104, P=0.005), liver invasion (20/44 vs. 21/104, P=0.003) and CBD invasion (25/44 vs. 5/104, P<0.001).



## SURVIVAL OF ALL SURGICALLY EXPLORED GBC PATIENTS

Median OS was 15.9 months (95% CI 11.2 – 20.6). Median OS of jaundiced patients was 7.7 months and significantly worse than the median OS of 26.1 months in non-jaundiced patients (Figure 3A, 3-year survival 14% vs. 42%, P<0.001).

## SURVIVAL OF RESECTED PATIENTS

In resected patients (N=148), median OS in jaundiced patients was 16.7 months vs. 36.4 months in non-jaundiced patients (P<0.001, Figure 3B). In the subgroup of patients who underwent an extended resection (≥3 liver segments or adjacent organ resection), jaundiced patients also showed significantly reduced survival compared to non-jaundiced patients (16 vs. 26 months, P = 0.010). When analyzing survival according to pN status, jaundice was associated with worse median OS in pN0 patients (jaundiced 18.9 vs. non-jaundiced 112.5 months, P<0.001), but not in pN1 patients (jaundiced 10.4 vs. non jaundiced 14.8 months, P=0.365). In patients who received an R0 resection, survival was worse in jaundiced compared to non-jaundiced patients (23.6 months vs. 104 months, P=0.001). In patients with an R1 resection, no differences in survival were found between jaundiced and non-jaundiced patients (13.7 vs. 8.2 months, P=0.888).

**Figure 2.** Comparison of histopathological characteristics of jaundiced vs. non-jaundiced patients that underwent resection. * Chi squared P-values <0.05.

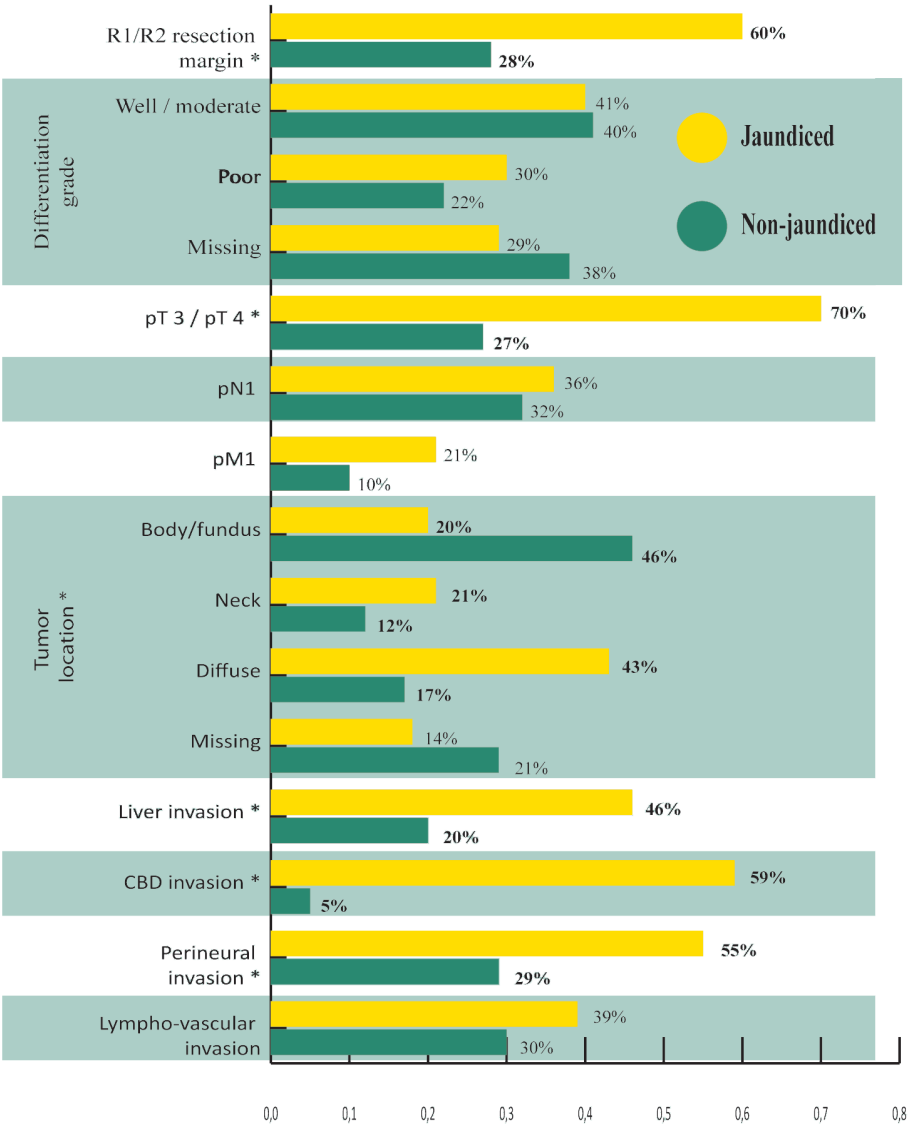
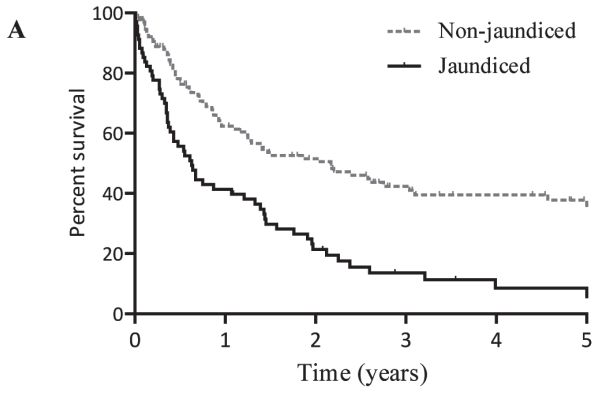
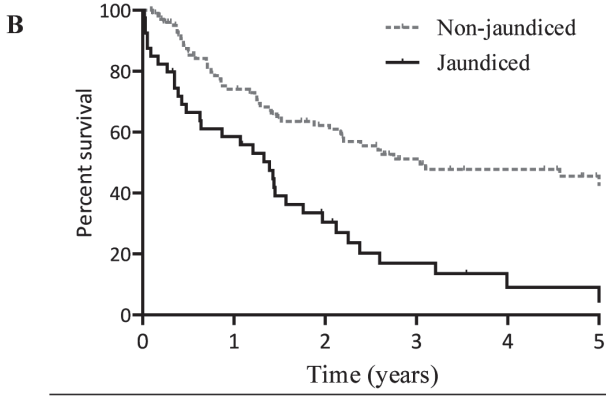


Figure 3. A: Survival of all jaundiced vs. all non-jaundiced patients. Log rank <0.001. B: Survival of resected jaundiced vs. resected non-jaundiced patients. Log rank <0.001.




| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Non jaundiced | N | 127 | 68 | 49 | 32 | 25 | 19 |
| Jaundiced | N | 75 | 27 | 13 | - | - | - |




| | | | | | | |
|---|---|---|---|---|---|---|
| N | 104 | 67 | 49 | 32 | 25 | 19 |
| N | 44 | 23 | 12 | 6 | - | - |

## PROGNOSTIC FACTORS FOR OS IN SURGICALLY EXPLORED PATIENTS

Age, jaundice, weight loss, T3/T4 disease on imaging and a diffuse tumor or a tumor located in the gallbladder neck on imaging were all associated with a poor prognosis on univariate analysis in all surgically explored patients (N = 202, Supplementary Table 1A). In multivariable Cox regression analysis, jaundice (HR 1.89, 95% CI 1.01–3.53, P=0.049) and T3/T4 disease on imaging (HR 2.02, 95% CI 1.03 – 3.97, P=0.041) remained significant poor prognostic factors for OS.

## PROGNOSTIC FACTORS FOR OS IN RESECTED PATIENTS

Jaundice, extent of resection (non extended vs. extended resection), pT-stage (pT1/2 vs. pT 3/4), pN-stage, CBD-invasion, perineural invasion, liver invasion and resection margin were predictive for prognosis on univariate analysis in resected patients (N = 148, Supplementary Table 1B). In multivariate analysis, only pN1 stage (HR 1.84, 95%CI 1.08 – 3.12, P=0.025), liver invasion (HR 2.23, 95% CI 1.29 – 3.88, P=0.004) and R1 resection (HR 2.76, 95% CI 1.58 – 4.84, P<0.001) were significant poor prognostic factors for OS. pT3/4 stage was borderline significant (HR 1.95, 95% CI 0.99 – 3.81, P=0.055). In multivariate regression analyses excluding all patients with peri-operative mortality <90 days (data not shown) and in patients without gallstones on imaging (supplementary table 1C) jaundice was also not a significant prognostic factor for survival.

## CHARACTERISTICS OF JAUNDICED, LONG-TERM SURVIVORS.

After resection, 12/44 (27%) jaundiced patients lived beyond two years; their characteristics are summarized in Table 3. Five patients died due to tumor progression after a mean follow-up of 42 months. One patient died due to complications related to end-stage liver cirrhosis and PSC, one patient died due to another malignancy, one patient died from postsurgical liver failure after extended right hemihepatectomy for a recurrence. Four patients were still alive at the time this study was conducted. Notably, all patients who remained free of recurrence (N=6) had a tumor located in the body or the fundus of the gallbladder and showed no liver invasion on pre-operative imaging. Additionally, five out of six patients had a pT1b or pT2 tumor and four patients did not have any perineural or lymphovascular invasion. Gallstones were present in three out of six patients without a recurrence. CBD invasion was not present in any of the patients without a recurrence.

Table 3. Characteristics of long-term (≥2 years) survivors of patients with GBC and jaundice.

| Age, gender, ASA | Ca 19.9 | Gallstones, imaging | Liver / lymph node invasion, imaging | Tumor location, imaging | Procedure | pTNM | Resection margin, grade, PNVI | Organ invasion | Follow-up (months) | Vital status (+COD) |
|---|---|---|---|---|---|---|---|---|---|---|
| 64, F, ASA 2 | - | Yes | Yes (regional lymph nodes) | Unspecified | Right hepatectomy | T2 N0 Mx | R1, GR x, PNVI+ | None | 25 | Alive without recurrence |
| 81, F, ASA 2 | - | Yes | No | Unspecified | Classic cholecystectomy | T3 Nx Mx | Rx, GR 2, PNVI - | None | 25 | Dead, tumor progression |
| 68, M, ASA 1 | - | Yes | No | Unspecified | Radical cholecystectomy + Extended right hemihepatectomy due to recurrence (26 months later) | T2 N0 M0 | R0, GR 2, PNVI - | None | 26 | Dead, liver failure after extended right hemi hepatectomy |
| 79, F, ASA 2 | - | Yes | Yes (liver) | Diffuse | Radical cholecystectomy, bile duct excision, Whipple | T2 N1 M0 | R1, GR 2, PNVI + | None | 29 | Dead, tumor progression |
| 62, F, ASA 1 | 234 | Yes | Yes (regional lymph nodes) | Diffuse | Radical cholecystectomy and bile duct excision | T4 N1 M0 | R0, GR 2, PNVI + | None | 31 | Dead, other malignancy |
| 63, M, ASA 2 | - | No | No | Unspecified | Whipple / PPPD | T4 N1 M0 | R1, GR 1, PNVI + | Pancreas | 38 | Dead, tumor progression |
| 82, F, ASA 2 | - | No | No | Corpus | Classic cholecystectomy | T2 N0 M0 | R0, GR 2, PNVI - | None | 42 | Alive without disease |
| 46, M, | 90 | No | No | Diffuse | Right | T3 N1 | R0, GR 2, | Extrahepatic | 48 | Dead, tumor |

**Table 3.** Characteristics of long-term (≥2 years) survivors of patients with GBC and jaundice.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 48, M, ASA 2 | 70 | No | No | Diffuse | Right hemihepatectomy, bile duct excision and PPPD | T3 N1 M0 | R0, GR 2, PNVI + | Extrahepatic bile duct | 45 | Dead, tumor progression |
| 77, F, ASA 2 | - | No | No | Unspecified | Radical cholecystectomy | T2 N0 M0 | R0, GR 1, PNVI - | None | 53 | Alive without recurrence |
| 69, F, ASA 3 | 28 | No | No | Unspecified | Right hemihepatectomy, bile duct excision and PPPD | T4 N1 M1 | R1, GR 1, PNVI + | Pancreas | 70 | Dead, tumor progression |
| 60, M, ASA 3 | - | No | No | Fundus | Cholecystectomy, local excision bile ducts | T1B N0 M0 | R0, GR x, PNVI - | None | 100 | Dead, liver cirrhosis (PSC) |
| 62, M, ASA 2 | - | No | No | Fundus | Classic cholecystectomy | T2 NX M0 | R0, GR 2, PNVI - | None | 166 | Alive without recurrence |

GR = tumor differentiation grade. 1 = good differentiation, 2 = moderate differentiation, 3 = poor differentiation, x = missing differentiation.
PNVI = Perineural or lymphovascular invasion.

# DISCUSSION

Jaundice as a presenting symptom for GBC is significantly associated with irresectability or extensive surgery. In resected GBC patients, jaundice is associated with significant postoperative morbidity, mortality and poor histopathological features. Median OS in jaundiced patients was 7.7 months versus 26.1 months in non-jaundiced patients. Pre-operative jaundice was significantly correlated with poor OS in surgically explored patients. However, multivariable analysis showed that jaundice was not an independent predictor of poor outcome in patients who underwent resection when adjusting for N status, liver invasion and resection margin. All jaundiced patients who remained free of recurrence had a tumor located outside of the gallbladder neck and did not show liver invasion on pre-operative imaging. The present study is the first to assess imaging characteristics of GBC long-term survivors who presented with jaundice.

Previous studies investigating surgery in jaundiced GBC patients report dismal median survival and virtually no survival beyond two years.[7, 16-18] In the present series median OS was 7.7 months and 12/44 resected jaundiced patients survived beyond two years. Major postoperative complications occurred in 41% of jaundiced patients in our study, which is less than other studies; complication rates in literature range from 52% to 83%.[9, 11, 19-24] These results support the notion that long-term survival in jaundiced patients is achievable after surgical resection.



In the present study, jaundice was no longer significantly associated with poor survival in resected patients in multivariate analysis. This finding implies that jaundice in itself does not preclude resection. Rather, jaundice in GBC is indicative of advanced disease. When considering surgery one should be aware of the limited survival benefit of surgery for advanced GBC in general. [25]

Multiple explanations arise for the correlation between jaundice, advanced GBC and poor survival. Tumors growing in the neck of the gallbladder rapidly result in obstructive jaundice by either direct invasion in the hepatic hilum or compression of the CBD. In contrast, tumors arising from the fundus or corpus of the gallbladder are more likely to invade the hepatic parenchyma. Gallbladder neck tumors frequently require an (extended) hemihepatectomy to obtain tumor-free surgical margins, whereas fundus or corpus type tumors may be entirely resected by a non-anatomical wedge resection. The difference in morbidity and mortality between extended resections and wedge resections may account for the difference in survival between jaundiced and non-jaundiced patients.

Another explanation may be that jaundiced patients represent a biologically more aggressive subset of tumors. In our cohort jaundiced patients showed higher rates of perineural and hepatic invasion; known poor prognostic factors. [16, 26] Additionally, malignant biliary obstruction and hyperbilirubinemia are associated with increased risks in liver surgery due to hepatic and systemic inflammation . [27]

Although patient selection appears to be key when considering surgery in jaundiced patients, no reported pre-operative selection criteria are definitive. In our cohort, resected patients with jaundice who did not develop recurrent disease did not show liver invasion on pre-operative imaging and did not have a tumor located in the gallbladder neck. All of these patients had R0 resection margins and did not have lymph node metastases. In case jaundiced patients do not show liver involvement on pre-operative imaging, have a tumor that is confined to the fundus or corpus of the gallbladder and no lymph node metastases are detected, surgery may provide favorable survival outcomes.

In addition to pre-operative imaging, staging laparoscopy (SL) is a helpful tool for pre-operative evaluation and patient selection. [28] Although 41% of jaundiced patients had unresectable disease during surgical exploration, only 20% underwent SL. SL could have potentially prevented several futile laparotomies and should be recommended in jaundiced GBC patients.

This study has several limitations that need to be considered when interpreting results. First and foremost, all pitfalls of retrospective data are applicable to this study; selection bias may significantly influence our results. Propensity score matching to adjust for bias was attempted but the number of available matches was too low to draw any conclusions. Additionally, many pre-operative characteristics potentially associated with prognosis could not be investigated due to missing data. Imputation would not have been feasible since these data were most likely not missing at random. Finally, in some included patients with a small (T1/T2) tumor, jaundice may not have been caused by CBD invasion but rather by proximity of the tumor to the CBD, nodal metastases or even Mirizzi syndrome and inflammation. Because it is difficult to identify the cause of jaundice retrospectively, we chose to include all jaundiced patients as this an accurate reflection of clinical practice.

Strengths of this study include the nation-wide design. Previous studies stem from high-volume single center experiences or expert center collaborations. Our results

reflect actual, nation-wide outcomes in a low incidence country with per center less experience in the treatment of GBC and generalizability is likely high. Additionally, patients in whom unresectable disease was discovered during surgical exploration were included in our survival analysis. Some studies excluded these patients from analysis since they did not undergo resection. [9] However, we feel that the inclusion of these patients provides a more realistic reflection of the median survival of all patients with jaundice and GBC. Analyzing only resected patients may induce treatment-selection bias because patients with smaller tumors (and thus a more favorable prognosis) are more likely to receive a resection.

In summary, jaundice primarily indicates the presence of advanced GBC; a disease for which the benefit of resection is questionable in general. Moreover, jaundice is associated with increased postoperative morbidity, mortality and poor overall survival. In cases with limited disease, a tumor located outside of the gallbladder neck and no lymph node metastases on pre-operative imaging, long-term survival may be achieved. When considering surgery for patients with jaundice, careful pre-operative evaluation is required. Multiple pathways may result in biliary obstruction and not all these mechanisms are directly associated with the presence of unresectable disease. Identifying the root cause of jaundice is key when selecting patients for surgery.

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**Supplementary Table 1A.** Prognostic value for survival of pre-operative characteristics of patients with GBC that underwent surgical exploration (N=202).

| | Univariable analysis | | | Multivariable analysis | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | P value | HR | 95% CI | P value |
| CA 19.9 [a] | 1 | 1 - 1 | 1.000 | | | |
| Lymph node invasion, imaging | 1.70 | 1.01 – 2.85 | **0.048** | [c] | | |
| Liver invasion, imaging | 1.27 | 0.86 – 1.87 | 0.232 | | | |
| **T stage 3/4 imaging** | **2.09** | **1.13 – 3.99** | **0.019** | **2.02** | **1.03 – 3.97** | **0.041** |
| **Age (years)** | 1.02 | 1.01 – 1.04 | **0.008** | [c] | | |
| Weight loss | 1.69 | 1.15 – 2.48 | **0.008** | [c] | | |
| **Jaundice** | **2.24** | **1.58 – 3.18** | **<0.001** | **1.89** | **1.01 – 3.53** | **0.049** |
| ASA > 2 | 1.21 | 0.81 – 1.80 | 0.343 | | | |
| Tumor location imaging[b] | | | | | | |
| Fundus/Body | 1 | | | *** | | |
| Neck | 2.30 | 1.19 – 4.43 | **0.013** | | | |
| Diffuse | 2.12 | 1.24 – 3.63 | **0.006** | | | |

**Supplementary Table 1B.** Prognostic value for survival of surgical and histopathological characteristics of patients with GBC that underwent resection (N = 148).

| | Univariable analysis | | | Multivariable analysis | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | P value | HR | 95% CI | P value |
| Jaundice | 2.78 | 1.78 – 4.35 | <0.001 | [a] | | |
| Extended resection | 1.78 | 1.15 – 2.75 | <0.001 | [a] | | |
| pT | | | | | | |
| 1/2 | 1 | | | 1 | | |
| 3/4 | 5.76 | 3.70 – 8.99 | <0.001 | 1.94 | 0.99 – 3.81 | 0.055 |
| pN | | | | | | |
| N0 | 1 | | | 1 | | |
| N1 | 2.80 | 1.68 – 4.69 | <0.001 | 1.84 | 1.08 – 3.12 | 0.025 |
| Nx | 1.83 | 1.03 – 3.25 | 0.039 | 2.10 | 1.15 – 3.83 | 0.016 |
| CBD invasion | 3.02 | 1.89 – 4.84 | <0.001 | [a] | | |
| Liver invasion | 4.02 | 2.56 – 6.30 | <0.001 | 2.23 | 1.29 – 3.88 | 0.004 |
| Perineural invasion | 2.54 | 1.64 – 3.93 | <0.001 | [a] | | |
| R1 resection | 4.92 | 3.08 – 7.89 | <0.001 | 2.76 | 1.58 – 4.84 | <0.001 |

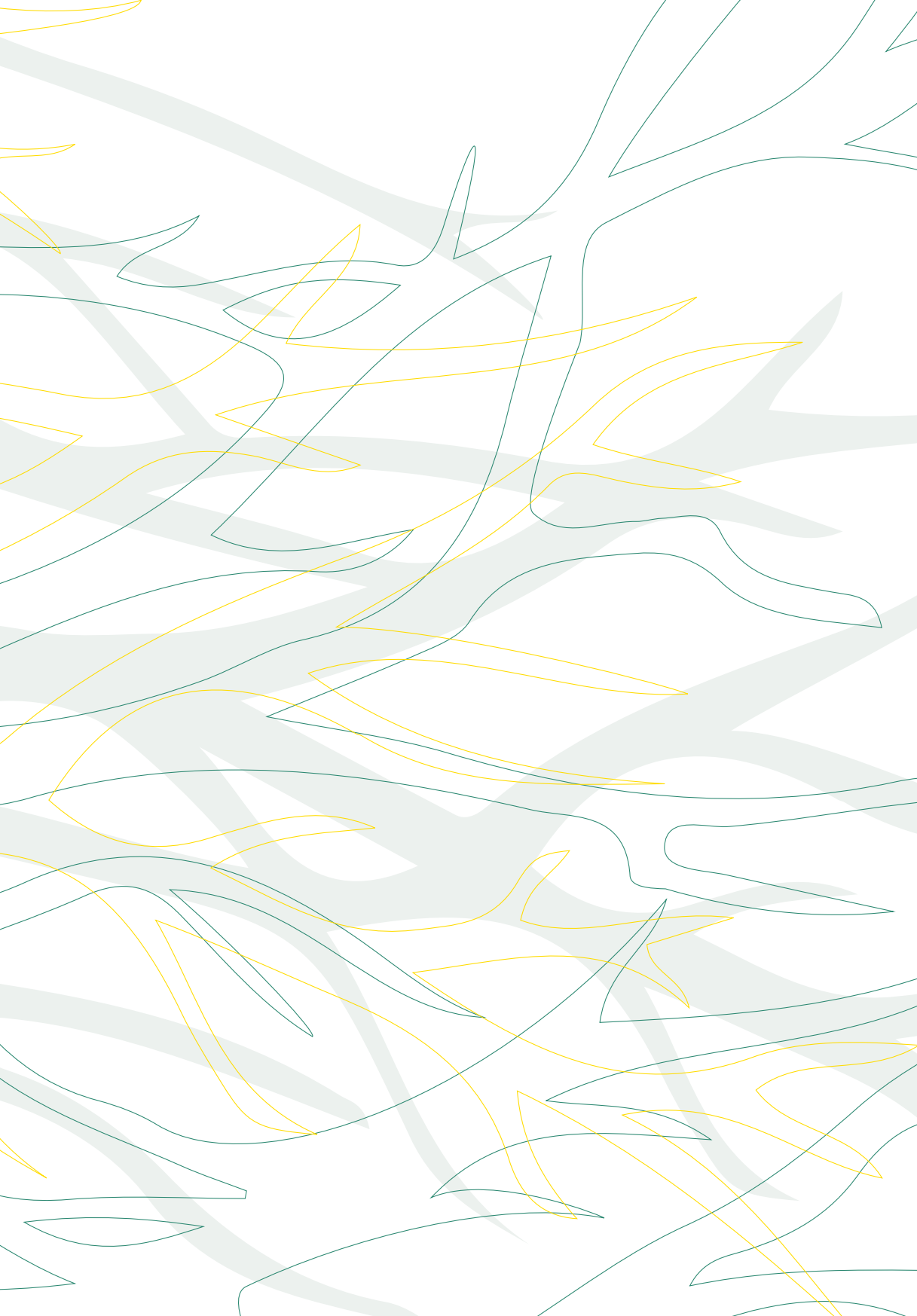
[a] Removed in backward selection.

**Supplementary Table 1C.** Prognostic value for survival of surgical and histopathological characteristics of GBC patients without gallstones on imaging that underwent resection (N = 134).

| | Univariable analysis | | | Multivariable analysis | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | P value | HR | 95% CI | P value |
| Jaundice | 4.84 | 2.19 – 10.67 | **<0.001** | [a] | | |
| Extended resection | 1.20 | 0.95 – 4.20 | **0.069** | | | |
| pT | | | | | | |
| 1/2 | 1 | | | [a] | | |
| 3/4 | 7.77 | 3.50 – 17.23 | **<0.001** | [a] | | |
| pN | | | | | | |
| N0 | 1 | | | [a] | | |
| N1 | 4.83 | 1.93 – 12.11 | **0.001** | [a] | | |
| Nx | 2.06 | 0.69 – 6.14 | 0.196 | [a] | | |
| CBD invasion | 4.61 | 2.18 – 9.75 | **<0.001** | [a] | | |
| **Liver invasion** | **9.52** | **4.26 – 21.26** | **<0.001** | **8.43** | **3.49 – 20.38** | **<0.001** |
| Perineural invasion | 2.54 | 1.64 – 3.93 | **<0.001** | [a] | | |
| **R1 resection** | **5.65** | **2.60 – 12.28** | **<0.001** | **4.49** | **1.95 – 10.34** | **<0.001** |

[*] Removed in backward selection.

# 6



CHAPTER 7

# Adjuvant treatment for resected biliary tract cancer: a SEER-Medicare analysis

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*Submitted*

# ABSTRACT


## BACKGROUND

In patients with resected biliary tract cancer (BTC), the role of adjuvant chemotherapy (aCT) remains ill-defined. This study evaluates the value of aCT for BTC and assesses response according to tumor stage.

## METHODS

Patients with resected BTC diagnosed from 2004-2015 were identified using the Surveillance, Epidemiology and End Results (SEER)/Medicare linked database. After propensity score matching, survival of patients treated with aCT was compared to survival of patients who did not receive aCT using Kaplan-Meier and Cox proportional hazards analysis.

## RESULTS

Of 3511 patients identified with resected BTC, 876 (25%) received aCT. In the full cohort of 1554 propensity-score matched patients, survival did not differ between patients treated with aCT (24.3 months) and without aCT (24.2 months, P=0.486). Subgroup analysis showed that survival was significantly better after aCT in T3/T4 disease (18.5 vs. 12.4 months, P<0.001 and node-positive disease (aCT 20.5 vs. no aCT 13.3 months, P<0.001). Interaction analysis showed that benefit of aCT was primarily seen in combined T3/T4, node-positive disease (HR 0.56,P<0.001).

## CONCLUSIONS

In this large cohort of patients with resected BTC, aCT was not associated with increased survival. However, aCT does seem to provide a survival benefit in patients with T3/4 tumors or node-positive disease.

# INTRODUCTION

Biliary tract cancer (BTC) has an extremely poor prognosis.[1-3] In resected patients, recurrence rates are as high as 65%, and 5-year overall survival (OS) is only 15-30%.[4-6] Initial recurrence after resection is often locoregional, but distant relapse in the form of liver spread also occurs frequently.[6, 7] Adjuvant chemotherapy (aCT) could hypothetically grant a significant survival benefit by providing both locoregional and distant control.

Unfortunately, high-quality evidence supporting the benefit of aCT in BTC is sparse.[8, 9] Only the BILCAP trial (adjuvant capecitabine vs. observation alone) showed a statistically significant increase in survival in the per-protocol analysis alone (53 vs. 36 months, P=0·028). The primary, intention-to-treat analysis did not show significant survival differences between treatment groups.[7, 10, 11] Some have argued that the lack of apparent efficacy may be because aCT is only effective in patients with poor prognostic factors such as node-positive and R1 disease, a subgroup which was highly represented in the BILCAP trial compared to other RCTs.[7] Identification of subgroup-specific effects in these RCTs is limited by statistical power as these effects are usually not taken into consideration when calculating the sample size of the study.

Population-based data provide an opportunity to analyze treatment and survival in a large number of patients and help overcome the challenge of small numbers that accompanies research focused on rare cancers. Although observational studies are subject to treatment selection bias, they have the advantage of large sample sizes that allow subgroup-specific effects to be estimated. Statistical methods such as propensity score matching can help to reduce the influence of treatment selection bias on results.[12] We describe a propensity score-matched analysis of data from the SEER (Surveillance, Epidemiology and End Results) registry. The objective of this study was to determine the association of aCT in the treatment of BTC with survival and to identify clinically relevant subgroups of patients that may benefit from aCT.



# METHODS

## DATA SOURCES AND STUDY DESIGN

The SEER (Surveillance- and Epidemiology End Results) program is a population-based database maintained by the National Cancer Institute (NCI), encompassing

approximately 34% of the population of the United States (https://seer.cancer.gov). Data provided by SEER can be supplemented by Medicare claims data in order to capture information not recoded by SEER, such as specific information on chemotherapy treatment. Medicare is a federally-funded health insurance program for citizens aged ≥65 years, with disabilities or end-stage renal disease. [13] The study protocol was approved by the University of Michigan Institutional Review Board and a waiver for informed consent was provided. This study was conducted in accordance with the declaration of Helsinki. This study was reported according to the STROBE guidelines. [14]

## STUDY POPULATION

The cohort was created using data from the 2018 SEER-Medicare release. The cohort was restricted to patients diagnosed from 2004 to 2015. Patients with resected, non-metastatic BTC (gallbladder cancer, intrahepatic, perihilar, and distal cholangiocarcinoma) were included using site and histology codes from the International Classification of Disease for Oncology, Third Edition (ICD-O-3). Only patients with non-metastatic disease who underwent resection of the primary tumor site were included. Patients with overlapping lesions or uncommon histologies were excluded (Supplementary Table 1). The cohort was limited to patients aged 65 years or older with Medicare part A and B coverage and no Health Maintenance Organization (HMO) enrollment during 12 months prior and 6 months after diagnosis (or until death) in order to assure completeness of Medicare claims.

## DEMOGRAPHIC AND CLINICOPATHOLOGIC VARIABLES

The following demographic variables were analyzed; age, race (White vs Black vs Asian/Pacific vs Alaskan/Native American), year of diagnosis, zip-code level percentage of residents with a high-school education (in quartiles), zip-code level median household income (in quartiles) and percentage living in poverty by zip-code. The number of Elixhauser comorbidities was derived from the outpatient and inpatient claims data. [15, 16]

Clinicopathologic characteristics included for analysis were tumor location, differentiation grade, nodal status (N0 vs. N+) and pT-stage. Tumor T, N and M stage were reported according to the 7$^{th}$ edition of the AJCC-staging manual. [17]

## SURGERY, CHEMOTHERAPY, AND RADIOTHERAPY TREATMENT IDENTIFICATION

The date of surgery was identified using Current Procedural Terminology (CPT) codes, Healthcare Common Procedure Coding System (HCPCS) codes, and International Classification of Diseases version 9 (ICD-9) procedure codes. Chemotherapy administration was identified using CPT codes, HCPCS codes and ICD-9 procedure codes. Oral equivalents of chemotherapeutic drugs were identified using National Drug Codes. Radiotherapy (RT) was identified using CPT and revenue center codes for radiation therapy. All codes are available upon request to the authors.

## ADJUVANT TREATMENT CLASSIFICATION

Patients were classified into two groups according to the initial treatment strategy; aCT versus no aCT. aCT was defined as a claim for chemotherapy within six months of surgery, similar to other studies. [18, 19] A single claim for chemotherapy was used to reflect that a patient had received aCT. Claims for radiation therapy spanning ≥7 days were used to reflect that a patient had received RT.

## STATISTICAL ANALYSIS

Categorical variables were reported as counts with percentages and compared using the chi-squared test or Fisher's exact test, where appropriate. Numeric variables were reported as means with ranges and compared using the student's T-test or Mann-Whitney U test, where appropriate. The primary outcome of all analyses was overall survival. For all survival analyses, patients whom died within 30 days of surgery were excluded in order to correct for immortal-time bias. Sensitivity analysis in patients who survived >6 months was conducted to further reduce treatment selection and survivor bias since they likely had poor performance status or significant postoperative complications, resulting in a poor prognosis and precluding them from receiving aCT. Survival was assessed using Kaplan-Meier curves and compared using the log-rank test.

Propensity-score matching (PSM) was used to adjust for treatment selection bias and compare overall survival (OS) of patients treated with aCT to that of patients with no aCT. The conditional probability of receiving chemotherapy (i.e. propensity score) was estimated using a multivariable logistic regression model including age, gender, education, median household income, Elixhauser score, tumor site, tumor size, tumor grade, pT/pN classification, tumor location and extent of lymph node resection. One-to-one nearest-neighbor PSM without replacement (caliper width 0.1) was then used to create a balanced cohort. Standardized mean differences were used to conduct

balance diagnostics; all had a value of <0.1, indicating good balance according to Austin et al. [12]

In the matched cohort, additional sensitivity analyses were conducted to investigate the association between N-classification and T-classification with treatment effect of aCT and survival. Cox-regression analysis modelling the interaction between N-classification, T-classification, differentiation grade, receipt of adjuvant chemotherapy and survival was conducted. To this end, patients were grouped according to pT/pN classification and differentiation grade. A model was composed using the combined stage/grade groups as an interaction term. A p-value of <0.05 was considered statistically significant. All analyses were performed using SAS/STAT software, version 9.4 of the SAS system for Windows (Copyright © 2013, SAS Institute Inc., Cary, NC, USA).

# RESULTS

## PATIENT CHARACTERISTICS

A total of 31.465 patients with BTC was identified, of whom 3.511 underwent resection and met the inclusion criteria (Figure 1, Table 1). Of these patients, 875 (25%) were treated with aCT. Median follow-up of patients alive at last follow-up was 49 months. Median age at diagnosis was 77 years, and most patients presented with AJCC T1/T2 (N= 2156, 61%) and N0 (N=2542, 72%) disease.

Patients who received aCT were younger and had a lower Elixhauser comorbidity score compared to patients that did not receive aCT. Patients that did receive aCT were diagnosed with more advanced tumors and more node-positive disease as opposed to patients that did not receive aCT. Adjuvant RT was more frequently administered in patients that received aCT compared to patients that did not receive aCT. After propensity score matching, 1.628 cases were matched: 814 patients with aCT and 814 patients who did not receive aCT. Baseline characteristics of the matched cohort did not differ significantly between patients with and without aCT except tumor grade (Table 1, Supplementary Table 2). Mean standardized difference in propensity score was 0.91 before matching and <0.1 across all variables after matching, indicating good balance (Supplementary Figure 1).

Table 1. Baseline characteristics of resected patients with BTC, 2004-2015.

| | Unmatched cohort | | | Matched cohort | | |
|---|---|---|---|---|---|---|
| Characteristic | No adjuvant therapy (N=2639, 75%) | Adjuvant chemotherapy (N=872, 25%) | P-value | No adjuvant chemotherapy (N=814) | Adjuvant chemotherapy (N=814) | P-value |
| Age | | | | | | |
| 65-70 | 391 (15%) | 217 (25%) | | 236 (29%) | 195 (24%) | |
| 70-75 | 525 (20%) | 280 (32%) | | 207 (25%) | 260 (32%) | |
| 75-80 | 621 (24%) | 218 (25%) | <0.001 | 202 (25%) | 212 (26%) | 0.983 |
| 80-84 | 586 (22%) | 111 (13%) | | 117 (14%) | 103 (13%) | |
| 85+ | 516 (20%) | 46 (5%) | | 52 (6%) | 44 (5%) | |
| Elixhauser comorbidity score | | | | | | |
| 0-2 | 644 (24%) | 272 (31%) | | 219 (27%) | 212 (26%) | |
| 3-4 | 690 (26%) | 227 (26%) | 0.002 | 250 (31%) | 254 (31%) | 0.924 |
| =>5 | 1305 (50%) | 373 (43%) | | 345 (42%) | 348 (43%) | |
| Gender, male | 1019 (39%) | 433 (50%) | <0.001 | 359 (44%) | 393 (48%) | 0.091 |
| Tumor site | | | | | | |
| Gallbladder | 1840 (70%) | 419 (48%) | | 423 (52%) | 404 (50%) | |
| Intrahepatic | 262 (10%) | 129 (15%) | <0.001 | 128 (16%) | 120 (15%) | 0.467 |
| Perihilar | 375 (14%) | 176 (20%) | | 155 (19%) | 162 (20%) | |
| Distal | 162 (6%) | 148 (17%) | | 108 (13%) | 128 (16%) | |
| Tumor size | | | | | | |
| ≤5 cm | 1480 (56%) | 544 (62%) | | 488 (60%) | 509 (63%) | |
| >5 cm | 350 (13%) | 139 (16%) | <0.001 | 133 (16%) | 129 (16%) | 0.526 |
| Unknown | 809 (31%) | 189 (22%) | | 193 (24%) | 176 (22%) | |
| pT stage | | | | | | |
| T1 | 653 (25%) | 115 (13%) | | 149 (18%) | 114 (14%) | |
| T2 | 1070 (41%) | 318 (36%) | | 285 (35%) | 314 (39%) | |
| T3 | 781 (30%) | 361 (41%) | <0.001 | 349 (43%) | 345 (42%) | 0.058 |
| T4 | 104 (4%) | 65 (7%) | | 31 (4%) | 41 (5%) | |
| Tx | 31 (1%) | 13 (2%) | | 0 (0%) | 0 (0%) | |
| Regional lymph node surgery | | | | | | |
| No nodes removed | 1356 (51%) | 272 (31%) | | 278 (34%) | 258 (31%) | |
| 1-3 nodes removed | 693 (26%) | 269 (31%) | <0.001 | 249 (30%) | 259 (32%) | 0.739 |
| 4+ nodes | 555 (21%) | 317 (36%) | | 284 (35%) | 294 (36%) | |
| Unknown | 35 (1%) | 14 (2%) | | 11 (1%) | 11 (1%) | |
| pN stage | | | | | | |
| N0 | 2056 (78%) | >480 (>55%) | | >485 (>60%) | >460 (>56%) | |
| N+ | 500 (19%) | 377 (43%) | <0.001 | 316 (39%) | 342 (42%) | 0.2563 |
| Nx | 83 (3%) | <11 (<1%) | | <11 (<1%) | <11 (<1%) | |

Table 1. Baseline characteristics of resected patients with BTC, 2004-2015.

| | Unmatched cohort | | | Matched cohort | | |
|---|---|---|---|---|---|---|
| Characteristic | No adjuvant therapy (N=2639, 75%) | Adjuvant chemotherapy (N=872, 25%) | P-value | No adjuvant chemotherapy (N=814) | Adjuvant chemotherapy (N=814) | P-value |
| **Differentiation grade** | | | | | | |
| Well | 434 (16%) | 93 (11%) | | 122 (15%) | 91 (11%) | |
| Moderate | 1145 (43%) | 363 (42%) | <0.001 | 361 (44%) | 343 (42%) | 0.0116 |
| Poor/ Undifferentiated | 820 (31%) | 348 (40%) | | 268 (33%) | 330 (40%) | |
| Unknown | 240 (9%) | 68 (8%) | | 63 (8%) | 58 (7%) | |
| **Type of chemotherapy** | | | | | | |
| Gemcitabine-based [a] | NA | 349 (40%) | | NA | 347 (43%) | |
| Gemcitabine + cisplatin based | NA | 139 (16%) | | NA | 117 (14%) | |
| 5-FU-based | NA | 313 (36%) | | NA | 279 (34%) | |
| Other | NA | 71 (8%) | | NA | 71 (9%) | |
| **Surgery** [b] | | | | | | |
| Cholecystectomy | 2194 (83%) | 650 (75%) | <0.001 | 606 (74%) | 613 (75%) | 0.6892 |
| Liver resection | 712 (27%) | 292 (33%) | <0.001 | 311 (38%) | 280 (34%) | 0.1101 |
| Bile duct resection | 454 (17%) | 240 (28%) | <0.001 | 197 (24%) | 226 (28%) | 0.1012 |
| PD/Whipple | 296 (11%) | 185 (21%) | <0.001 | 162 (20%) | 159 (20%) | 0.8518 |
| Not specified | 90 (3%) | 24 (3%) | 0.342 | 36 (4%) | 19 (2%) | 0.0197 |
| **Radiotherapy (yes)** [c] | 198 (8%) | 362 (41%) | <0.001 | 98 (12%) | 321 (39%) | <0.001 |

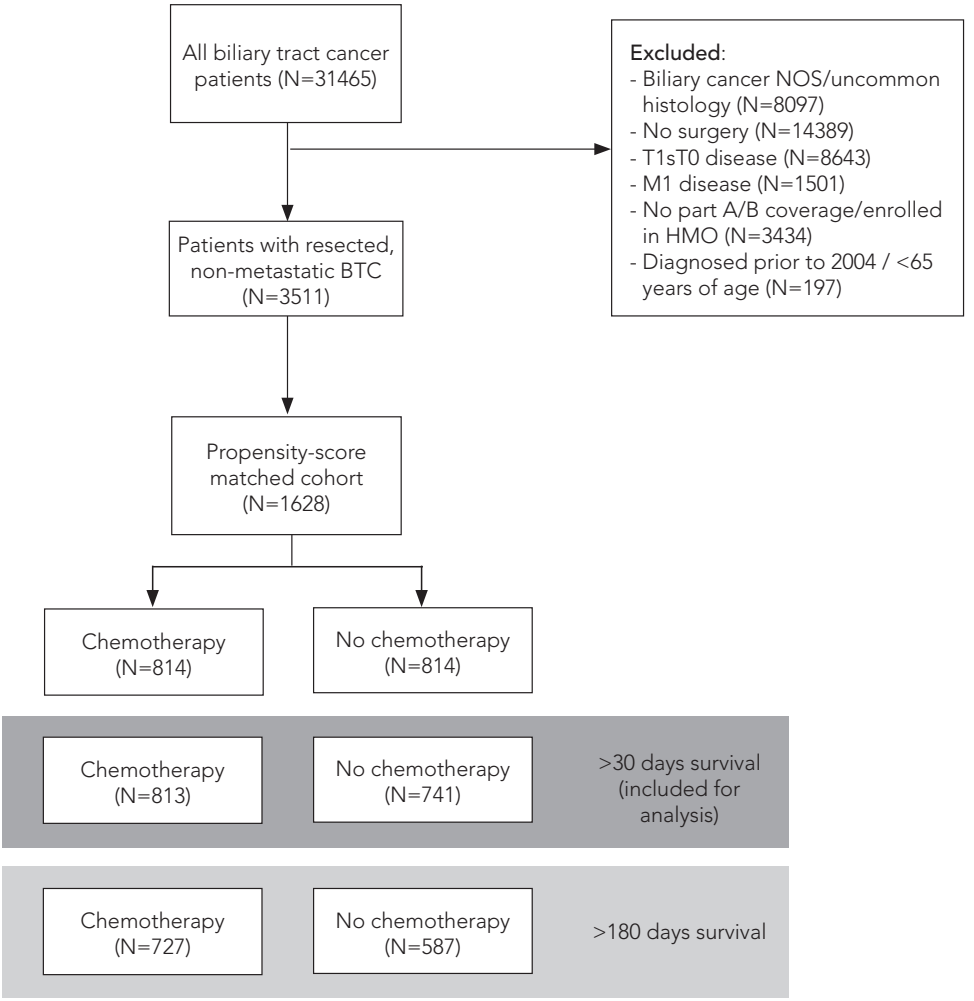
Patients were matched on age, gender, Elixhauser comorbidity score, date of diagnosis, tumor location, tumor size, pT- and pN stage, differentiation grade, education, income and poverty status. [a] 21 of these patients received 5-FU and gemcitabine.
[b] Patients may have received multiple procedures; i.e. both a liver resection and a bile duct resection.
[c] This variable was not used during propensity score matching

## SURVIVAL IN PATIENTS WITH AND WITHOUT ACT BEFORE AND AFTER PROPENSITY SCORE MATCHING

In the unmatched cohort median OS was 23.5 months and 5-year survival was 20%. Median OS of patients that received aCT was longer than survival in patients with no aCT (24.3 months vs. 23.2 months, P=0.026).

In the matched cohort included for survival analysis (N=1554), median OS was 24.3 months and 5-year survival was 35%. Survival did not differ between patients treated

**Figure 1.** Cohort selection.




with aCT (24.3 months) compared to patients with no aCT (24.2 months, P=0.486, Figure 2A). In subgroup analysis only including patients with survival >180 days postoperatively, survival was 27.0 months in patients that received aCT (N=727), versus 30.9 in patients without aCT (N=587) (Supplementary Figure 2A, P=0.014). In adjusted Cox Regression analysis, chemotherapy was a positive prognostic factor for survival (HR 0.87, $CI_{95\%}$ 0.76 - 0.99, P=0.04, Supplementary Table 3).

## SUBGROUP ANALYSIS OF SURVIVAL IN PATIENTS WITH AND WITHOUT CHEMOTHERAPY ACCORDING T-CLASSIFICATION AND N-CLASSIFICATION.

In the subgroup of patients with T1/T2 disease, no statistically significant difference between patients treated with aCT (29 months) and without aCT (43 months, P= 0.157, Figure 2B) was found. In T3/T4 disease, median OS in patients with aCT was 18.5 months, versus 12.4 months in patients without (P=0.001) (Figure 2B). In node-positive disease, survival was also significantly higher (P<0.001) in patients treated with aCT (20.5 months) compared to patients without (13.3 months, Figure 2C). In patients with N0 disease no difference in survival could be demonstrated. In patients with postoperative survival of >180 days, median OS in patients with T1/T2 disease with aCT was 30.9 months and without aCT 37.9 months (P=0.005, Supplementary Figure 2B). In patients with N0 disease, survival with aCT was 29.1 months, versus 36.5 months without aCT (P<0.001, Supplementary Figure 2C). In patients with T3/T4 and N+ disease surviving >180 days postoperatively, no significant differences in survival were seen between treatment groups.

## EFFECT OF DIFFERENT CHEMOTHERAPY REGIMENS

In uncorrected analysis, there was a significant (P=0.005) difference in median OS between different aCT regimens; FU-based 23.9 months, gemcitabine-based 27.0 months, gemcitabine-cisplatin 48.6 months and other chemotherapy combinations 14.4 months. In adjusted multivariable analysis, no differences in survival between aCT regimens remained (Table 2).

## INTERACTION ANALYSIS OF COMBINED T-/N- CLASSIFICATION AND DIFFERENTIATION GRADE WITH ACT IN THE MATCHED COHORT

An association with superior OS after treatment with aCT was seen in patients with node-positive disease (HR 0.67, 95% CI 0.55-0.81) (Supplementary Table 4) and T3 disease (HR 0.74, 95% CI 0.62-0.89). Patients were grouped according to different combinations of T-classification (T1/T2 vs. T3/T4) and N-classification (N0 vs. N+) to analyze their interaction with chemotherapy and survival (Table 3). Multivariable analysis showed that a survival benefit of aCT was only seen in patients with a combination of T3/T4 and N+ disease (HR 0.56, Table 3). Sensitivity analysis in patients with survival of >180 days revealed similar results (HR 0.73).

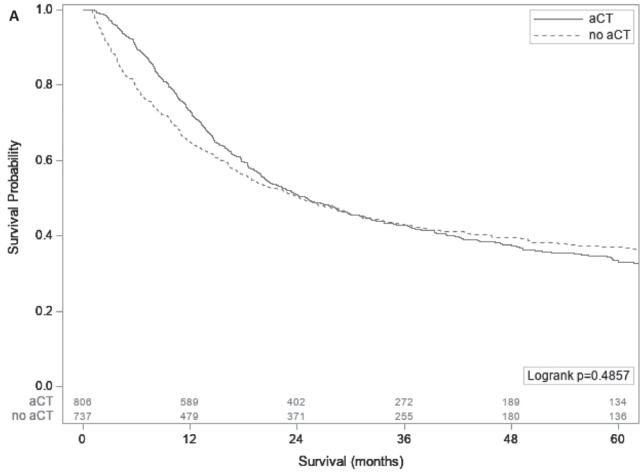
Table 2. Cox regression of prognostic factors for patients that received chemotherapy in the matched cohort.

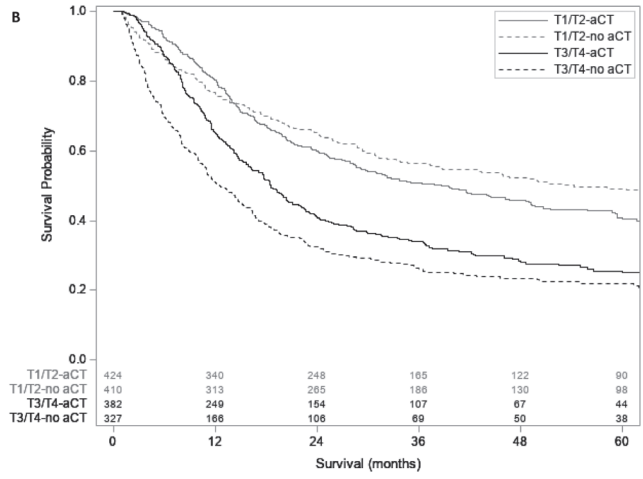
| Factor | | HR | 95% CI | P-value |
|---|---|---|---|---|
| Age (per year) | | 1.03 | 1.017 - 1.04 | <.001 |
| Elixhauser comorbidity score | 0-2 | 1 | | 1 |
| | 3-4 | 0.85 | 0.68 - 1.07 | 0.166 |
| | =>5 | 1.10 | 0.89 - 1.36 | <.001 |
| Differentiation grade | Well | 1 | | 1 |
| | Moderate | 1.05 | 0.79 - 1.40 | 0.742 |
| | Poor | 1.35 | 1.01 - 1.81 | 0.045 |
| | Undifferentiated | 1.21 | 0.64 - 2.27 | 0.556 |
| Poverty indicator | 0-5% | 1 | | 1 |
| | 5-10% | 1.07 | 0.84 - 1.37 | 0.567 |
| | 10-20% | 1.29 | 1.02 - 1.62 | 0.034 |
| | >20% | 1.13 | 0.85 - 1.50 | 0.411 |
| Race | White | 1 | | 1 |
| | Black | 0.85 | 0.58 - 1.25 | 0.410 |
| | Alaskan/Native American | 0 | 0 – 1000.00 | 0.956 |
| | Asian/pacific islander | 0.72 | 0.52 - 0.98 | 0.037 |
| Year of diagnosis | 2004-2005 | 1 | | 1 |
| | 2006-2007 | 1.03 | 0.76 - 1.39 | 0.860 |
| | 2008-2009 | 1.20 | 0.90 - 1.61 | 0.216 |
| | 2010-2011 | 1.08 | 0.80 - 1.47 | 0.612 |
| | 2012-2014 | 0.81 | 0.59 - 1.12 | 0.209 |
| Tumor location | Gallbladder | 1 | | 1 |
| | Distal bile ducts | 0.68 | 0.49 - 0.94 | 0.019 |
| | Intrahepatic bile ducts | 0.78 | 0.56 - 1.09 | 0.141 |
| | Perihilar bile ducts | 0.82 | 0.64 - 1.05 | 0.116 |
| pN-stage | N0 | 1 | | 1 |
| (yes vs. no) | N1/N2 | 1.10 | 0.91 - 1.33 | 0.312 |
| pT-stage | T1 | 1 | | 1 |
| | T2 | 1.28 | 0.96 - 1.75 | 0.086 |
| | T3 | 1.87 | 1.38 - 2.51 | <0.001 |
| | T4 | 2.41 | 2.41 - 3.75 | <0.001 |
| Tumor Size | <5cm | 1 | | 1 |
| | >5cm | 1.04 | 0.78 - 1.39 | 0.776 |
| Radiotherapy (yes) | | 0.73 | 0.59 – 0.91 | 0.004 |
| Type of chemotherapy | Gemcitabine-based | 1 | | |
| | 5FU-based | 1.11 | 0.88 - 1.41 | 0.372 |
| | Gemcitabine-cisplatin | 0.83 | 0.61 - 1.12 | 0.220 |
| | Other | 1.37 | 0.98 - 1.90 | 0.062 |

When adding differentiation grade into the model, benefit of aCT was seen in the following subgroups: T1/T2, N+, poorly/undifferentiated disease (HR 0.54, Supplementary Table 5), T3/T4, N+, well/moderately differentiated disease (HR 0.71) and T3/T4, N+, poorly/undifferentiated disease (HR 0.49).

**Table 3**. Interaction analysis between T-/N- classification and receipt of aCT and survival in patients who survived >30 days and >180 days postoperatively.

| Group | aCT (N) | No aCT (N) | HR | 95% CI | P-value |
|---|---|---|---|---|---|
| T1/T2, N0, >30 days | 284 | 293 | 1.09 | 0.86 - 1.39 | 0.466 |
| T1/T2, N0, >180 days | 264 | 259 | 1.22 | 0.93 - 1.61 | 0.147 |
| T1/T2, N1/N2, >30 days | 140 | 117 | 0.84 | 0.62 - 1.15 | 0.270 |
| T1/T2, N1/N2, >180 days | 129 | 99 | 0.90 | 0.64 - 1.28 | 0.566 |
| T3/T4, N0, >30 days | 180 | 158 | 0.97 | 0.75 - 1.25 | 0.810 |
| T3/T4, N0, >180 days | 154 | 115 | 1.26 | 0.93 - 1.71 | 0.134 |
| T3/T4, N1/N2, >30 days | 202 | 169 | 0.56 | 0.44 - 0.71 | <0.001 |
| T3/T4, N1/N2, >180 days | 180 | 114 | 0.70 | 0.53 - 0.93 | 0.014 |



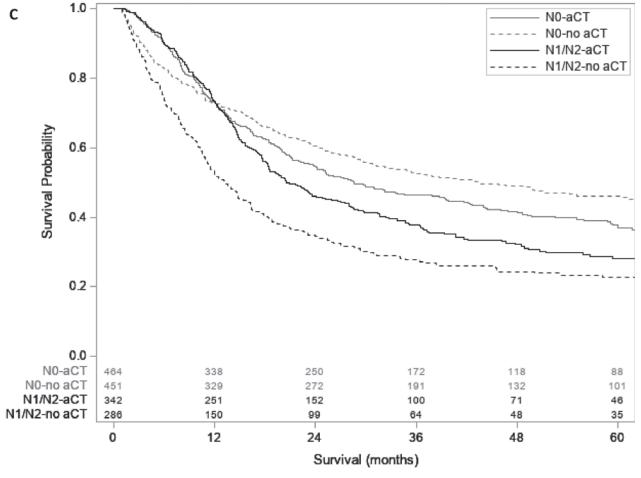




**Figure 2.** 2A. Survival of matched cohort excluding mortality <30 days (N=1554, P=0.544). B. Survival of matched cohort excluding mortality <30 days, stratified by T-stage. Log rank P=0.157 in T1/T2, aCT vs. no aCT. Log rank P=0.001 in T3/T4, aCT vs. no aCT. C. Survival of matched cohort excluding mortality <30 days, stratified according to N stage. Log rank P=0.072 in N0, aCT vs. no ACT. Log rank P<0.001 in N1/N2, aCT vs. no aCT.

# DISCUSSION

Tumor recurrence after resection of BTC occurs in up to 65% of patients and ultimately determines survival.[6] Although RCT's show that adjuvant chemotherapy reduces recurrence rates in other cancers, robust evidence to support its use in BTCs has not existed. Given the conflicting results from RCTs, we sought to inform this debate by performing an analysis of SEER registry data on patients with resected BTC. In this propensity-score matched cohort of patients with resected BTC survival was not significantly associated with receipt of aCT. However, subgroup analysis revealed that patients with T3/T4, node-positive disease showed longer OS after administration of aCT.

The few published randomized trials investigating the efficacy of aCT in BTC have shown conflicting results. Of three high-quality trials completed recently, only the BILCAP trial (adjuvant capecitabine compared to observation alone) showed positive results in the per-protocol analysis alone (53 vs. 36 months, P=0.028) and not in the primary, intention to treat analysis. [7, 20] It is important to note that in the BILCAP trial, a relatively high number of patients with R1 resections (38%) or node-positive disease (46%) was included; both factors are known poor prognostic factors and are associated with increased response to chemotherapy. [18, 20, 21] We aimed to delineate clinically relevant subgroups of patients that may benefit from chemotherapy and found that chemotherapy may only be beneficial in patients with T3/T4 disease or when lymph node metastases are present. Although our results suggested that patients with higher tumor grade may also benefit from chemotherapy regardless of T-classification, this was not seen when only including patients who survived >180 days postoperatively and were thus likely fit enough to start an initial course of chemotherapy. In patients with low-risk (i.e. T1/T2, node-negative, well-differentiated) disease aCT even appeared harmful in patients with >180 days survival postoperatively. This harmful effect was, however, not seen in adjusted survival analysis and can potentially also be attributed to the fact that patients with irradical resection are more frequently treated with chemotherapy. Since other studies show that irradical resection is a poor prognostic factor, we suggest that aCT for low-risk patients should potentially only be considered in case of positive resection margins. [22]

After the aforementioned RCT's, the next highest level of evidence is a recent meta-analysis of 21 studies including 6,712 patients, of which 1,797 were treated with chemotherapy, radiotherapy or a combination of both. [23] The meta-analysis showed a positive effect of adjuvant therapy in all patients with BTC (OR 0.74), contradicting our

finding that aCT is only beneficial in patients with high-risk (i.e. T3/T4, N+) features. However, only one RCT was included and all other studies were retrospective, single-center experiences, which are likely subject to selection and immortal-time bias. Additionally, a large grade of heterogeneity was seen, and the authors were unable to report hazard ratio's (adjusted for survival time) since many studies did not report actual survival times. Finally, a significant portion of the included studies had a high (>50%) rate of R1 resection or lymph-node positivity, explaining the high effect of aCT in their study. We used propensity score matching and exclusion of patients who deceased <30 days to account for both forms of bias, which may explain the lack of efficacy of aCT across the full cohort in our study.

Since the publication of the ABC-02 trial in 2010, gemcitabine and cisplatin (Gem-Cis) has been the regimen of choice in the treatment of locally advanced or metastatic BTC. [24, 25, 26] However, its efficacy has not been proven in the adjuvant setting. [27, 28] The only chemotherapeutic agent which has demonstrated to increase survival in resected patients compared to observation alone in a randomized controlled study is capecitabine. [11] Most studies investigating other agents or combination regimens were single-arm studies or compared to observation alone and no studies have directly compared capecitabine to other commonly used agents. Therefore, it is difficult to establish whether other treatment regimens may be more effective than capecitabine. In the present study, after covariate adjustment all chemotherapeutic regimens (gemcitabine monotherapy, 5-FU, Gem-Cis or other combinations) were comparable in terms of association with median OS. This lack of demonstrated survival differences may not necessarily mean that their efficacy is comparable. BTC is a heterogenous disease; gallbladder cancer, proximal, intrahepatic and distal cholangiocarcinoma all have their own staging systems and differ in genomic alterations. The expression of specific molecular profiles (depending on tumor location) may be associated with extremely good response to certain forms of (targeted) therapy. [29, 30] Future research should focus on identifying specific molecular profiles and their response to chemotherapy as opposed to analyzing all forms of BTC together.

The primary limitation of this study is the non-random allocation of treatment. Propensity-score matching and exclusion of subjects whom were deceased within 30 days of surgery were used to limit the impact of selection bias and immortal time bias. Secondly, SEER does not register margin status after resection, which may be both a marker of disease biology and a risk factor for recurrence. Since Medicare is primarily limited to patients aged 65 years or older, it was impossible to assess the efficacy

of chemotherapy in a younger population. However, biliary tract cancer is typically a disease present in the elderly population; only 29% of patients is under 65 years of age. [31] Younger patients also typically have other hepatobiliary comorbidities (such as PSC ) and consequently their tumor biology may differ significantly from the typical BTC patient. [32] Finally, it is possible that the use of oral chemotherapeutic agents, especially capecitabine, is not fully captured because not all patients had Part D coverage. However, if anything this likely leads to an underestimation of treatment efficacy.

This study has multiple strengths. Primarily, our results provide an overview of outcomes of a very large cohort of patients with BTC treated with aCT. Due to the use of population-based data and the inclusion of elderly patients with comorbidities, this paper provides an excellent reflection of contemporary clinical practice and outcomes of patients with BTC. In contrast to most retrospective and registry studies that do not include information on patient comorbidity, we used Medicare claims data to calculate the Elixhauser Comorbidity Score. This method is viable to assess and correct for performance status in statistical models. [33, 34] Finally, we were able to compare different chemotherapeutic regimens.

# CONCLUSION

These data shows that adjuvant chemotherapy may provide a survival benefit in high-risk patients with advanced BTCs, including T3/T4 tumors and node-positive disease. Future research efforts should focus on improving the selection of BTC patients who might have a higher likelihood of benefitting from adjuvant treatment.

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**Supplementary Table 1:** Location codes.

| Code | Location |
|---|---|
| C22.0 | Liver [a] |
| C22.1 | Intrahepatic bile duct [b] |
| C23.9 | Gallbladder |
| C24.0 | Extrahepatic bile duct [c] |

| Code | Histology |
|---|---|
| 8000 | Neoplasm, malignant |
| 8001 | Tumor cells, malignant |
| 8010 | Carcinoma, NOS |
| 8020 | Carcinoma, undifferentiated, NOS |
| 8050 | Papillary carcinoma, NOS |
| 8052 | Papillary squamous cell carcinoma |
| 8070 | Squamous cell carcinoma, NOS |
| 8140 | Adenocarcinoma, NOS |
| 8141 | Scirrhous adenocarcinoma |
| 8144 | Adenocarcinoma, intestinal type |
| 8145 | Adenocarcinoma, diffuse type |
| 8160 | Cholangiocarcinoma |
| 8162 | Klatskin tumor |
| 8210 | Adenocarcinoma in adenomatous polyp |
| 8211 | Tubular adenocarcinoma |
| 8260 | Papillary adenocarcinoma |
| 8480 | Mucinous adenocarcinoma |
| 8481 | Mucin producing adenocarcinoma |
| 8560 | Adenosquamous carcinoma |
| 8570 | Adenocarcinoma with squamous metaplasia |
| 8576 | Hepatoid adenocarcinoma |

[a] Combined with histology code 8160 recoded to proximal cholangiocarcinoma and 8160 to intrahepatic cholangiocarcinoma. [b] Combined with histology code 8162 recoded to proximal cholangiocarcinoma. [c] Use site-specific factor 25 to differentiate between proximal and distal cholangiocarcinoma.

**Supplementary Table 2.** Additional patient characteristics used for PSM.

| | No adjuvant therapy (N=2639) | Adjuvant chemotherapy (N=872) | P-value | No adjuvant chemotherapy (N=814) | Adjuvant chemotherapy (N=814) | |
|---|---|---|---|---|---|---|
| **Percentage >24 years old with highschool education** | | | | | | |
| 1st quartile | 661 (25%) | 218 (25%) | | 192 (24%) | 211 (26%) | |
| 2nd quartile | 688 (26%) | 220 (25%) | 0.632 | 222 (27%) | 207 (25%) | 0.539 |
| 3rd quartile | 632 (24%) | 227 (26%) | | 193 (24%) | 203 (25%) | |
| 4th quartile | 658 (25%) | 207 (24%) | | 207 (25%) | 193 (24%) | |
| **Median household income** [a] | | | | | | |
| 1st quartile | 659 (25%) | 204 (23%) | | 212 (26%) | 187 (23%) | |
| 2nd quartile | 650 (25%) | 215 (25%) | 0.791 | 195 (24%) | 208 (25%) | 0.395 |
| 3rd quartile | 671 (25%) | 231 (27%) | | 212 (26%) | 205 (25%) | |
| 4th quartile | 657 (25%) | 222 (25%) | | 194 (24%) | 214 (26%) | |
| **Poverty** | | | | | | |
| <5% | 596 (23%) | 236 (27%) | | 224 (28%) | 216 (27%) | |
| 5 - 10% | 711 (27%) | 238 (27%) | | 216 (27%) | 222 (27%) | |
| 10 - 20% | 745 (28%) | 257 (29%) | 0.001 | 247 (30%) | 242 (30%) | 0.9763 |
| >20% | 563 (21%) | 137 (16%) | | 124 (15%) | 131 (16%) | |

2 missing values

**Supplementary Table 3** Cox-regression analysis of prognostic factors in a matched cohort of patients with resected BTC.

| Factor | | HR | 95% CI | P-value |
|---|---|---|---|---|
| Age (per year) | | 1.02 | 1.01 - 1.04 | 0.006 |
| Elixhauser comorbidity score | 0-2 | 1 | | 1 |
| | 3-4 | 0.99 | 0.84 - 1.17 | 0.907 |
| | =>5 | 1.45 | 1.24 - 1.70 | <.001 |
| Differentiation grade | Well | 1 | | 1 |
| | Moderate | 1.21 | 0.98 - 1.49 | 0.079 |
| | Poor | 1.72 | 1.40 - 2.15 | <0.001 |
| | Undifferentiated | 1.89 | 1.21 - 2.94 | 0.005 |
| Poverty | 0-5% | 1 | | 1 |
| | 5-10% | 1.12 | 0.94 - 1.33 | 0.192 |
| | 10-20% | 1.19 | 1.01 - 1.41 | 0.036 |
| | >20% | 1.27 | 1.04 - 1.56 | 0.020 |
| Race | White | 1 | | 1 |
| | Black | 0.94 | 0.72 - 1.23 | 0.649 |
| | American indian / alaskan native | 0.53 | 0.13 - 2.19 | 0.383 |
| | Asian/pacific islander | 0.73 | 0.58 - 0.91 | 0.005 |
| Year of diagnosis | 2004-2005 | 1 | | 1 |
| | 2006-2007 | 0.88 | 0.71 - 1.08 | 0.215 |
| | 2008-2009 | 0.99 | 0.80 - 1.22 | 0.918 |
| | 2010-2011 | 0.97 | 0.77 - 1.22 | 0.800 |
| | 2012-2013 | 0.69 | 0.55 - 0.88 | 0.002 |
| | 2014 | 0.27 | 0.20 - 0.36 | <0.001 |
| Tumor location | Gallbladder | 1 | | 1 |
| | Distal bile ducts | 0.78 | 0.62 - 0.99 | 0.037 |
| | Intrahepatic bile ducts | 0.79 | 0.62 - 1.00 | 0.047 |
| | Perihilar bile ducts | 0.96 | 0.81 - 1.14 | 0.662 |
| pN-stage | N0 | 1 | | 1 |
| | N1/N2 | 1.22 | 1.06 - 1.39 | 0.005 |
| | Nx | 1.71 | 0.92 - 3.17 | 0.090 |
| pT-stage | T1 | 1 | | 1 |
| | T2 | 1.40 | 1.13 - 1.75 | 0.003 |
| | T3 | 2.12 | 1.71 - 2.64 | <0.001 |
| | T4 | 2.32 | 1.66 - 3.23 | <0.001 |
| Tumor Size | <5cm | 1 | | 1 |
| | >5cm | 1.28 | 1.04 - 1.56 | 0.017 |
| | unknown | 1.34 | 1.15 - 1.56 | <0.001 |
| Chemotherapy (yes) | | 0.87 | 0.76 - 0.99 | 0.040 |
| Radiotherapy (yes) | | 0.81 | 0.70 - 0.93 | 0.003 |

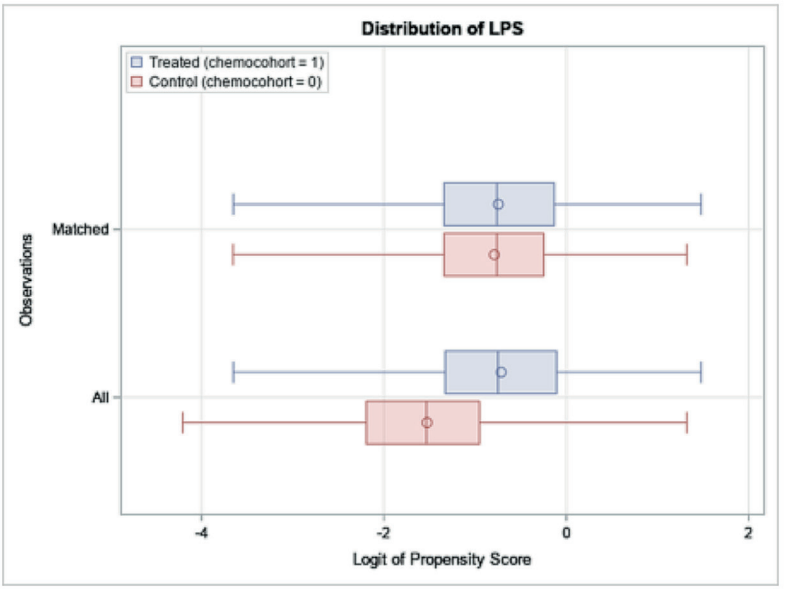
**Supplementary Table 4.** Adjusted interaction between receipt of chemotherapy and T/N-classification in the matched cohort in patients with survival >30 days postoperatively.

| Factor | | HR | 95% CI | P-value |
|---|---|---|---|---|
| pN-stage*chemotherapy | N0 | 1.08 | 0.90 - 1.29 | 0.787 |
| (yes vs. no) | N1/N2 | 0.67 | 0.55 - 0.81 | <0.001 |
| pT-stage*chemotherapy | T1 | 1.27 | 0.88 - 1.85 | 0.206 |
| (yes vs. no) | T2 | 0.99 | 0.88 - 1.23 | 0.130 |
| | T3 | 0.74 | 0.62 - 0.89 | <0.001 |
| | T4 | 0.78 | 0.48 - 1.29 | 0.295 |

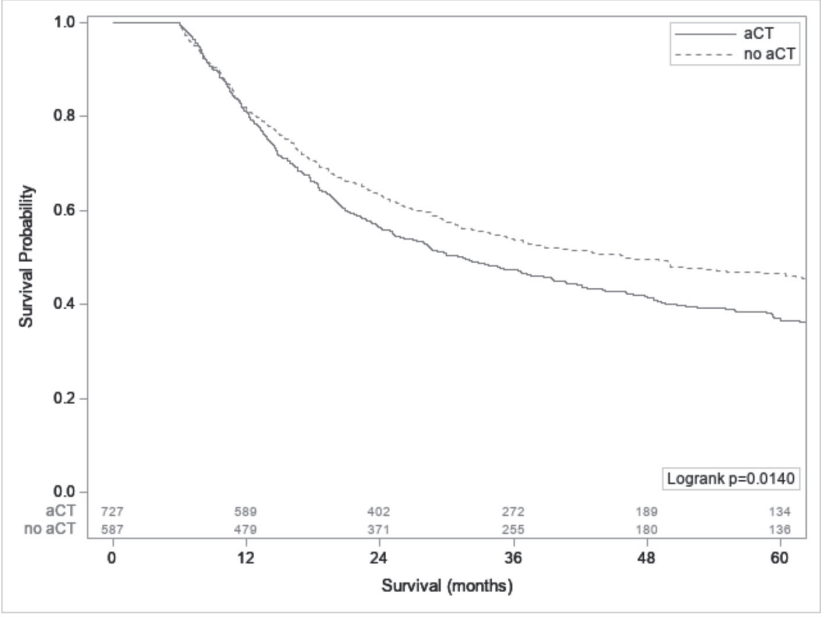
**Supplementary Table 5.** Adjusted interaction analysis between T-/N- classification, differentia grade, receipt of aCT and survival in patients with survival >30 days postoperatively.

| Group | aCT (N) | No aCT (N) | HR | 95% CI | P-value |
|---|---|---|---|---|---|
| T1/T2, N0, well/moderate | 167 | 203 | 1.32 | 1.32 - 0.97 | 0.073 |
| T1/T2, N0, poor/undifferentiated | 91 | 58 | 0.77 | 0.77 - 0.50 | 0.240 |
| T1/T2, N1/N2, well/moderate | 78 | 62 | 1.22 | 1.20 - 0.78 | 0.414 |
| T1/T2, N1/N2, poor/ undifferentiated | 58 | 51 | 0.54 | 0.53 - 0.33 | 0.008 |
| T3/T4, N0, well/moderate | 84 | 96 | 0.96 | 0.99 - 0.69 | 0.959 |
| T3/T4, N0, poor/undifferentiated | 80 | 54 | 0.89 | 0.85 - 0.57 | 0.431 |
| T3/T4, N1/N2, well/moderate | 95 | 84 | 0.71 | 0.70 - 0.49 | 0.037 |
| T3/T4, N1/N2, poor/ undifferentiated | 97 | 73 | 0.49 | 0.48 - 0.34 | <0.001 |

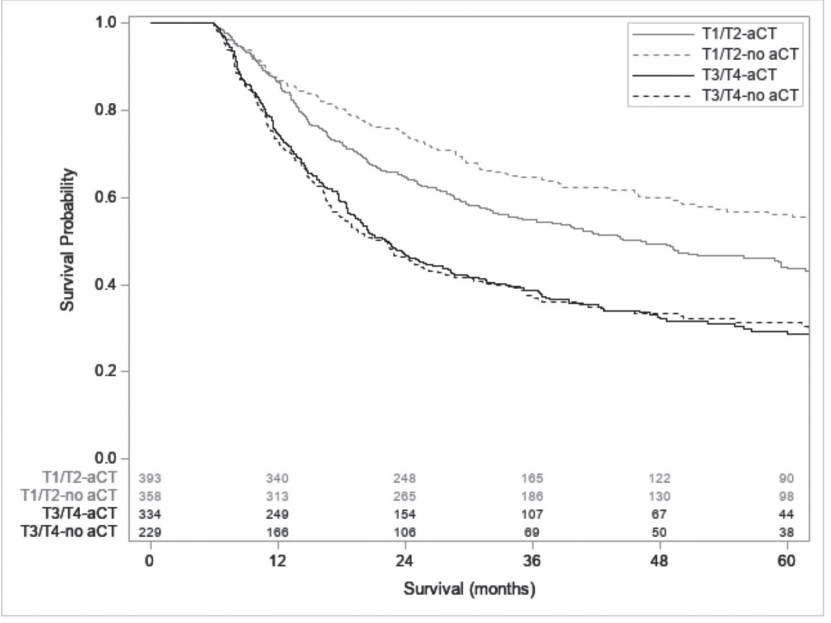
**Supplementary Figure 1.** Distribution of the logit propensity score, before and after matching.



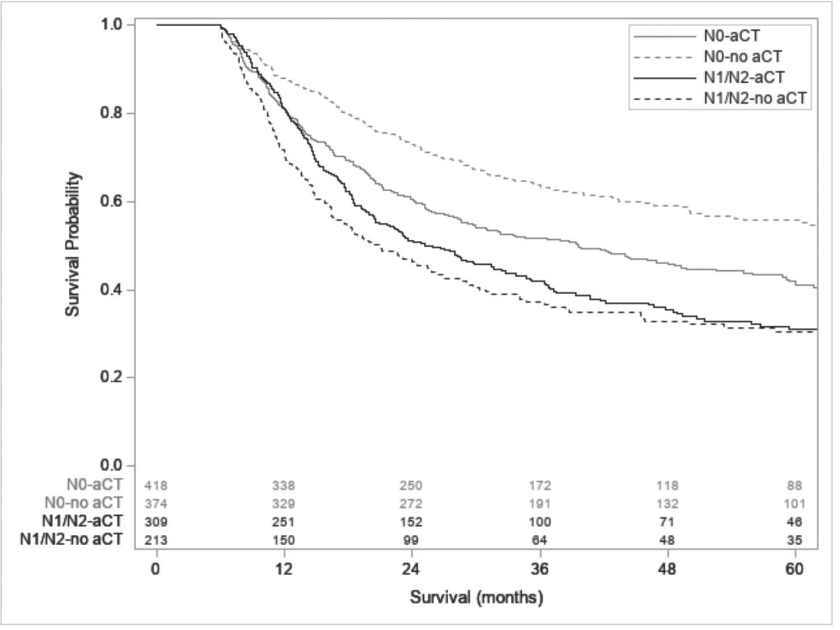

**Supplementary Figure 2A.** Survival of matched cohort excluding mortality <180 days.



**Supplementary Figure 2B.** Survival of matched cohort excluding mortality <180 days, stratified according to T stage. Log rank P=0.005 in T1/T2, aCT vs. no ACT. Log rank P=0.713 in T3/T4, aCT vs. no aCT.

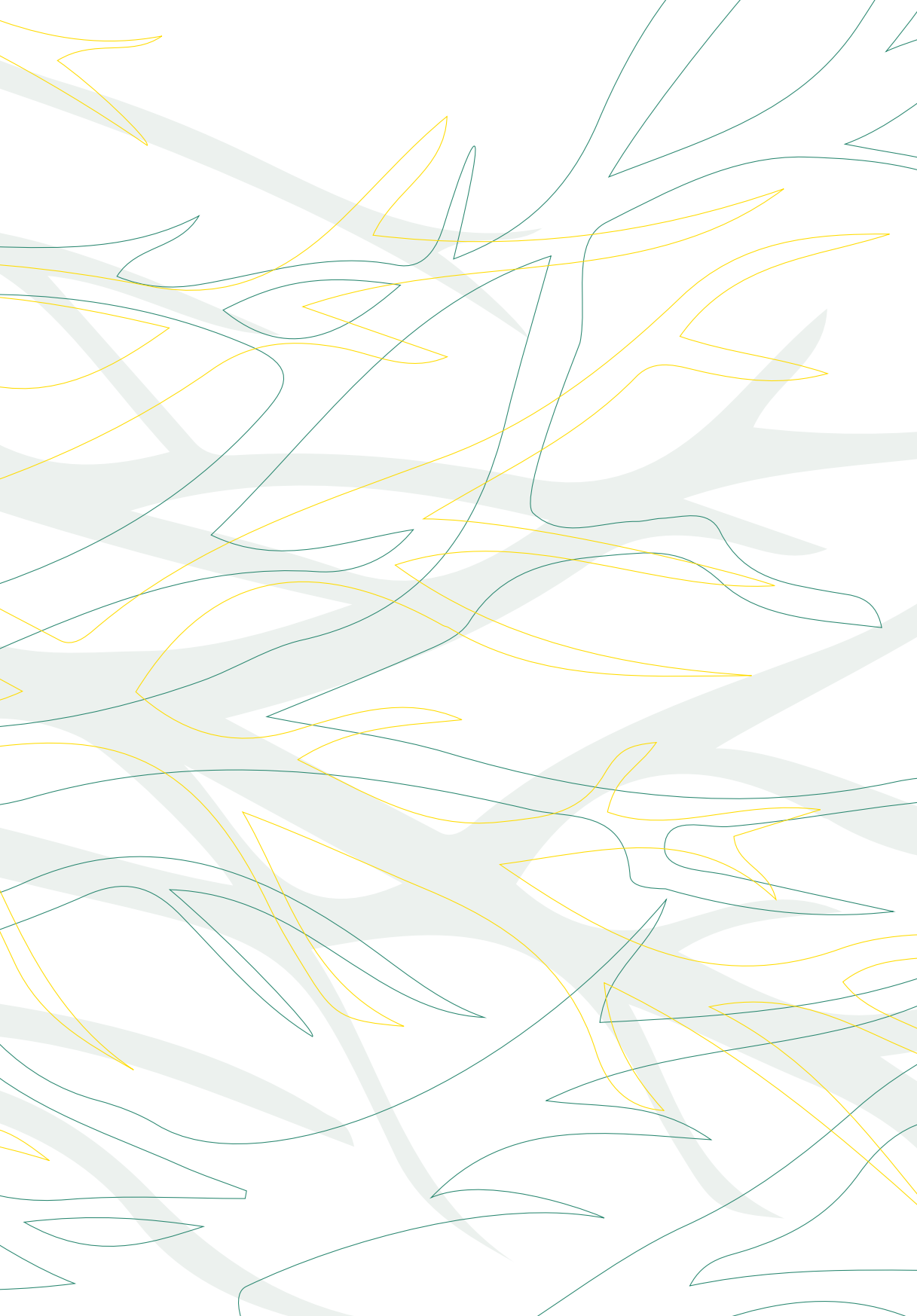


**Supplementary Figure 2C**. Survival of matched cohort excluding mortality <180 days, stratified according to N stage. Log rank P<0.001 in N0, aCT vs. no ACT. Log rank P=0.0938 in N1/N2, aCT vs. no aCT.



# PART 3

# HISTOPATHOLOGY AND PROGNOSIS



CHAPTER 8

# Re-resection in incidental gallbladder cancer; survival and the incidence of residual disease

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# ABSTRACT


## BACKGROUND

Re-resection for incidental gallbladder cancer (iGBC) is associated with improved survival but little is known about residual disease (RD) and prognostic factors. In this study survival after re-resection, RD and prognostic factors are analyzed.

## METHODS

Patients with iGBC were identified from the Netherlands Cancer Registry (NCR) and pathology reports of re-resected patients were reviewed. Survival and prognostic factors were analyzed.

## RESULTS

463 patients were included; 24% (n=110) underwent re-resection after a median interval of 66 days. RD was present in 35% of patients and most frequently found in the lymph nodes (23%). R0 resection was achieved in 93 patients (92%).

Median OS of patients without re-resection was 13.7 months (95% CI 11.6-15.6) compared to 52.6 months (95% CI 36.3-68.8) in re-resected patients (P<0.001). After re-resection, median OS was superior in patients without RD vs. patients with RD (not reached vs. 23.1 months, P<0.001). In patients who underwent re-resection, RD in the liver (HR 5.54, P<0.001) and lymph nodes (HR 2.35, P=0.005) were the only significant prognostic factors in multivariable analysis. Predictive factors for the presence of RD were pT3 (HR 25.3, P=0.003) and pN1 (HR 23.0, p=0.022) stage.

## CONCLUSIONS

Re-resection for iGBC is associated with improved survival but remains infrequently used and is often performed after the optimal timing interval. Residual disease is the only significant prognostic factor for survival after re-resection and can be predicted by pT- and pN- stage.

# INTRODUCTION

Gallbladder cancer (GBC) is the most prevalent biliary tract malignancy and the sixth most common gastrointestinal malignancy worldwide. [1] Due to an asymptomatic course in early stages, patients are frequently diagnosed in an advanced stage and prognosis is extremely poor. [2-5]

However, long-term survival does occur in patients with early-stage tumors. [6] These patients are most frequently diagnosed incidentally (iGBC), after cholecystectomy for presumed benign gallbladder disease. [7-9] Due to the growing number of laparoscopic cholecystectomies performed, iGBC is an increasingly relevant clinical issue. [10, 11] Noticeably, especially in the Western world many GBCs are detected as an incidental finding. [2, 12]

In order to prevent early locoregional recurrence, re-exploration and definitive resection is currently recommended for patients with tumors invading the muscle layer and no evidence of disseminated disease. [10] Re-resection involves a partial hepatectomy of segments 4b/5, either as a full segmentectomy or wedge excision, and resection of the hepatoduodenal lymph nodes. [13]

Re-resection is associated with improved survival in retrospective studies. However, it is still controversial whether resecting residual disease actually improves survival or whether it merely enables more complete staging and consequently provides more accurate estimation of survival.

Prognosis after re-resection appears to be primarily determined by the presence of residual disease (RD) and lymph node metastases. [6, 14-16] Interestingly, although the likelihood of detecting RD increases concurrently with T-stage, a study including 135 patients found that survival did not differ between T2 and T3 tumors in patients in whom no RD was detected. [15] This finding suggests that rather than T-stage, the presence of RD after re-resection appears to be the primary predictor for survival.
Evidently, identifying patients at risk for RD after re-resection could greatly improve candidate selection for additional surgery. Patients whom are likely to have RD could potentially benefit from more aggressive surgery. On the other hand, in patients at low risk of RD a more conservative approach could be justified.

The aim of this study was to assess survival of iGBC patients with and without re-resection. Secondly, we assessed the prognostic value of histopathological characteristics after re-resection on survival.

# METHODS

This is a retrospective, nation-wide cohort study. This study was approved by the NCR ethical review board and a waiver for ethical approval was provided by the Medical Ethics Review Committee of the region Arnhem-Nijmegen (CMO A-N, nr. 2017-3912). The STROBE statement for reporting of observational cohort studies was followed. [17]

## PATIENT SELECTION AND VARIABLE DEFINITIONS

All patients diagnosed with iGBC from 2000 – 2016 were identified from the Netherlands Cancer Registry (NCR). The NCR contains data on all newly diagnosed malignancies, including year of diagnosis, patient age and -gender and tumor characteristics (cTNM and pTNM stage [18]). Notification sources are the nation-wide network and registry of histo- and cytopathology in the Netherlands (PALGA [19]) and data from the National Registry of Hospital Discharge Diagnoses. Follow-up data on vital status (complete until February 2018) were provided by linkage to the automated Municipal Personal Records Database. iGBC was defined as GBC diagnosed based on postoperative histopathological examination. All patients with pre- or perioperative suspicion of GBC (defined as suspicion of gallbladder cancer on pre-operative imaging or findings suspect for malignancy during surgery) were excluded since the NCR categorizes these patients as suspected GBC. Patients with T1a disease or metastatic disease (detected by imaging during postoperative re-staging or during re-exploration within 6 months of diagnosis) were excluded from analysis since these patients have no indication for additional radical surgery.

Re-resection was defined as any additional, gallbladder cancer-directed surgery within 6 months after primary surgery. A retrospective review of the complete pathology reports of re-resected patients was performed using data supplied by PALGA. For patients that received a re-resection the pTNM-stage as reported after primary surgery was used to reconstruct the initial TNM stage. Because the location of the tumor was frequently not reported, no differentiation between serosal- and liver side tumors could be made, and all tumors were classified according to the 7$^{th}$ edition of the AJCC-staging manual. [18] Adjuvant chemo(radio)therapy is not considered standard of care in the Netherlands and was not administered to any of the patients throughout the study period.

For re-resected patients of whom complete histopathological reports were available, the following variables were extracted from the report of the primary surgery: type of surgery (laparoscopic cholecystectomy, open cholecystectomy, other, unspecified), pTNM stage, tumor size, tumor differentiation, presence of perineural/perivascular/ lymphatic growth and radicality (R0 defined as no microscopically present tumor <1mm from resection margin). The following variables were assessed in the re-resection report: cystic duct stump resection (yes/no), lymphadenectomy (yes (number of lymph nodes resected)/no), liver resection (no/gallbladder bed/ one segment/two segments/ ≥3 segments), presence and location of RD (defined as findings of microscopic liver/lymph node/cystic duct involvement in the pathological examination after radical surgery) and radicality of the re-resection.

## STATISTICAL ANALYSIS

Patient- and tumor characteristics were described using counts and percentages for discrete variables and means and ranges for continuous variables. Patients who underwent re-resection were categorized as having T1b, T2 or T3/Tx disease, based on the T-stage after primary resection (no patients with T4 disease received a re-resection). All analyses for patients with a re-resection were conducted using the T-stage as assessed after primary resection. $\chi$ – square testing or Fisher's exact test, where appropriate, were used to assess differences in the extent of re-resection performed and the presence and location of RD. Kaplan-Meier curves were used to calculate median survival times. Survival was defined as time in days from date of diagnosis (primary surgery) until date of death from any cause or the date of end of follow-up. [20] Log-rank testing and Cox-Regression analysis were used to compare survival between groups of patients. To deal with immortal time-bias of patients who underwent re-resection, patients with a follow-up duration of <90 days after resection were excluded from all comparative survival analyses. Additionally, to reduce treatment selection bias in the calculation of median survival times, the Kaplan Meier method was repeated in patients under 65 years of age. Cox regression analysis was used to calculate hazard ratios for potential prognostic factors in patients who underwent re-resection and logistic regression was used to identify factors predictive for RD. Covariates were selected based on literature and entered in the multivariable model when statistically relevant ($p < 0.1$) on univariable analysis. A stepwise forward selection approach was used. Missing data was determined to be Missing at Random (unrelated to the outcome, potentially related to other parameters) and complete case analysis was used to assess covariates. [20, 21] P-values < 0.05 were considered statistically significant. All tests of significance were two-tailed. Statistical analyses were conducted using the SPSS 25.0 statistical package (SPSS, Inc., Chicago, IL).

# RESULTS

## PATIENT- AND TUMOR CHARACTERISTICS

A total of 463 patients with iGBC was included (figure 1), of whom 110 patients (23%) underwent re-resection. Patient- and tumor characteristics are displayed in Table 1. Patients with a re-resection were significantly younger; the mean age difference was 10 years and 43% of patients ≤65 years received a re-resection as opposed to 15% in patients 66 years or older (P<0.001). Furthermore, re-resected patients were more likely to have T2 disease (67% vs. 52%, P=0.020) and node-positive disease (12% vs. 6%, P=0.001).

**Table 1.** Baseline patient and tumor characteristics.

| | Re-resection (N = 110) | No re-resection (N = 353) | P value |
|---|---|---|---|
| **Age** | 62.9 (36 - 81) | 72.2 (25 - 97) | **<0.001** |
| **Gender (male)** | 33 *(30.0%)* | 93 *(26.3%)* | 0.452 |
| **Tumor differentiation grade** | | | |
| Well | 16 *(14.5%)* | 38 *(10.8%)* | |
| Moderately | 47 *(40.9%)* | 124 *(35.1%)* | 0.244 |
| Poor | 22 *(20.0%)* | 101 *(28.6%)* | |
| Unknown | 27 *(24.5%)* | 90 *(25.5%)* | |
| **pT-stage** | | | |
| T1b | 10 *(9.1%)* | 47 *(13.3%)* | |
| T2 | 74 *(67.3%)* | 185 *(52.4%)* | 0.020 |
| T3/T4 | 24 *(21.8%)* | 90 *(25.5%)* | |
| Tx | 2 *(1.8%)* | 31 *(8.8%)* | |
| **pN-stage** | | | |
| N0 | 22 *(20.0%)* | 132 *(37.4%)* | **0.001** |
| N1-2 | 13 *(11.8%)* | 22 *(6.2%)* | |
| Nx | 75 *(68.2%)* | 199 *(56.4%)* | |
| **Resection margin** | | | |
| R0 | 73 *(66.4%)* | 160 *(45.3%)* | **<0.001** |
| R1/R2 | 32 *(29.1%)* | 95 *(26.9%)* | |
| Unknown | 5 *(4.5%)* | 98 *(27.8%)* | |

## RE-RESECTION PROCEDURES AND HISTOPATHOLOGY ASSESSMENT

Complete histopathology reports were available in 102 patients who underwent re-resection. Primary surgery of these 102 patients was laparoscopic cholecystectomy in 26 (25%) patients, open cholecystectomy in 4 (4%) patients, subtotal cholecystectomy in 6 (6%) patients and unspecified in 66 (65%) patients. Median interval between primary surgery and re-resection was 66 days (IQR 47 – 83). An overview of conducted re-resection procedures and incidence of residual disease is provided in figure 2. Ninety-seven patients underwent dissection of the hepatoduodenal ligament with a median

Figure 1. Selection of the included patients. GBC gallbladder cancer, iGBC incidental gallbladder cancer.

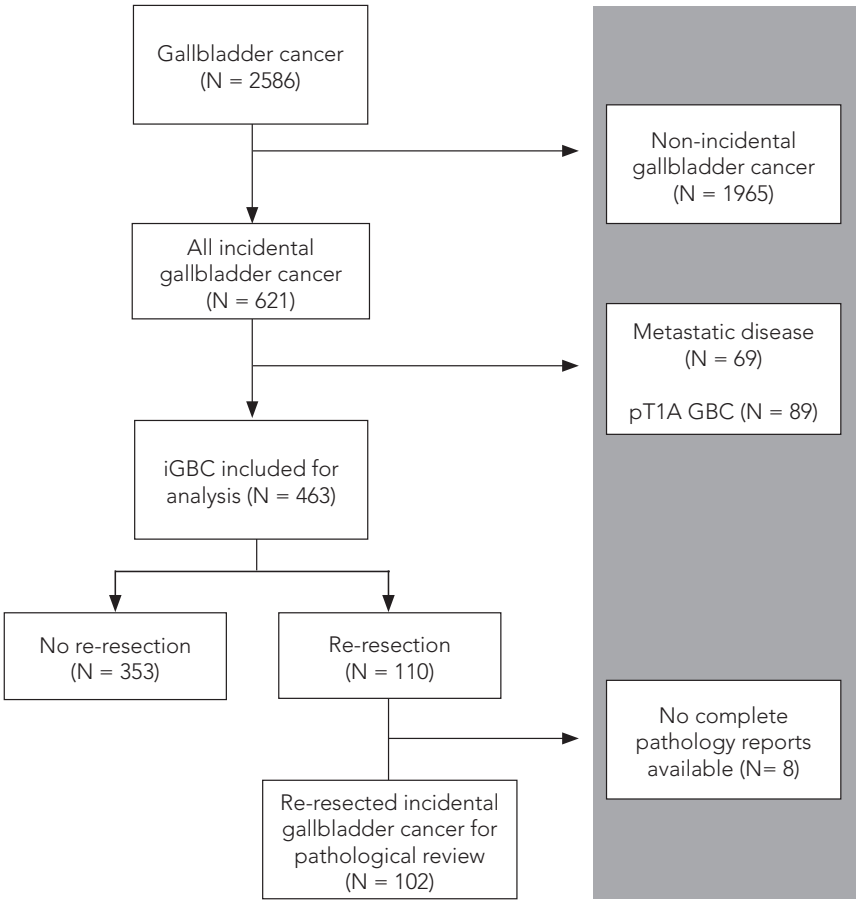
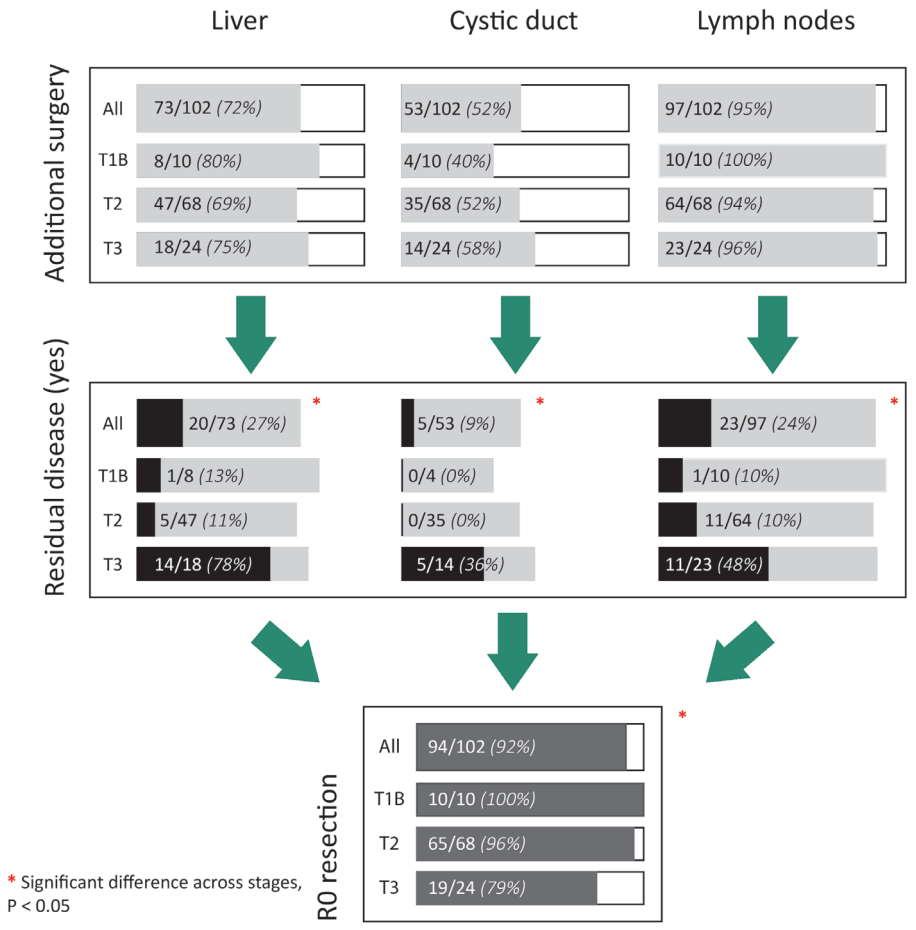
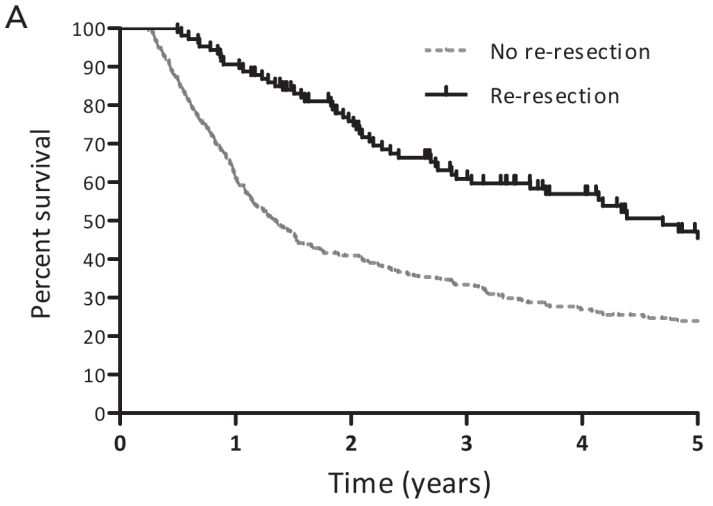


**Figure 2.** Extent of resection and incidence of residual disease according to T stage.

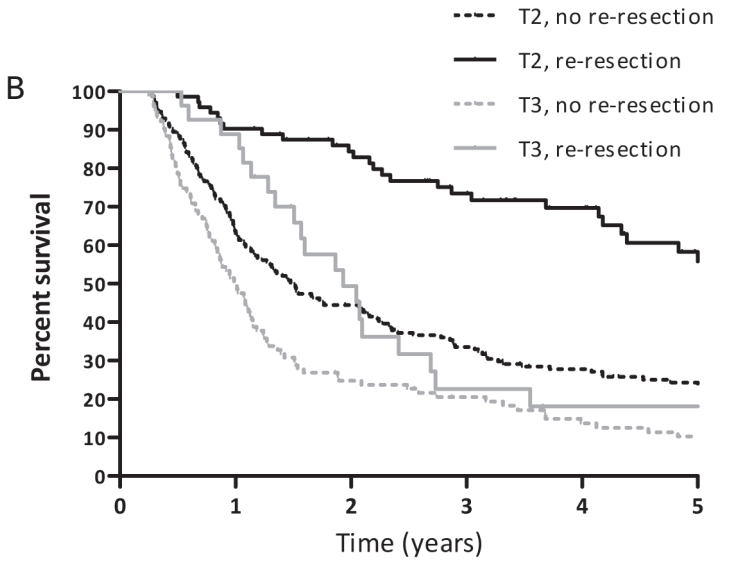



lymph node harvest of 3 (range 2 – 20). Seventy-three patients underwent resection of the liver parenchyma; gallbladder bed resection in 55 (75%) patients, gallbladder bed resection plus resection of segment 4 and 5 in 17 (23%) patients and right hemihepatectomy in one (1%) patient. Fifty-three (52%) patients underwent resection of the cystic duct stump, of whom eight (8%) also underwent extrahepatic bile duct resection.

Figure 3. **A:** Overall survival of patients with iGBC (N = 423 after exclusion of follow-up of < 90 days), by re-resection (yes, no). Log-rank p < 0.001. **B:** Overall survival of patients with T2 (N = 243) and T3/Tx (N = 130) iGBC after exclusion of follow-up of < 90 days, by re-resection.




| | | | | | | | |
|---|---|---|---|---|---|---|---|
| No re-resection | N | 316 | 195 | 127 | 99 | 75 | 62 |
| Re-resection | N | 107 | 98 | 74 | 56 | 41 | 27 |



No significant difference in extent of resection was found between T-stages (figure 2). RD was significantly more often present in re-resection specimens of patients with T3 disease. R0 re-resection was achieved in 92% of patients across the re-resected cohort but only in 79% of patients with T3 disease (P<0.001).

## SURVIVAL IN IGBC

Median OS was 18.3 months (95% CI 14.1 – 22.4). Median OS of iGBC patients without re-resection was 13.7 (95% CI 11.6-15.6) compared to 52.6 months (95% CI 36.3-68.8) in patients who underwent re-resection (P<0.001). When patients with a follow-up duration of < 90 days from primary surgery were excluded from analysis, survival was 16.1 months (95% CI 13.7 – 18.5) in patients without re-resection and 56.3 (95% CI 49.0 – 63.5) months in re-resected patients (figure 3a, P<0.001). When selecting patients under the age of 65 years and with a follow-up duration of ≥ 90 days, re-resection was still associated with superior survival (18 vs. 77 months, P<0.001).

In multivariable analysis including patients with ≥90 days of follow-up and controlling for age, T-stage, nodal status, resection margin and tumor grade, re-resection remained a significant predictor for superior survival (HR 0.47, 95% CI 0.34 - 0.65, P<0.001, Supplementary Table 1).

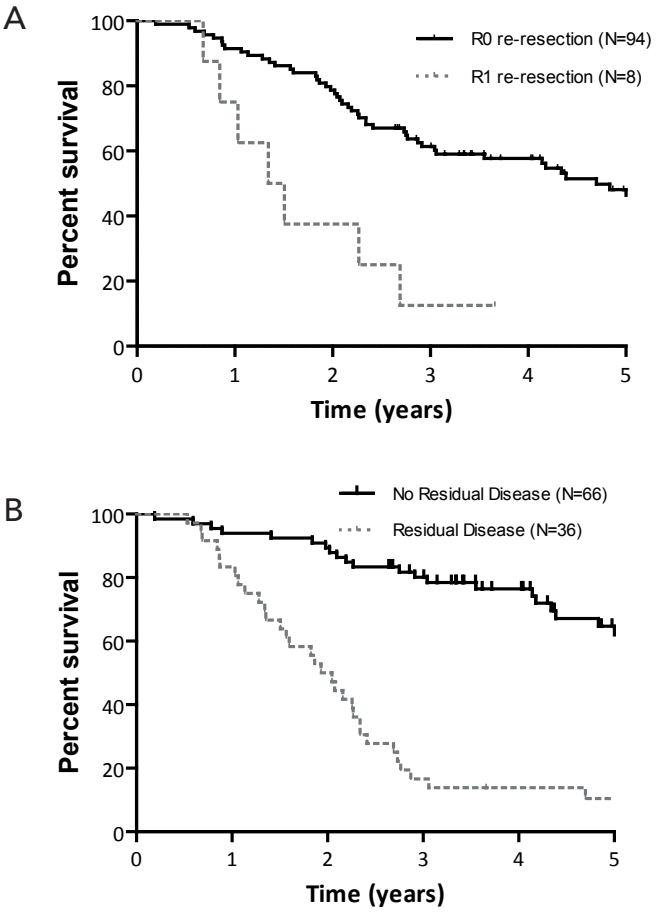
In a subgroup analysis of all patients who had tumor-free resection margins at the primary resection (N = 226) median OS was 25.9 months in patients without re-resection (95% CI 14.3-37.5) vs. 83.8 months (95% CI 41.6 – 125.9) in patients whom received a re-resection (P<0.001). After excluding patients with <90 days of follow-up, this difference persisted: 28.2 months (R0) versus 90.0 months (R1) (P<0.001). Median OS (after exclusion of patients with a follow-up duration of <90 days) in re-resected T2 disease was 60.0 months, versus 18.1 months (P<0.001) in non re-resected T2 disease (figure 3b). Median OS in re-resected T3 disease was 23.1 months versus 12.1 months (P<0.015) in non re-resected T3 disease (figure 3b). Re-resection in T1b iGBC was not significantly associated with longer survival (median OS re-resected T1b = 56.0 months vs. no resection T1b = 60.0 months, P=0.705).

## PROGNOSTIC FACTORS AND SURVIVAL AFTER RE-RESECTION

In the patients who received a re-resection and for which complete pathology reports were available (n=102), median OS was 56.3 (95%CI 32.3 – 80.2) months in patients with tumor-free resection margins versus 18.0 (95% CI 13.1 – 23.0) months in patients with tumor-positive resection margins in re-resection specimens (P<0.001, figure 4a). No significant survival difference was seen between patients who did and did not receive any form of liver resection (i.e. gallbladder bed, segmentectomy or hemihepatectomy),

neither across the entire cohort (50.0 vs. 52.6 months respectively, P=0.601) nor stratified according to T-stage (data not shown). Median OS in patients without RD (N=66) in the re-resection specimen was not reached, versus 23.1 months (95% CI 18.8 – 27.5) in patients in whom RD was present (N=36) (P<0.001, figure 4b). No survival differences were found between different locations of RD; patients with RD in the liver only (N=13) had a median OS of 22.9 months, versus 24.5 months in patients with RD in the lymph nodes alone (N=16) and 22.3 months in patients with RD in both liver and

Figure 4. A: Overall survival of patients with iGBC after re-resection (N = 102), by a) margin status and b) residual disease. Log-rank p < 0.001.



lymph nodes (N=7) (P=0.257). In patients with RD, no significant difference in median OS was found between patients with tumor-free resection margins (R0, N=8, median OS = 24.5 months) in the re-resection specimen and patients in which re-resection margins were not clear (R1, n=28, median OS 16.1 months) (P=0.447).
On univariable screening, significant prognostic factors associated with worse outcome after re-resection were; pT3-stage, irradical (R1/R2) resection margins after re-resection, perineural- and lymphovascular invasion and the presence of RD in the lymph nodes, cystic duct and liver (Table 2). In the multivariable Cox Proportional Hazards model, only presence of RD in lymph nodes (HR 2.35, p=0.005) or liver (HR 5.54, p<0.001) remained significant prognostic factors (Table 2).

## PREDICTIVE FACTORS FOR RD

On univariable screening N1 disease, T3 disease, R1/R2 resection margins at primary cholecystectomy, lymphovascular invasion and perineural invasion were predictive for RD in re-resected patients (Table 3). When entered into a multivariable model, only N1 (HR 23.0, p=0.022) and pT3 disease (HR 25.3, P=0.003) remained predictive of the presence of RD.

Table 2. Prognostic factors for survival after re-resection in patients with iGBC (N=102).

| | Univariable Cox regression | | | Multivariable Cox regression | | |
|---|---|---|---|---|---|---|
| Characteristic | HR | 95% CI | P value | HR | 95% CI | P value |
| Age (year) | 1.02 | 0.99 – 1.05 | 0.156 | | | |
| Pathological N stage | | | | | | |
| N0 | 1 | | | | | |
| N1/N2 | 0.72 | 0.38 – 1.35 | 0.303 | | | |
| Nx | 1.38 | 0.80 – 2.37 | 0.247 | | | |
| Pathological T stage | | | | | | |
| T1 | 1 | | | [c] | | |
| T2 | 1.42 | 0.50 – 4.01 | 0.512 | [c] | | |
| T3/Tx | 4.09 | 1.39 – 12.04 | 0.011 | [c] | | |
| Radicality re-resection | | | | | | |
| R0 | 1 | | | [c] | | |
| R1/R2 | 3.93 | 1.74 – 8.88 | 0.001 | [c] | | |
| Tumor differentiation grade | | | | | | |
| Well | 1 | | | | | |
| Moderate | 0.81 | 0.37 – 1.78 | 0.606 | | | |
| Poor | 1.20 | 0.52 – 2.80 | 0.668 | | | |
| Unknown | 0.82 | 0.34 – 1.95 | 0.648 | | | |
| Residual disease, lymph node (yes) | 3.18 | 1.84 – 5.52 | <0.001 | **2.35** | **1.30 – 4.23** | **0.005** |
| Residual disease, liver (yes) | 7.08 | 3.57 – 14.05 | <0.001 | **5.54** | **2.70 – 11.37** | **<0.001** |
| Residual disease, cystic duct (yes) | 5.82 | 2.17 – 15.57 | <0.001 | [c] | | |
| Lymphovascular invasion (yes)[a] | 2.31 | 1.36 – 3.91 | 0.002 | [c] | | |
| Perineural invasion (yes)[b] | 1.86 | 1.06 – 3.27 | 0.031 | [c] | | |

All variables with P < 0.10 on univariable analysis were entered into the multivariable model. [a] Missing values in 10 cases. [b] Missing values in 13 cases. [c] Not significant during forward selection.

Table 3. Predictive factors for the presence of residual disease after re-resection in patients with iGBC (n=102).

| | Univariable logistic regression | | | Multivariable logistic regression | | |
|---|---|---|---|---|---|---|
| Characteristic | HR | 95% CI | P value | HR | 95% CI | P value |
| Tumor differentiation grade | | | | | | |
| Well | 1 | | | [c] | | |
| Moderate | 2.49 | 0.62 – 9.96 | 0.197 | [c] | | |
| Poor | 5.03 | 1.17 – 21.59 | 0.030 | [c] | | |
| Unknown | 1.56 | 0.26 – 9.47 | 0.632 | [c] | | |
| Pathological N stage | | | | | | |
| 0 | 1 | | | 1 | | |
| 1 /2 | 25.00 | 2.36 – 264.80 | 0.008 | 23.00 | 1.57 – 337.44 | **0.022** |
| Unknown | 1.00 | 0.284 – 3.53 | 1.000 | 1.053 | 0.14 – 4.18 | 0.763 |
| Pathological T stage | | | | | | |
| T1 | 1 | | | 1 | | |
| T2 | 0.93 | 0.20 – 5.44 | 0.966 | 1.22 | 0.18 – 8.52 | 0.838 |
| T3/Tx | 11.60 | 3.04 – 131.73 | 0.002 | 25.33 | 2.98 – 215.69 | **0.003*** |
| Time since index surgery (days) | 1.00 | 0.99 – 1.01 | 0.788 | | | |
| Radicality primary resection | | | | | | |
| R0 | 1 | | | [c] | | |
| R1/R2 | 4.80 | 1.97 – 11.70 | 0.001 | [c] | | |
| Unknown | 1.14 | 0.11 – 11.87 | 0.911 | [c] | | |
| Socioeconomic status (deciles) | 1.03 | 0.88 – 1.19 | 0.743 | | | |
| Hospital of diagnosis | | | | | | |
| Community | 1 | | | | | |
| Academic | 0.21 | 0.03 – 1.73 | 0.146 | | | |
| Lymphovascular invasion (yes) [a] | 4.25 | 1.77 – 10.18 | 0.001 | [c] | | |
| Perineural invasion (yes) [b] | 3.18 | 1.25 – 8.09 | 0.015 | [c] | | |

All variables with P < 0.10 on univariable analysis were entered into the multivariable model. [a] Missing values in 10 cases. [b] Missing values in 13 cases. [c] Not significant during forward selection.

# DISCUSSION

The present study demonstrates that re-resection was associated with increased survival in patients with T2 and T3 iGBC and that re-resection remained an independent favorable prognostic factor in multivariable analysis. In patients who underwent re-resection, RD was more often found in patients with a higher pT-stage and the presence of RD was the primary determinant of worse survival.

Although international guidelines recommend radical cholecystectomy for all iGBC patients except those with T1a disease, the management of T1b iGBC remains controversial. Results from literature are conflicting; some studies do not report a survival benefit [22-24] whereas other series show an increase in 5-year survival of up to 30% after radical cholecystectomy. [25, 26] Interestingly, although a general survival benefit was shown across the entire re-resected cohort, patients with T1b disease did not show superior survival after re-resection. Potentially, re-resection in T1b disease is not beneficial due to the low prevalence of RD; only 1 out of 10 T1b patients had RD. Another explanation might be that the extent of surgery in these patients was too small to provide a survival benefit: in all T1b patients, only a 1 – 2 cm, non-anatomic wedge resection of the gallbladder bed was performed and median lymph node harvest was only 2. This may have resulted in understaging and undertreatment and masked the potential benefit of radical cholecystectomy. Finally, our cohort was potentially too small to detect a significant difference in survival.

On the other hand, re-resection for T3 patients is currently not considered standard practice in the Dutch national guideline due to a lack of perceived benefit. [27] In our cohort median OS was one year in patients without re-resection and 1.9 years in re-resected patients with T3 disease (landmark at 90 days). Four out of 24 (17%) patients with T3 disease had tumor-free margins at primary surgery. After re-resection, tumor-free margins were achieved in 19/24 patients (79%). Survival in GBC is primarily determined by the ability to achieve tumor-free margins. [10] Therefore, the increased rate of R0 resections after re-resection is the most likely cause for the higher survival in re-resected T3 disease.

International guidelines recommend re-resection for all patients with ≥T1b iGBC fit to undergo surgery within 4 – 8 weeks from initial cholecystectomy. [13, 28] Worrisomely, in our cohort only 23% of patients received a re-resection and the median time interval between index surgery and re-resection was over 9 weeks. In a recent

publication from Sweden, 121/201 (60%) non-metastatic iGBC patients received a re-resection. [24] In 27 out of 201 (13%) patients, a re-resection was not performed due to comorbidities. Another study included 218 iGBC patients and re-resection was attempted in 188 (86%). [16] Only 17(8%) patients did not undergo a re-resection due to low performance status. Unfortunately, due to the nature of our study we were not able to assess the rationale for not performing a re-resection in our cohort. However, it is evident from other studies that comorbidities do not frequently preclude re-resection. Other factors such as physician unawareness of or ambiguity regarding the efficacy of re-resection may account for the low number of re-resected patients in our cohort. Moreover, our results show considerable practice variation concerning the extent of re-resection performed. International guidelines recommend gallbladder bed resection for all patients as well as lymphadenectomy with a minimum count of 6 nodes. [13] In our cohort only 72% of patients received any form of liver resection and the median lymph node harvest count was 3. Evidently, guideline adherence is suboptimal and more extensive surgery than currently performed is necessary to improve outcomes

Additionally, our results raise concerns on the accuracy of staging of iGBC, especially in T2 disease. In the case of two-stage procedures, it is impossible to differentiate between metastatic disease and underestimation of T stage at initial assessment.
Residual disease was found in 35% of patients who underwent re-resection and was most frequently located in the lymph nodes (23%) and liver (20%). RD in the extrahepatic bile duct was found in 4 out of 8 patients (50%). These findings conflict recent literature, in which a higher rate (up to 60%) of RD was found. [6, 16] However, our finding that RD is mostly found in T3 disease and (regardless of site) is the primary determinant of survival after re-resection is in line with previously published literature. The finding that RD is the primary determinant of survival raises questions about the value of re-resection in iGBC. The goal of re-resection is to clear the patient of residual local or regional disease and consequently improve survival. However, survival in patients in which RD is found is poor, even when resection margins are clear. Moreover, no significant survival difference was found between patients with RD which received R0 versus R1 re-resection. This contradicts the notion that the increase in survival seen after re-resection stems from complete tumor clearance. Potentially, re-resection is beneficial solely for patients in whom only microscopic RD, undetected by the pathologist, is present. When macroscopic RD is found, the tumor may already have progressed beyond potential curation. The fact that survival between patients with different locations of RD did not differ suggests that the presence of RD acts as the clinical and prognostic equivalent of metastatic disease.

The fact that patients with RD appear unlikely to benefit from surgical treatment alone gives rise to novel clinical challenges. Although data is lacking, patients with iGBC may benefit from (neo) adjuvant chemotherapy, especially when RD is present. Predicting which patients are likely to have RD could be a useful tool to identify potential candidates for neo-adjuvant treatment. Perineural- and lymphovascular invasion, R1/R2 margins at initial cholecystectomy, tumor grade, pT and pN stage were univariably associated with the presence of RD in our cohort. After multivariable analysis only pT and pN stage appeared predictive for RD, although confidence intervals were wide. The other factors may have remained significant if more patients would have been included. Two studies with larger cohorts produced similar results [29, 30]. However, confidence intervals were either not reported or very wide and pN stage was not included in their models. Future, larger cohorts are needed to further identify histopathological characteristics associated with RD.

This study has several limitations. Primarily, our results are sensitive to selection bias due to the retrospective study design. For example, improved survival after re-resection in T3 disease may very well be a result from treatment selection bias and immortal time bias rather than a potential therapeutic effect of re-resection. We attempted to address these biases by landmarking, multivariable analysis and subgroup analysis in younger patients. However, some bias may still be present. Secondly, pathology reports were reviewed but no revision of the actual resection specimens was performed. Review by an expert hepatobiliary pathologist may have altered our results. Finally, survival according to T-stage in non re-resected patients may have been underestimated due to understaging.
A strength of this study is that our results are based on actual nation-wide outcomes and generalizability is therefore likely high. Moreover, our study is the only study that used landmark and stratification techniques when investigating the value of re-resection in iGBC, thus reducing the effects of the aforementioned biases.



# CONCLUSION

There is substantial surgical undertreatment of iGBC in the Netherlands. Re-resection is associated with improved survival in T2 and T3 iGBC. Presence of RD is the main prognostic factor for survival after re-resection and can be predicted by pT- and pN-stage. Additional histopathological research is necessary to identify candidates most likely to benefit from additional surgery and possible neo-adjuvant chemotherapy.

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Supplementary Table 1.

| | Univariable cox regression | | | Multivariable cox regression | | |
|---|---|---|---|---|---|---|
| Characteristic | HR | 95% CI | P value | HR | 95% CI | P value |
| Age (year) | 1.03 | 1.02 – 1.04 | <0.001 | **1.02** | **1.01 – 1.03** | **<0.001** |
| Re-resection (yes) | 0.43 | 0.32 – 0.58 | <0.001 | **0.47** | **0.34 – 0.65** | **<0.001** |
| Pathological T stage | | | | | | |
| T1 | 1 | | | 1 | | |
| T2 | 1.47 | 0.99 – 2.18 | 0.057 | 1.45 | 0.97 – 2.16 | 0.069 |
| T3/T4 | 2.84 | 1.89 – 4.28 | <0.001 | **2.14** | **1.39 – 3.28** | **0.001** |
| Pathological N-stage | | | | | | |
| N0 | 1 | | | 1 | | |
| N1 | 3.85 | 2.15 – 6.91 | <0.001 | 2.53 | **1.39 – 4.62** | **0.002** |
| Nx | 3.31 | 1.98 – 5.57 | <0.001 | 2.23 | **1.31 – 3.81** | **0.003** |
| Radicality primary resection | | | | | | |
| R0 | 1 | | | 1 | | |
| R1/R2 | 2.47 | 1.90 – 3.22 | <0.001 | **2.02** | **1.59 – 2.78** | **<0.001** |
| Unknown | 1.63 | 1.22 – 2.16 | <0.001 | 1.18 | 0.87 – 1.59 | 0.292 |
| Differentiation grade | | | | | | |
| Well | 1 | | | [a] | | |
| Moderate | 0.86 | 0.58 – 1.29 | 0.469 | [a] | | |
| Poor | 1.70 | 1.14 – 2.55 | 0.009 | [a] | | |
| Unknown | 1.11 | 0.73 – 1.67 | 0.632 | [a] | | |

Cox-regression analysis for survival in patients with iGBC. [a] Not significant during forward selection.

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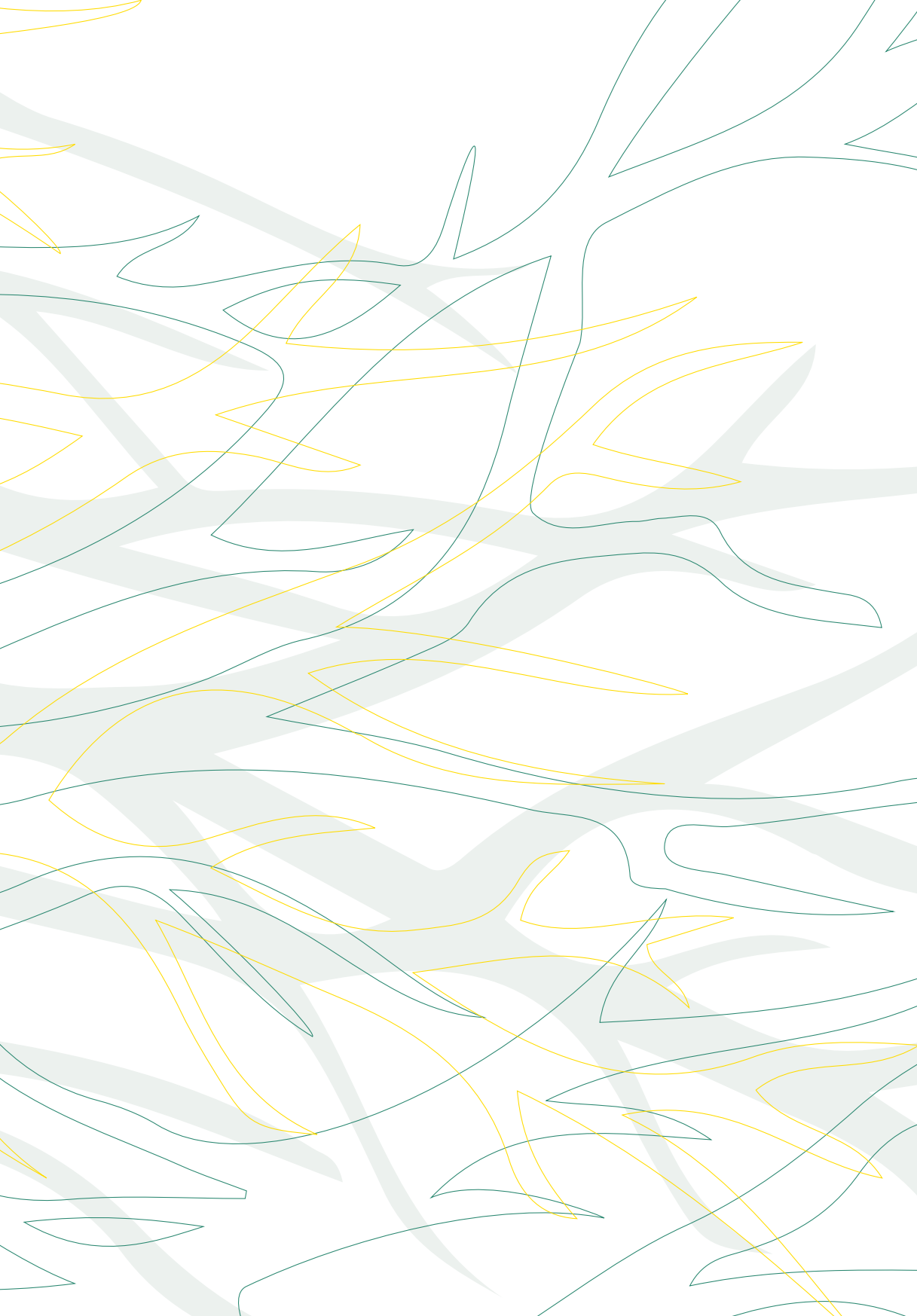

CHAPTER 9

# Development and external validation of a model to predict overall survival in patients with resected gallbladder cancer: an international validation study

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# ABSTRACT

## OBJECTIVE

The aim of this study was to develop and validate a clinical prediction model to predict overall survival (OS) in patients with non-metastatic, resected gallbladder cancer (GBC).

## BACKGROUND

Although several tools are available, no optimal method has been identified to assess survival in patients with resected resected GBC.

## METHODS

Data from a Dutch, nation-wide cohort of patients with resected GBC was used to develop a prediction model for survival. The model was internally validated and a cohort of Australian GBC patients who underwent resection was used for external validation. The performance of the AJCC staging system and the present model were compared.

## RESULTS

In total, 446 patients were included; 380 patients in the development cohort, and 66 patients in the validation cohort. In the development cohort median OS was 22 months (median follow-up 75 months). Age, T/N classification, resection margin, differentiation grade and angio invasion were independent predictors of OS. The discriminative ability of the present model after internal validation was superior to the ability of the AJCC staging system (Harrell's C-index 0.71, (95%CI 0.69-0.72) versus 0.59 (95%CI 0.57-0.60)). External validation resulted in a c-index and calibration slope of respectively 0.75 (95%CI 0.69-0.80) and 1.22 (95%CI 0.72-1.72), implying good discriminatory capacity and reasonable calibration.

## CONCLUSIONS

The proposed model for the prediction of OS in patients with resected GBC demonstrates good discriminatory capacity, reasonable calibration and outperforms the authoritative AJCC staging system. This model is a useful tool for physicians and patients to obtain information about prognosis after resection and is available from https://gallbladderresearch.shinyapps.io/Predict_GBC_survival/.

# INTRODUCTION

Gallbladder cancer (GBC) constitutes 80% of biliary tract cancers, with an estimated global incidence of 219,000 cases in 2018. [1, 2] Radical resection is the only curative treatment option. [3] Unfortunately, over 50% of patients will suffer from local or distant recurrence and 5-year survival after radical resection is only 20%-30%. [4-9]

Multiple factors associated with survival have been identified in GBC. [10] The American Joint Committee on Cancer (AJCC) staging system has been the primary method used to establish prognosis. [11, 12] However, there is significant within-stage variation in survival and the most recent edition of the AJCC only has modest prognostic value. [9, 13, 14] Recently, multiple predictive models for survival of GBC have been composed using additional independent prognostic characteristics such as resection margin and differentiation grade. [13, 15-17] However, these models have either been derived from small, non-Western single-center cohorts or from large registries. Single center data often have limited general applicability and registry data is often lacking in detail.

Evidently, there is a need for a high-quality, accessible tool for risk stratification for clinical trials, to facilitate comparison of outcomes across centers by enabling adjustment for potential confounders, and to better inform patients about their prognosis after resection. In this study, reviewed clinicopathological data from a nation-wide cohort is used to establish a tool for the prediction of OS of patients with non-metastatic, resected GBC. Additionally, the performance of the model was validated in an external, Australian dataset.

# METHODS

## STUDY DESIGN AND PATIENT SELECTION

This study uses two datasets: a development and a validation set. The development dataset was derived from a nation-wide, Dutch cohort based on data from two linked prospective registries. The validation dataset was derived from an Australian, single-institute cohort.

Inclusion criteria for both cohorts were all patients with non-metastatic gallbladder adenocarcinoma who underwent curative intent resection, defined as (radical) cholecystectomy, hemi-hepatectomy or (hepato)pancreatoduodenectomy with macr-

oscopically negative margins (R0/R1 resection). Patients that were not suspected of GBC pre-operatively but instead diagnosed with GBC per- or postoperatively were also included if they underwent curative intent resection. Patients aged <18 years, with Tis disease and patients in whom the resection specimen was not available for histopathological review were excluded. Patients who died <30 days postoperatively were excluded as the model was intended for use in the outpatient clinic.

## DEVELOPMENT COHORT

The registries used to establish the development cohort are the Netherlands Cancer Registry (NCR) and the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA). The NCR is a nation-wide database containing clinical data on all patients diagnosed with in situ or invasive tumors from 1989 onwards. PALGA contains the histopathological records of all residents of the Netherlands from 1991 onwards. Data concerning patient, demographic, clinical and tumor characteristics are routinely collected from the medical records by trained data managers. All patients who underwent curative intent resection of an adenocarcinoma of the gallbladder (International Classification of Diseases for Oncology, Third Revision [ICD-O-3] location code C239) diagnosed from 2000-2017 were identified from the NCR.

## VALIDATION COHORT

The validation cohort is composed of patients with GBC from the Northern Campus of the Upper Gastro-Intestinal Surgical Unit (a tertiary referral center) which includes Royal North Shore Hospital (RNSH) and North Shore Private Hospital (NSPH) diagnosed from October 1999 to March 2018.

## DATA COLLECTION AND VARIABLE DEFINITIONS

For the development cohort, data on patient age and gender, surgical procedures, adjuvant treatment and presence of distant metastases were supplied by the NCR. During the study period, only one patient received adjuvant chemotherapy (gemcitabine). (Neo-)adjuvant radiotherapy was not administered. Clinical information on indication for surgery and type of resection was obtained from the histopathological records provided by PALGA. For the validation cohort, all clinical data required for analysis were retrieved from the patients' medical files.

Data on follow-up and vital status were obtained for the development cohort by linkage to the Municipal Personal Records Database, which contains the vital status of all residents of the Netherlands. For the validation cohort, data were drawn from the patients' medical files and supplemented by the Ryerson Index. Survival was defined

as the number of days between surgery and end of follow-up (1st of February 2019) or death, whichever occurred first.

## QUALITY CONTROL AND HISTOPATHOLOGICAL REVIEW

To reduce the influence of inter-observer variability and ensure the quality of our data, formalin-fixed paraffin-embedded tissue blocks or slides from resection specimens of all included patients of the development cohort were requested through PALGA. Histopathology was reviewed by an expert pathology team (RP, EVB, IN) with >5 years of experience in gastrointestinal pathology. Additionally, resection specimens of all patients that were included in the validation cohort were sent to the Netherlands for review by the same expert pathology team. Histopathological characteristics that were scored include TNM stage, histology, differentiation grade, presence of vascular, perineural and lymphatic invasion and resection margin. The most recent, 8th edition of the AJCC staging system was used for TNM classification. [11] Patients were classified according to the following groups: T stage (T1 vs. T2 vs. T3/T4), N-stage (Nx vs. N0 vs N1/N2), resection margin (R0 vs. R1), tumor differentiation grade (well vs. moderate vs. poor differentiation), tumor histology (biliary vs. intestinal adenocarcinoma), and presence of vascular, lymphatic or perineural invasion. Whenever a factor could not be assessed properly due to insufficient material provided for staging, it was classified as ''unknown".

## CONSENT AND ETHICAL CONSIDERATIONS

A waiver for informed consent was provided by the Medical Ethics Committee of the NCR, PALGA and the participating hospitals ethical board (METC region Arnhem-Nijmegen, 2017-3912) as well as the Northern Sydney Local Health District Human Research Ethics Committee (ETH10589) since all data used for this study was anonymized. This study was reported according to the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) guidelines. [18]



## STATISTICAL ANALYSIS

### BASELINE CHARACTERISTICS, MISSING DATA AND SURVIVAL

Descriptive statistics were used to summarize patient, tumor and treatment characteristics. Patients with <30 days postoperative survival were excluded from analysis. Survival analysis was conducted using Kaplan Meier methodology. Median follow-up was calculated using reverse Kaplan-Meier estimation of non-deceased patients. Since all resection specimens were reviewed, there were no true ''missing"

histopathological data in this study. Rather, all missing data were actually ''unknown/ not assessable" data and were thus categorized as such. Statistical analysis was performed using R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) using R package 'rms' and SAS/STAT software, version 9.4 of the SAS system for Windows (Copyright © 2013, SAS Institute Inc., Cary, NC, USA). All p-value of <0.05 was considered statistically significant. All tests were 2-tailed.

### SURVIVAL OUTCOMES

The survival outcomes of interest were survival after 1, 3 and 5 years postoperatively.

### MODEL DEVELOPMENT

To establish the predictive model, potential predictors based on literature were entered into a Cox regression model. Backward model selection was performed based on AIC values. The sample size was calculated according to the methods described by Riley et al. [19, 20] With 5-year survival as the primary outcome, the estimated event ratio was 0.25 and median follow-up was 6.25 years. An r-squared value of .15 was used as suggested. [19] This resulted in the possibility of including 10 potential predictors for a sample size of ~400 patients.

### INTERNAL VALIDATION

Overfitting is a known issue in models based on multivariable regression analyses and may lead to false predictions in other datasets. Therefore, we used internal validation with bootstrapping to assess potential overfitting. This technique is used to generate a shrinkage factor to adjust regression coefficients. [21] Bootstrapping was performed by drawing five hundred random samples (with replacement) from the development dataset. The shrinkage factor was then calculated and applied to the model. A calibration plot for predictions at 1, 3 and 5 years was used to assess calibration. Harrell's C was used to assess the model's discriminatory capacity (i.e. dead or alive). [22] A value of 0.5 indicates poor discriminative ability, whilst 1 indicates perfect discriminative ability (≤0.5 poor;0.6-0.7 fair;0.7-0.8 good;0.8-0.9 very good;≥0.9 excellent).

### EXTERNAL VALIDATION

Calibration of the model after external validation was assessed by calculating the calibration slope of the prognostic index. A slope of 1.0 indicates perfect calibration. A calibration slope below 1.0 indicates overestimation and a slope above 1 indicates underestimation of the estimated probability of the model. Furthermore, patients were divided into three risk groups (i.e. low, medium and high risk of death), stratified

by their prognostic index. These risk groups corresponded to an estimated 60% (low risk), 30% (medium risk) and 10% (high risk) chance of survival at 5 years. Predicted and observed (Kaplan-Meier estimated) survival probabilities of each tercile were then compared. To compare the viability of our model to the currently used AJCC staging system, a model using only the AJCC stage as the prognostic indicator was created.

### PREDICTION TOOL

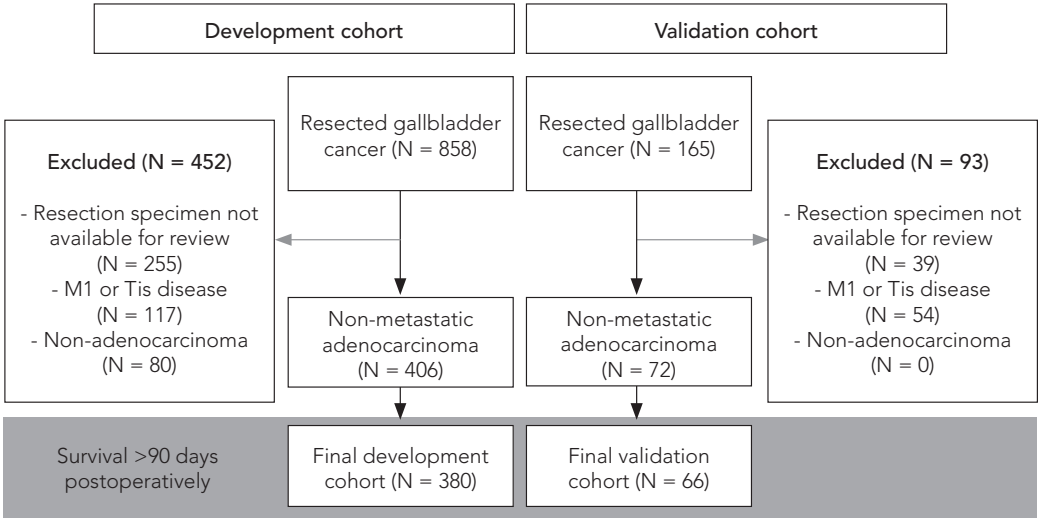
A prediction tool was created. Prediction tools are easily accessible applications to estimate an individual's survival and are well-suited for every day, clinical use. The application is available at https://gallbladderresearch.shinyapps.io/Predict_GBC_survival/.

## RESULTS

### PATIENTS

In total, 858 patients with resected gallbladder cancer were identified through the NCR of which 380 patients were included in the development cohort (Figure 1). For the validation cohort, 165 patients were identified from the prospective database and 66 were included for analysis.

**Figure 1.** Patient flow of the development and validation cohort.



## BASELINE CHARACTERISTICS

Baseline characteristics of both cohorts are reported in Table 1. Patients in the development cohort were more frequently treated with simple cholecystectomy (27.6% vs. 7.6%) and had a higher rate of T1 (12.4% vs. 7.6%), T2 (59.7% vs. 53.0%), Nx (59.7% vs. 36.4%) disease. In the validation cohort, a higher rate of T3 (34.9% vs. 27.4%) and node-positive disease (30.3% vs. 16.3%) was observed.

## SURVIVAL OUTCOMES

In the development cohort, median OS was 22 months and 1, 3- and 5-year survival rates were 65%, 37% and 28%, respectively. Median OS in the validation cohort was 24 months and 1, 3- and 5-year survival rates were 68%, 42% and 30%, respectively. Median follow-up of event-free patients was 75 months (95% CI 71 - 85) in the development cohort and was not reached in the validation cohort.

## MODEL PERFORMANCE AJCC-STAGING

A model using the AJCC staging system was created using data from the development cohort (Supplementary Figure 1 shows survival of patients in the development cohort according to AJCC stage and Supplementary Figure 2 shows the calibration plot at 5 years). The C-index for the prediction of survival was 0.59 (95% CI 0.58-0.61). In summary, this indicates a moderate discriminatory capacity with good calibration.

## DEVELOPMENT AND VALIDATION OF A PREDICTIVE MODEL FOR SURVIVAL IN PATIENTS WITH RESECTED GBC

An overview of the univariable and multivariable analysis of factors potentially associated with survival in the development cohort is presented in Supplementary table 1. Higher age, higher T-classification, node-positive or Nx disease, irradical or unknown resection margins, higher tumor grade and presence of vascular invasion were independently associated with poor survival. All independent predictors were entered into the final model (see Table 2).

Internal validation provided a shrinkage factor of 0.91. After application of the shrinkage factor, the C-index was 0.71 (95%CI 0.70 - 0.72). The calibration plots at 1, 3 and 5 years are provided in Figure 2A-C. These results imply a fair discriminative capacity of the model and good calibration with a slight tendency towards underestimation of survival probabilities in low-risk patients and overestimation of survival chances in high-risk patients.

Table 1. Baseline patient and tumor characteristics.

| Characteristic | Development cohort (N=380) | Validation cohort (N=66) |
|---|---|---|
| Age, mean ± SD | 69.9 (±11.4) | 71.2 (±9.6) |
| Gender (male) | 106 (27.9) | 22 (34.3) |
| Indication for surgery | | |
| Cholecystitis | 151 (39.7) | 9 (13.6) |
| Cholecystolithiasis | 126 (33.1) | 7 (10.6) |
| Polyp | 18 (4.7) | 0 (0.0) |
| Carcinoma | 150 (39.4) | 18 (27.3) |
| Other/Not specified | 77 (20.3) | 44 (66.7) |
| Procedure | | |
| Simple cholecystectomy | 105 (27.6) | 5 (7.6) |
| Extended cholecystectomy [a] | 77 (20.3) | 22 (33.3) |
| Other | 28 (7.3) | 14 (21.2) |
| Not specified | 170 (44.7) | 25 (37.7) |
| pT-classification | | |
| 1 | 47 (12.4) | 5 (7.6) |
| 2 | 227 (59.7) | 35 (53.0) |
| 3 | 104 (27.4) | 23 (34.9) |
| 4 | 2 (0.5) | 3 (4.6) |
| pN-classification | | |
| N0 | 91 (24.0) | 22 (33.3) |
| N1/N2 | 62 (16.3) | 20 (30.3) |
| Nx | 227 (59.7) | 24 (36.4) |
| Resection margin | | |
| R0 | 243 (64.0) | 40 (60.6) |
| R1 | 90 (23.7) | 19 (28.8) |
| Rx | 47 (12.4) | 7 (10.6) |
| Differentiation grade | | |
| Well | 43 (11.3) | 5 (7.6) |
| Moderate | 209 (55.0) | 40 (60.6) |
| Poor | 128 (33.7) | 21 (31.8) |
| Perineural invasion (yes) | 130 (34.2) | 18 (27.3) |
| Lymphatic invasion (yes) | 181 (47.6) | 25 (37.9) |
| Vascular invasion (yes) | 138 (36.3) | 26 (39.4) |
| Histology | | |
| Biliary adenocarcinoma | 242 (71.6) | 49 (74.2) |
| Intestinal adenocarcinoma | 138 (28.4) | 17 (25.8) |

[a]Including 57 patients with re-resection after incidentally discovered GBC.

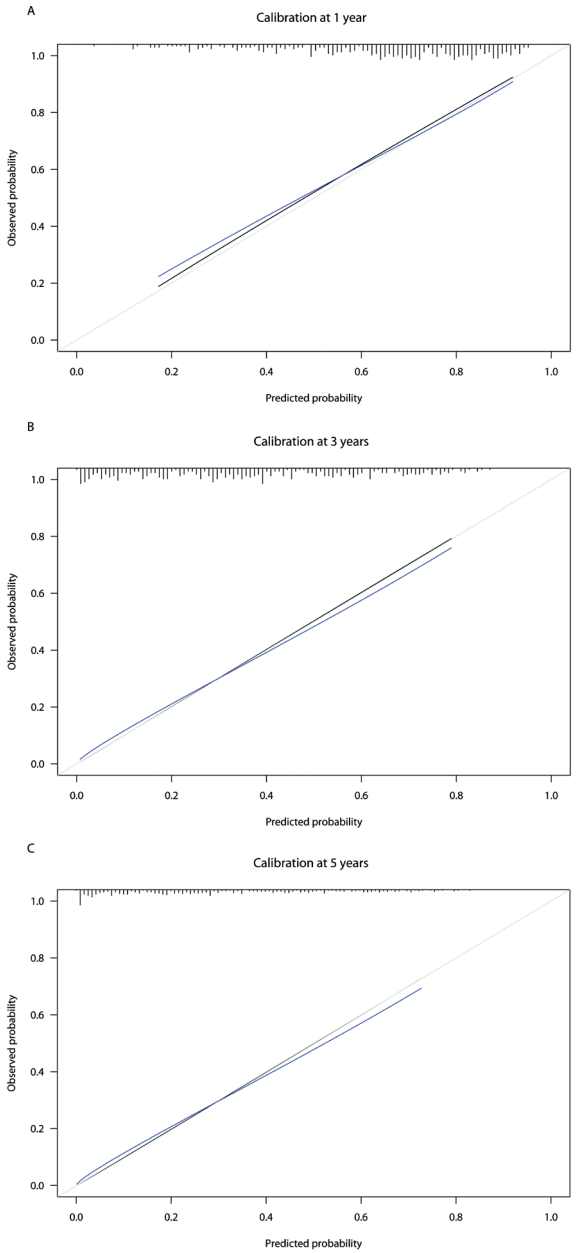
Table 2. Multivariable analysis of predictors for survival after resection in GBC.

| Clinicopathological parameter | Regression coefficient (β) after internal validation | Hazard Ratio (95% CI) |
|---|---|---|
| Age | 0.03 | 1.61 (1.34 - 1.94)[a] |
| pT-classification | | |
| T1 | 1 | 1 |
| T2 | 0.43 | 1.61 (1.01 - 2.56) |
| T3/T4 | 0.82 | 2.47 (1.47 - 4.14) |
| pN-classification | | |
| N0 | 1 | 1 |
| N1/N2 | 0.55 | 1.84 (1.22 - 2.77) |
| Nx | 0.53 | 1.78 (1.27 - 2.50) |
| Resection margin | | |
| R0 | 1 | 1 |
| R1 | 0.47 | 1.67 (1.24 - 2.25) |
| Rx | 0.59 | 1.91 (1.29 - 2.80) |
| Differentiation grade | | |
| Well | 1 | 1 |
| Moderate | 0.17 | 1.21 (0.75 - 1.96) |
| Poor/undifferentiated | 0.54 | 1.82 (1.10 - 3.02) |
| Vascular invasion (yes) | 0.33 | 1.44 (1.10 - 1.86) |

[a] IQR-hazard ratio

External validation resulted in a C index of 0.75 (95%CI 0.69-0.80) and a slope of 1.21 (95%CI 0.72-1.70). Patients were divided into three terciles according to linear predictor value. These terciles represent patients with low, moderate and high risk of death. Figure 3 shows the predicted survival in the development and validation cohort compared to the actual Kaplan-Meier estimated survival in the validation cohort in the three risk strata. Figure 4 provides an overview of the Kaplan-Meier estimated survival in the development and validation cohort in the three risk strata. Overall, the external validation indicates a fair discriminatory capacity and good calibration with a tendency towards underestimation of survival in patients with moderate risk. Baseline survival rates of the development and validation cohorts were comparable (Supplementary figure 3).

Figure 2. Calibration plots of the prediction model for survival after internal validation. The blue line shows the actual (Y-axis) versus predicted (X-axis) survival of the patients according to the model. A: Calibration at one year. B: Calibration at three years. C: calibration at five years.



Finally, an online calculator was created which can be used to predict survival in patients with non-metastatic, resected gallbladder cancer, available at https://gallbladderresearch.shinyapps.io/Predict_GBC_survival/

**Figure 3.** Calibration plot of the predicted survival in the development and validation cohort versus the observed survival in the validation cohort in three risk strata.

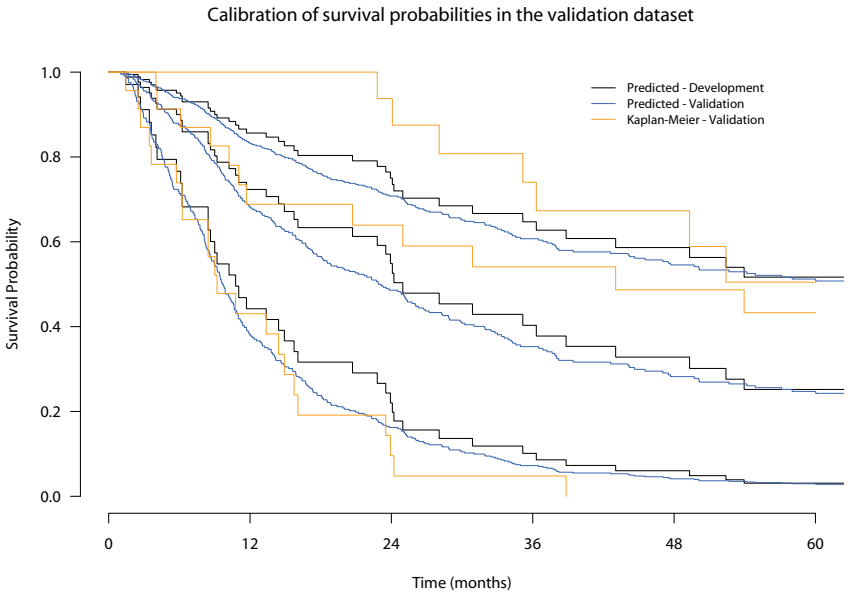
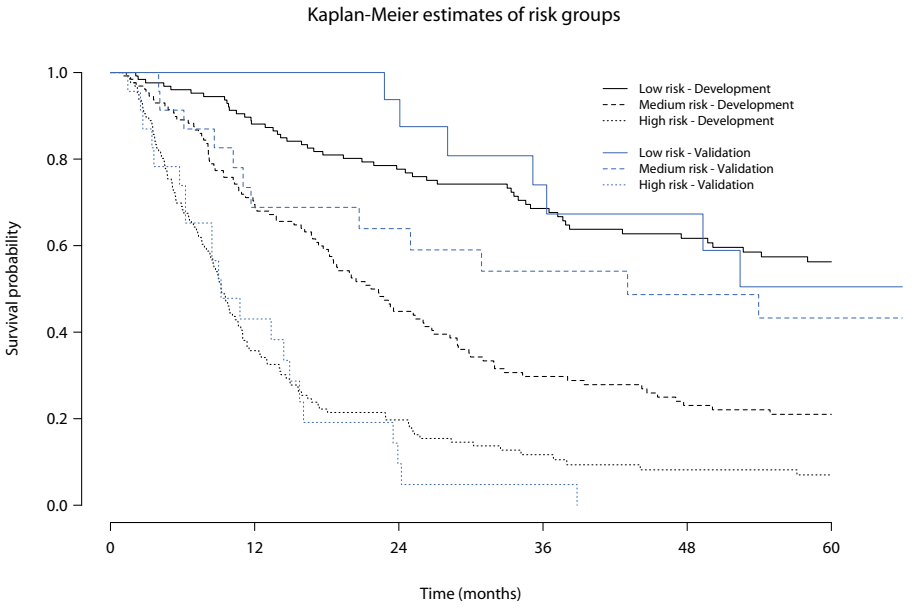


**Figure 4.** Plot of Kaplan-Meier estimated survival in the development and validation cohort in three risk strata.



# DISCUSSION

In this study, we used clinical and histopathological data of a nation-wide cohort of 380 Western patients with non-metastatic, resected adenocarcinoma of the gallbladder to create a model designed to predict survival after curative intent resection. The model incorporates easily accessible characteristics which should be provided in every histopathology report. Using age, pT-/pN stage, differentiation grade, resection margin and the presence of vascular invasion, the model has a good discriminatory capacity and clearly outperforms the authoritative AJCC staging system. External validation in a cohort of 66 Australian patients shows that calibration is reasonable, especially in high-risk patients.

The AJCC TNM staging system is the most commonly used method to estimate survival after resection. [23] However, the AJCC model does not have a great prognostic value since it only incorporates extent of invasion and lymph node status, ignoring other prognostic factors. [24, 25] Hence, several newer tools have been proposed to estimate survival. [24] Our data show that age, resection margin, tumor grade and the presence of vascular invasion are predictive of median OS. Potential differences between the outcome of our study and other models can be explained by the fact that other studies have not investigated the relative value of these prognostic factors in multivariable analysis. [16, 17, 25] For example, although perineural invasion was univariably associated with survival in our cohort, multivariable cox regression revealed that it was not an independent predictor for survival. This finding is in contrast with other cancers, in which the presence of perineural invasion independently predicts survival. [26, 27] To our knowledge, our study is the first study to describe the presence of vascular invasion as an independent prognostic factor in GBC.

The prognosis of GBC is poor as recurrence rates remain over 50% after radical resection. Although in other cancers adjuvant chemotherapy (aCT) is used to prevent recurrence and increase survival, its role in gallbladder cancer remains ill-defined. Multiple RCTs have been conducted in patients with all forms of biliary tract cancer, of which only one trial (BILCAP) showed a survival benefit of adjuvant capecitabine in the per-protocol analysis alone (17 months, P=0.034). Experts argue that aCT, rather than being an indisputable standard, should only be considered in patients with a high risk of recurrent disease and poor prognosis. [28, 29] However, no consensus regarding the definition of ''high risk disease" exists. The proposed prediction tool can be used to help identify patients at high risk of recurrence and thus select candidates for aCT.

For example, in patients with T3, N0, R0 disease predicted 5-year survival is 32%, which is higher than the 28% 5-year survival of the entire cohort. On the other hand, in T1, N0, R1 disease, 5-year survival is only 16%. Ignoring prognostic factors other than T- and N-stage when selecting patients for chemotherapy is evidently suboptimal, as this may result in high-risk patients not being offered chemotherapy. The present tool could also be used to identify patients with predicted 5-year survival of >75%, who may not benefit from aCT. This subgroup includes patients with T1, N0, R0, well-differentiated disease and represents >10% of patients with gallbladder cancer.

Four other nomograms for patients with non-metastatic, resected gallbladder cancer have been developed. Two of those have been established using data supplied by the Surveillance, Epidemiology and End Results (SEER) registry. However, the first model was established using only node-negative patients, reducing its applicability in the clinical setting. [30] The authors of the second model did not use internal validation to account for overfitting and did not validate their findings on an external dataset.[17] Two other models have been established using data derived from single-institute cohorts. [16, 31] Both studies included under 170 patients, far less than the 400 patients required to obtain a sufficient sample size and no external validation was performed. [20] Moreover, all patients were derived from non-Western, single-institute cohorts, severely limiting the applicability of both models.

This study has several limitations. First, the cohort used for external validation consisted of 66 patients. Ideally, a minimum of 100 events is required to perform an adequately powered external validation. [32] However, a C-statistic of 0.74 with small confidence interval indicates the discriminatory capacity is likely satisfactory. It is important to note that no other Western multi-institutional series of patients with GBC have previously been published, since the extremely low incidence of gallbladder cancer makes it challenging to gather large cohorts of patients. Experts increasingly recognize that in research involving rare cancers sometimes even potentially imprecise evidence may be better than no evidence at all. [33] Additionally, calibration in the middle-risk group was suboptimal. Suboptimal calibration is relatively common in external validation since patient and tumor characteristics and especially management can vary considerably across cohorts. The relatively high number of extended compared to simple cholecystectomies in the validation cohort compared to the development cohort may explain the suboptimal calibration in the middle-risk group; management in the validation cohort was likely more aggressive and may therefore have led to better outcomes compared to the development cohort.

A major strength of this study is the fact that this is the first study using nation-wide, multi-institutional data. Moreover, excluding registry data our cohort is not only by far the largest but also the only Western cohort used to generate a prediction model. Finally, all histopathological samples, including those of the validation cohort, were reviewed by an expert pathology team which greatly increases the quality of our data.

# CONCLUSION

The proposed model can be used to predict survival in patients with non-metastatic, resected gallbladder cancer based on age, T- and N- classification, differentiation grade, resection margin and the presence of perivascular invasion and outperforms traditional AJCC staging. The provided, easily accessible prediction application can be used by physicians to inform patients about their prognosis, guide decisions concerning adjuvant chemotherapy and can assist risk stratification for future randomized trials.

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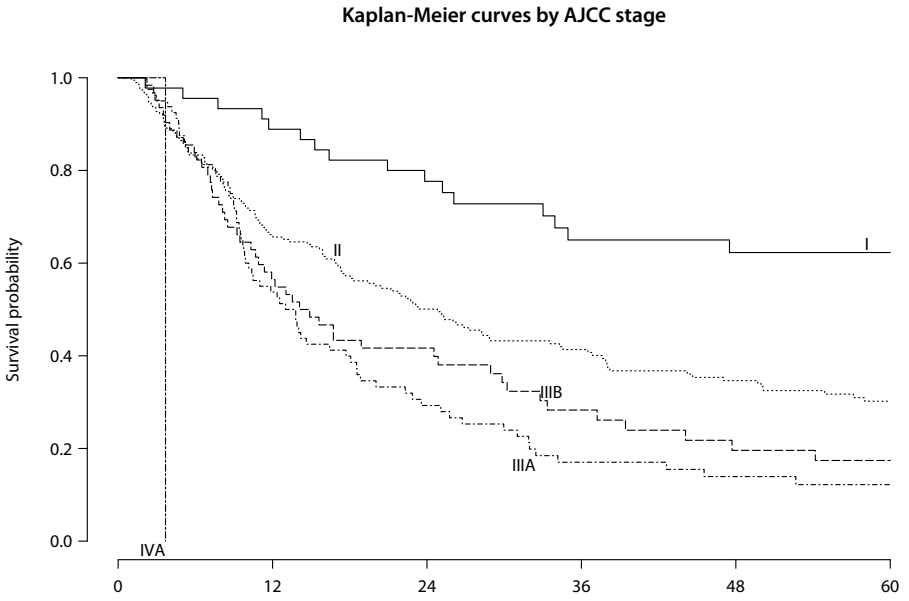
**Supplementary Table 1.** Univariable and multivariable analysis of factors potentially associated with survival in the development cohort.

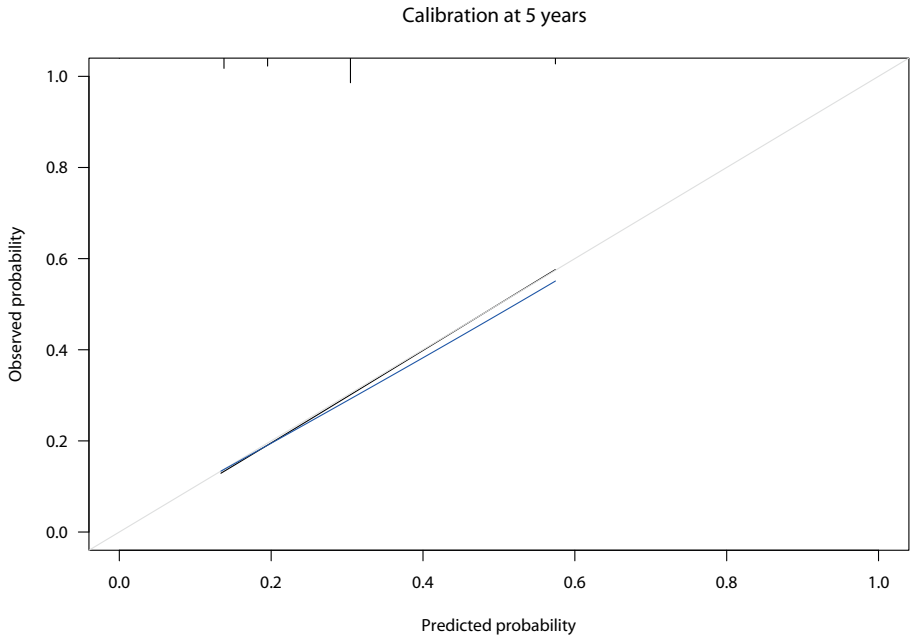
| Clinicopathological parameter | Univariable analysis HR (95% CI) | P value | Multivariable analysis HR (95% CI) | P value |
|---|---|---|---|---|
| Age | 1.70 (1.45 - 2.01) a | <0.001 | 1.60 (1.33 - 1.94)[a] | <0.001 |
| pT-classification | | | | |
| T1 | 1 | | 1 | |
| T2 | 2.04 (1.33 – 3.14) | 0.001 | 1.57 (0.99 - 2.51) | 0.057 |
| T3/T4 | 3.48 (2.21 – 5.48) | <0.001 | 2.28 (1.32 - 3.92) | 0.003 |
| pN-classification | | | | |
| N0 | 1 | | 1 | |
| N1/N2 | 2.43 (1.62 – 3.65) | <0.001 | 1.81 (1.20 - 2.72) | 0.005 |
| Nx | 2.20 (1.58 – 3.06) | <0.001 | 1.81 (1.29 - 2.54) | 0.001 |
| Resection margin | | | | |
| R0 | 1 | | 1 | |
| R1 | 2.43 (1.85 – 3.20) | <0.001 | 1.63 (1.21 - 2.21) | 0.001 |
| Rx | 2.16 (1.51 – 3.10) | <0.001 | 1.90 (1.28 - 2.81) | 0.002 |
| Differentiation grade | | | | |
| Well | 1 | | 1 | |
| Moderate | 1.81 (1.15 – 2.87) | 0.011 | 1.22 (0.75 - 1.97) | 0.424 |
| Poor/undifferentiated | 3.19 (1.99 – 5.10) | <0.001 | 1.83 (1.09 - 3.04) | 0.021 |
| Perineural invasion (yes) | 1.96 (1.53 – 2.52) | <0.001 | [b] | |
| Lymphatic invasion (yes) | 1.77 (1.40 – 2.25) | <0.001 | [b] | |
| Vascular invasion (yes) | 2.20 (1.72 – 2.81) | <0.001 | 1.40 (1.05 - 1.87) | 0.021 |

[a] IQR-hazard ratio [b]Not significant during backward selection

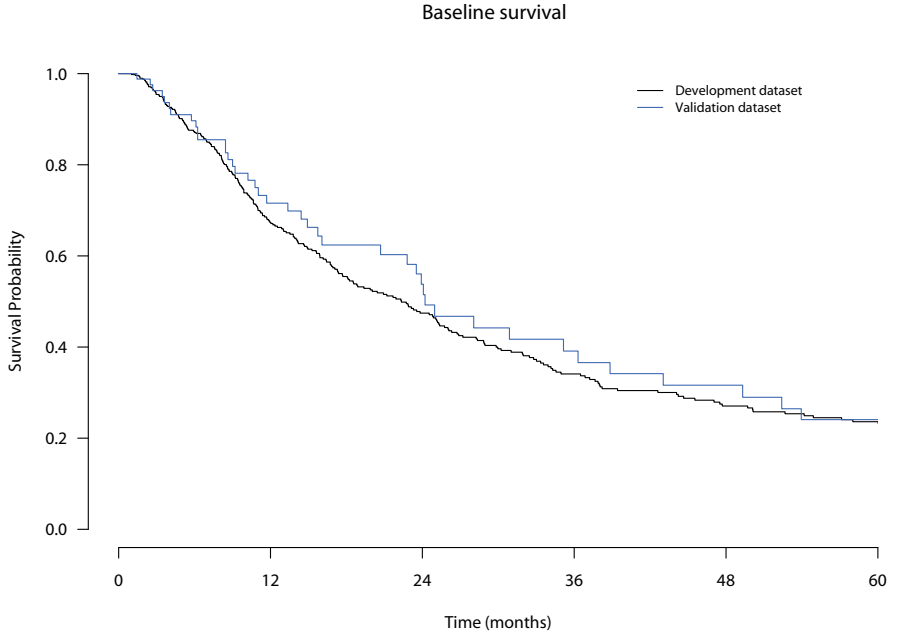
**Supplementary Figure 1.** Survival according to AJCC stage in the development cohort.



**Supplementary Figure 2.** Calibration plot of predicted versus observed survival at five years according to the AJCC staging model.
The blue line shows the actual (Y - axis) versus predicted (X - axis) survival of the patients according to the AJCC staging model.




**Supplementary figure 3.** Baseline survival in the development and validation cohorts.



# PART 4

## GENERAL DISCUSSION, SUMMARY AND APPENDICES

CHAPTER 10

# General discussion and future perspectives

The central aim of this thesis was to increase the knowledge of development, treatment and prognosis of gallbladder cancer in order to facilitate a more personalized treatment approach for gallbladder cancer patients. To this end, this thesis provides an overview of current knowledge on etiology and treatment approach of gallbladder and biliary tract tumors. These insights may improve care for gallbladder cancer patients by promoting early detection of gallbladder cancer and increasing awareness on treatment options amongst clinicians.

# ETIOLOGY: INFLAMMATION TO MUTATION AND ACTIONABLE GENES

Though chronic inflammation appears to be the primary culprit for gallbladder cancer development, the exact sequence of molecular events remains elusive. During chronic inflammation a multitude of inflammatory mediators such as growth factors, cytokines, reactive oxygen species and prostaglandins are at play which can all induce oncogenesis through (epi)genetic alteration of oncogenes or tumor suppressor genes. This is reflected by the finding that the rate of mutations in inflamed microenvironment vastly exceeds that of normal tissue. [1] *Salmonella* serovar Typhi bacteria can cause chronic inflammation of the gallbladder through the formation of biofilm on gallstones and are associated with an increased risk of gallbladder cancer. [2, 3] However, in **Chapter 2** the association between non-typhoid *Salmonella* infection and the development of biliary tract cancer in a Dutch cohort was studied and no significant correlation was found. Studies show that the tumor biology differs significantly per region; for example, gallstones appear to be the primary risk factor for gallbladder cancer in Chile whereas an anomalous pancreatobiliary duct junction (APBJ) is almost exclusively reported in Japan. [4-6] Gallstone-associated gallbladder cancer is often paired with mutations in the *AT-rich interaction domain 1A* (*ARID1A*) gene, whereas *KRAS* mutations are often found in APBJ-associated gallbladder cancer.

# MOVING FORWARD; TRENDS IN TREATMENT

Gallbladder cancer treatment has typically consisted of radical surgery in resectable patients and palliative chemotherapy in patients with non-resectable or metastatic disease. **Chapter 4** demonstrates how treatment of gallbladder cancer patients in the Netherlands has changed in the past decade. Therapy appears increasingly aggressive, as the rate of extended surgery and use of (palliative) chemotherapy rose significantly. Unfortunately, these treatment changes do not appear to have a significant positive effect on overall survival. The lack of improvement in survival despite changing treatment strategies is a world-wide issue and displays the urgent need for more effective treatment strategies in order to improve the grim prospects of gallbladder cancer patients. [7]

## CANDIDATE SELECTION FOR SURGERY

The primary factor in ensuring improved survival outcomes after surgery is appropriate selection of surgical candidates. [8] Prognosis after surgery is primarily determined by depth of invasion and the presence of lymph node metastases. Currently, most guidelines recommend performing at minimum a contrast-enhanced multidetector CT (MDCT) of the abdomen to assess tumor stage, supplemented by contrast-enhanced MRI with magnetic resonance cholangiopancreatography (MRCP) if additional clarity regarding matters such as bile duct invasion is necessary. [9-11] In **Chapter 3,** we show that both MDCT and MRI appear relatively unreliable in the detection of lymph node metastases and highlight the need for more accurate imaging methods. PET-CT is a novel tool that appears to be valuable modality for the detection of lymph node metastases in other cancers. [12] Although detection of gallbladder cancer lymph node metastases by PET-CT is unfortunately subpar compared to MDCT, it appears to be a viable tool to detect occult metastatic disease which may be missed by MDCT. [13-15] In addition, maximum standardized uptake values (max SUV) are a prognostic factor survival after surgery and may be an additional tool to help guide treatment strategy. [16, 17 18]

Beyond tumor invasion, several other clinical factors may influence candidate selection for surgery. Obstructive jaundice as a presenting symptom has traditionally been regarded as a sign of advanced disease and associated with extremely poor outcome. Generally, jaundice is considered to preclude surgery and **Chapter 6** contests this belief.

We found that, although jaundice is a poor prognostic factor in all patients whom are potential candidates for surgery, jaundice is not an adverse prognostic factor in patients that actually underwent surgical resection. Our results show that with careful candidate selection for surgery within jaundiced patients, favorable survival outcomes can be obtained. Another study showed similar results and in addition found that when CA19.9 levels are low, survival outcomes after resection in jaundiced patients are favorable (50 months, vs. 14 months in patients with high CA 19.9).[19] To accommodate the need for the identification of additional prognostic factors, studies are looking into the validity of using biomarkers in combination with clinical characteristics to improve candidate selection for surgery. A recent meta-analysis shows that in addition to CA19.9, cancer antigens CEA and CA 242 have prognostic value for cancer recurrence after surgery.[20, 21] In the past few years, researchers have shown interest in analyzing the expression of certain genes involved in oncogenesis and prognosis through the detection of associated micro-RNA (miRNA) expression.[22] This research has provided several evaluable miRNA's such as miR-20a (involved in epithelial to mesenchymal transition) and miR-34a (involved in potentiation of DNA damage response and apoptosis) which can be used to estimate prognosis. Unfortunately, most studies investigating miRNA have been conducted using small cohorts of patients with primarily advanced disease. Future research should focus on patients with early-stage disease in order to validate the value of aforementioned biomarkers in selecting patients for surgery.

## EXTENT OF SURGERY AND CENTRALIZATION OF CARE

Survival of surgically treated gallbladder cancer patients has shown gradual improvement throughout the 21st century, which experts attribute to an increasingly aggressive surgical approach as well as advancements in surgical techniques and postoperative care in general.[23-25] Yet, the optimal extent of resection in both early (incidental) and advanced gallbladder cancer remains controversial.

Most patients with early (T1b/T2) gallbladder cancer are diagnosed incidentally (iGBC), after cholecystectomy for benign gallbladder disease. Treatment of early gallbladder cancer involves (re)resection of the gallbladder bed and the hepatoduodenal ligament as this most likely improves survival.[9] Surprisingly, in the Netherlands re-resection is only conducted in 23% of patients with iGBC (demonstrated in **Chapter 8**) whereas this percentage exceeds 80% in other countries.[26, 27] Although we were unable to assess the reason for not performing a re-resection, physician unawareness and ambivalence towards the efficacy of the procedure likely significantly contribute to the small number of re-resections in the Netherlands. It is important to note that non-adherence to

guidelines is not unique to gallbladder cancer but rather poses a problem in many rare cancers. [28] Physicians' lack of access to reliable information on diagnosis and treatment options results in higher rates of misdiagnosis and improper treatment management. [29] In recent years, centralization of care and (inter)national research collaboration have been successful tools in counteracting these challenges and improving outcomes. [28] The establishment of the DGCC is a first step to hopefully raise awareness of treatment options amongst Dutch clinicians and to incentivize nation-wide collaborative research efforts. An example of the potential of this collaborative is the OptiGO-trial, a multicenter cohort study with over 20 participating study sites. In this ongoing study, the role of additional surgery in patients with iGBC will be assessed.

Centralization of care has also proven to be a highly effective method in reducing morbidity and mortality associated with complex surgery. This is an especially pressing issue in patients with advanced gallbladder cancer, since extended procedures such as hepatopancreatoduodenectomy are often required to achieve tumor-free margins. [9] Unfortunately, morbidity and mortality rates after extended hepatobiliary resections in the Netherlands appear significantly higher than those reported by Japanese studies and outcomes remain poor (**Chapter 5**). [30, 31] The difference in morbidity and mortality can be explained by the fact that extended resections for advanced gallbladder cancer are a much rarer occurrence in the Netherlands compared to Japan. For example, in the Netherlands only 33 patients received extended surgery during a 15 year period across the entire nation whereas in the study by Shimada et al. a total of 126 patients was accrued in one center over a mere 10 year period. It is very likely that further centralization of care (potentially even on a European level) is likely to improve outcomes of patients with advanced, resected gallbladder cancer. [32, 33]

### SYSTEMIC THERAPY

In spite of valiant research efforts, much is still unclear regarding systemic treatment for patients with resected biliary tract cancer. Of all traditional cytotoxic therapies that have been investigated in the adjuvant setting, only capecitabine appears to effect survival. [34, 35] Potentially, RCT's investigating other agents had insufficient statistical power to show a significant survival difference or cytotoxic therapy may only be effective in patients with high risk features such as advanced T-stage or lymph node metastases (**Chapter 7**). At the time of writing of this thesis, multiple ongoing RCT's are investigating the association between certain prognostic factors and response to chemotherapy. Those results hopefully will provide tools for a more tailored approach to select candidates for chemotherapy. Since the publication of the ABC-02 trial in

2010, gemcitabine-cisplatin is considered standard of care in patients with advanced, unresectable biliary tract cancer as it significantly improves survival. Based on the assumption that gemcitabine-cisplatin may also be effective in the adjuvant setting, the ACTICCA-1 trial is an RCT investigating the efficacy of this regimen compared to capecitabine in patients with non-metastatic, resected biliary tract cancer. Randomization will be stratified according to lymph node status to account for the influence of lymph node positivity on survival. Hopefully, the ACTICCA-1 and similar RCT's will lead to the identification of adjuvant, systemic treatment strategies that reduce recurrence rates and improve overall survival.

# PROGNOSIS

Estimation of outcomes is of major importance in the field of oncology for physicians, patients and family members. The ability to reliably predict prognosis greatly increases patient autonomy and has proven helpful in the process of deciding between multiple treatment options. A realistic view of outcomes in light of complications may affect the decision to undergo surgery or to forgo treatment. In **Chapter** 8 we describe that the presence of residual disease (RD) after re-resection in patients with incidental gallbladder cancer severely impacts survival. Pre-operative identification of patients at risk for RD would expediate candidate selection for surgery and potentially prevent redundant re-resections. Although we only identified T- and N- stage as a predictive factor for the presence of residual disease, other studies found that irradical resection margins and the presence of perineural and lymphovascular invasion are also predictive factors. These findings highlight the importance of comprehensive histopathological assessment and reporting of resection specimens. Unfortunately, assessment of histopathological characteristics in biliary tract tumors is a complex task and as a result factors such as margin status or the presence of perineural invasion are insufficiently analyzed and reported. [36] Involvement of expert hepatobiliary pathologists and standardization of reporting greatly improves the quality of histopathology reports, and should be facilitated through the aforementioned centralization of care. More information in histopathological reports may impact clinical practice and treatment choices. If the results from adjuvant chemotherapy in patients with biliary tract cancer can be extrapolated to patients with incidental gallbladder cancer, high-risk features could be used to select candidates for systemic therapy.

Proper estimation of outcomes is not only important for treatment stratification but helps in counseling patients about their expected prognosis. This knowledge will help patients whom often have to make impactful decisions on personal and work-related matters, with consequences not only for themselves but also for those close to them. [37] To meet these demands, prediction models for survival have been created, however, no easily accessible tool for clinical use has been developed for gallbladder cancer so far. In **Chapter 9** we propose a simple to use, effective prediction model for survival after resection of gallbladder cancer based on T- and N- classification alongside several other histopathological characteristics. Although the proposed model is of reasonable quality, the margin of error in predicted survival rates is still considerable. This is a recurring issue in all prediction models for gallbladder cancer and is likely caused by the incorporation of only a limited set of prognostic factors. In other cancers, high-quality prognostic models have incorporated molecular markers associated with prognosis to predict survival, but this data is lacking for gallbladder cancer.

The few studies that have been performed however do show that there are several molecular markers which can be used to predict survival. For example, expression of ADAM-17 (also known as tumor necrosis factor-alpha converting enzyme), is significantly higher in patients with high-grade and higher pT-stage tumors compared to those with low-grade and lower pT-stage tumors. Increased expression of ADAM-17 was also correlated with significantly reduced overall survival. [38] Other prognostic markers include E-cadherin, CD24, CD133, CD147 and epidermal growth factor receptor (EGFR) expression. [39-42] Some of these markers (CD24, CD133) also predict response to chemotherapy. Finally, the latest research has been focusing on not only prognostic markers, but also the identification of actionable targets for focused, molecular therapy. Although gallbladder cancer is an extremely heterogeneous tumor, around 80% of patients will carry mutations in actionable genes such as A*RID1A, BRAF, EGFR, ERBB2-4, and TP53.* [43, 44] [45] Some gallbladder cancers express EGFR, Vascular Endothelial Growth Factor (VEGF) or HER2-neu, all mutations for which targeted agents are already available. [46, 47] There is an increasing number of trials investigating the efficacy of targeted therapy in gallbladder cancer. [48, 49] Whilst results so far have been mixed, mutation-matched personalized therapy is likely to improve patient outcomes and should be the primary focus of clinical trials investigating the efficacy of adjuvant treatment for gallbladder cancer patients moving forward. [50]

# FUTURE PERSPECTIVES

To promote research and improve care for gallbladder cancer patients, the Dutch Gallbladder Cancer Collaborative (DGCC) was founded in 2018. The DGCC is a multidisciplinary group of medical specialists and researchers involved in the study and management of gallbladder cancer. The DGCC collaborates closely with the Dutch Hepatocellular and Cholangiocarcinoma Group (DHCG); a Dutch research group for all hepatobiliary tumors. As of 2020, members of the DGCC have published multiple papers in scientific journals using nation-wide data. Additionally, the DGCC coordinates prospective registry studies for patients with gallbladder cancer (TULYP) and gallbladder polyps (POLYP). These studies aim to collect data on clinical outcomes as well as tissue samples for molecular analysis and have included over 350 patients as of August 2020. Although collaboration on a nation-wide level gives rise to a number of research opportunities, the incidence of gallbladder cancer is of such a low level that it is still difficult to perform adequately powered prospective research. The ultimate objective of the DGCC is therefore to join existing international collaborations involved in gallbladder cancer research. Large, global research networks are the ultimate tool to facilitate high-quality studies and to eventually improve the prospects of gallbladder cancer patients.

# CONCLUSION

Despite general improvement in oncological care, the prognosis of patients with gallbladder cancer remains poor and has not increased significantly in the past decade. Identification of risk factors and improved imaging techniques are required to facilitate early diagnosis. Aggressive surgery including liver resection is increasingly being used as a means of reducing recurrence rates and improving survival. Adjuvant chemotherapy, although potentially beneficial, is currently not yet considered standard of care in the Netherlands. Identification of high-risk patients whom may benefit from cytotoxic therapy and potential candidates for targeted therapy is a next step in improving outcomes of patients with gallbladder cancer after surgery. Known histopathological high-risk features include advanced T-classification, the presence of lymph node metastases and irradical resection. Future research should aim to identify additional, molecular prognostic factors which can be used to provide a personalized risk assessment and tailored treatment approach for individual gallbladder cancer patients. To meet this end, international collaboration is an essential tool to expedite research efforts and facilitate adequately powered RCT's.

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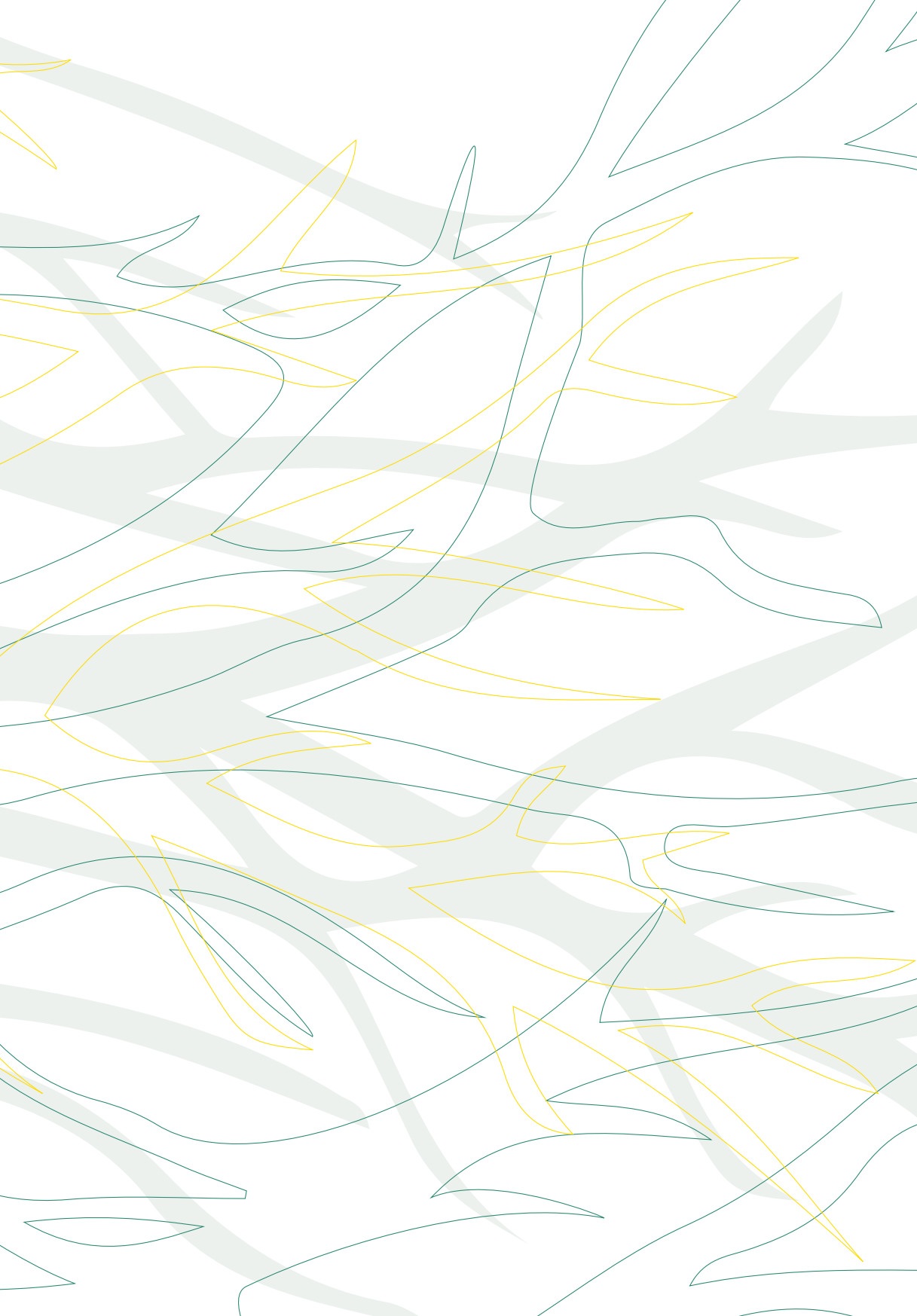

CHAPTER 11

# SUMMARY

# NEDERLANDSE SAMENVATTING

# SUMMARY

Gallbladder cancer is a rare disease with a dismal prognosis, caused by a combination of late detection and aggressive tumor biology. Although cancer survival in general has seen an upward trend in the past decade, survival of gallbladder cancer patients regrettably remains stagnant. The low incidence of the disease, especially in a resectable stage, makes it challenging to perform high-quality research. This thesis describes a multi-faceted effort to increase knowledge on gallbladder cancer etiology, treatment and prognostic factors with the ultimate aim of optimizing patient selection and improving treatment outcomes.

## PART I: ETIOLOGY AND DIAGNOSIS

Timely cancer diagnosis is critical to ensure favorable survival outcomes as tumors limited to the gallbladder without invasion into the liver have the best prognosis. The identification of risk factors has facilitated screening and early detection for certain cancers; a prime example is the screening program for cervical cancer, which is commonly caused by infection with the human papilloma virus. Although infectious agents are thought to be responsible for around 20% of the global cancer burden, little is known about the potential correlation between bacterial infections and risk of cancer. In **Chapter 2**, we investigated the correlation between infection with *Salmonella* or *Campylobacter* infection and the development of biliary tract cancer. Both species are capable of inflicting damage to the host cell's DNA through various mechanisms and have been associated with various forms of cancer. However, we were unable to demonstrate a significant correlation between infection with *Salmonella* or *Campylobacter* and the development of biliary tract cancer later in life. This finding may be especially surprising in *Salmonella* patients given the fact that many studies originating from non-Western countries show that *Salmonella* infection is a significant risk factor for the development of gallbladder cancer. Likely, the lack of significant correlation in our study is caused by the fact that the etiology of gallbladder and other biliary tract cancers differs per region.

In patients with potentially resectable gallbladder cancer, elaborate pre-operative assessment is necessary in order to establish whether a patient will benefit from curative intent surgery. Some factors, such as the presence of lymph node metastases or extensive invasion of surrounding organs, will render surgery futile as it will not provide a survival benefit if these factors are present in a patient. It is still unclear which imaging strategy proves to be the most reliable for pre-operative assessment of tumor

invasion. **Chapter 3** is a meta-analysis that elaborates on the value of MRI comparative to CT as a diagnostic tool for lymph node metastases in gallbladder cancer. Nine studies were included for narrative review and five studies were included for meta-analysis; four assessing MRI and one assessing CT. Sensitivity for the detection of lymph node metastases ranged from 0.25 to 0.93 in CT, from 0.60 - 0.92 in MRI and did not differ between the two modalities. Small (<10mm) lymph node metastases were most frequently missed by both CT and MRI. Novel imaging strategies may be better able to detect (small) lymph node metastases and improve candidate selection for surgery.

## PART II: SURGICAL AND SYSTEMIC TREATMENT

Guidelines for the treatment of gallbladder cancer are primarily based on data derived from small, high-volume single center cohort studies which do not necessarily reflect outcomes of contemporary clinical practice. Population-based studies often include much larger, more varied cohorts. Outcomes may be closer to reality and are vital to identify opportunities for improvement. However, population-based data on outcomes of patients with gallbladder cancer are scarce, especially from Western regions. In **Chapter 4** we describe the outcomes of over 1800 patients with gallbladder cancer in the Netherlands and survival between two time periods (2005-2009 and 2010-2016) is compared. The primary finding of this study is that only a very slight improvement in survival was seen across a period of over a decade, as median overall survival only improved from 5 months (2005-2009) to 6 months (2010-2016)($P<0.001$). Radical resection rates in early (T1b/T2) gallbladder cancer increased (12% to 26%) and radical resection was also associated with improved survival (re-resection 77 months vs. no re-resection 12 months, $P<0.001$). In patients with metastatic disease, administration rates of palliative chemotherapy rose significantly (11% to 29%) and use of palliative chemotherapy was also associated with higher median survival (chemotherapy 7 months vs. no chemotherapy 2 months, $P = 0.001$). These data show that although both radical surgery and palliative chemotherapy appear to improve prognosis, these treatment modalities remain under-utilized. A multi-disciplinary approach and the involvement of expert physicians during treatment may improve the prospects of gallbladder patients.

Extended resections (i.e. major hepatectomy or hepatopancreatoduodenectomy) are often required in patients with advanced gallbladder cancer in order to obtain tumor-free resection margins. However, surgeons are hesitant to perform these extensive procedures as it is unknown whether they actually provide a survival benefit,

especially in light of the high associated postoperative morbidity and mortality. Data on outcomes, especially from Western countries, are lacking. In **Chapter 5** we report on the outcomes of 33 patients with gallbladder cancer that underwent extended resection in the Netherlands. Radicality was achieved in sixteen (48%) patients. Major postoperative complications occurred in nineteen (58%) of patients and four (12%) patients died within 90 days postoperatively. Median overall survival was 13 months. Ten patients (30%) survived over 2 years postoperatively. Poor prognostic factors were common hepatic duct or liver invasion, perineural/perivascular growth and jaundice. Although morbidity and mortality are high, radical surgery may be beneficial in a select subset of patients and may provide a chance at long-term survival.

Jaundice is traditionally regarded as a poor prognostic factor and is thought to preclude surgery. Recent studies show that in some jaundiced patients, however, long term survival after resection is possible. **Chapter 6** evaluates the influence of obstructive jaundice on resectability rates and survival in patients with gallbladder cancer that underwent surgical exploration. The cohort consisted of 202 patients; 124 non-jaundiced patients (104 resected) and 75 (44 resected) jaundiced patients. We found in that in the group of jaundiced patients, there were higher rates of irresectable disease, extended resections, major postoperative complications, postoperative mortality and margin-positive resection. Median survival in jaundiced patients was also lower than in non-jaundiced patients (8 months vs. 26 months, $P<0.001$). However, in the cohort of patients that eventually underwent curative intent surgery, jaundice was not a poor prognostic factor in multivariable analysis. Rather, it appears that the presence of lymph-node or liver invasion and irradical resection are independent predictors of poor survival. These factors are all also associated with the presence of jaundice. In jaundiced patients with limited disease and no hepatic duct invasion on imaging, surgery should be considered as a potentially curative treatment option.

In **Chapter 7**, we analyzed the value of adjuvant chemotherapy (aCT) for patients with resected biliary tract cancer. Currently, it is questionable whether aCT provides a survival benefit, especially in patients with tumor-negative resection margins and no lymph node metastases. We analyzed the efficacy of aCT by using data from the SEER registry, the nationwide cancer registry of the United States, and linked these data to data from Medicare, the national insurance provider database. Propensity score matching and exclusion of patients whom died within 30 days postoperatively were used to account for treatment selection bias. In total, we included 1554 matched patients for survival analysis (813 patients with aCT, 741 patients without aCT). Median

overall survival was 24.3 months and did not differ between treatment groups (aCT 24.3 months, no aCT 24.2 months). However, in patients with T3/T4 or node-positive disease, survival was significantly longer in patients who received aCT (T3/T4 18.5 vs. 12.4 months, P<0.01 and N1/N2 20.5 vs. 13.3 months, P<0.001). In order to identify clinically relevant subgroups of patients with potential high response rates, we performed interaction analyses, combining T-stage, N-stage and differentiation grade. This analysis revealed that benefit of aCT was only seen in patients with T3/T4, node-positive disease. Our results show that aCT should be administered selectively and may only be beneficial in patients with high-risk features.

## PART III: HISTOLOGY AND PROGNOSIS

Guidelines recommend re-resection of the gallbladder bed and hepatoduodenal lymph nodes for all patients with incidentally diagnosed, muscle-invasive gallbladder cancer. Survival after re-resection varies greatly and little is known about prognostic factors in re-resected patients. **Chapter 8** describes survival, the incidence of residual disease and prognostic factors of patients with incidental gallbladder cancer that underwent re-resection. Patients were identified through the Netherlands Cancer Registry. In total, 463 patients were included, and 110 (24%) underwent re-resection. Median overall survival was 13.7 months in patients that did not undergo re-resection, compared to 52.6 months in re-resected patients. Residual disease was found in 35% of patients that underwent re-resection and was associated with poor survival after re-resection (residual disease 23.1 months versus median OS not reached in patients without residual disease). Pre-operative factors predictive for the presence of residual disease were high T-stage (T3 disease) and lymph-node positivity. We concluded that re-resection, although associated with improved survival, is only performed in a minority of patients with incidental gallbladder cancer. Presence of residual disease is the primary indicator for prognosis and is more prevalent in patients with T3, node-positive disease.

Accurate estimation of median survival after surgery is of vital importance for proper patient counseling, shared decision making and risk stratification for clinical trials. The AJCC staging system, currently the primary method to estimate survival, is criticized since it has poor discriminatory capacity and only includes a limited set of prognostic factors. In **Chapter 9**, we developed a novel dynamic prediction tool to estimate survival after resection of non-metastatic gallbladder cancer based on several histopathological prognostic factors. A nation-wide, multi-institute Dutch cohort of 380 patients with resected gallbladder cancer was used to establish the model and the model was validated in an external dataset of 66 Australian patients treated in a tertiary

referral center. Age, T/N classification, resection margin, differentiation grade and angio-invasion were independent predictors of median OS. The discriminative ability of the model was superior to the ability of the AJCC staging system (C-index 0.71 versus 0.59). External validation showed good discriminatory capacity (c-index 0.74) and reasonable calibration with slight underestimation of survival chances in medium-risk patients. This shows that the proposed model is a useful tool for physicians and patients to obtain an accurate estimation of survival chances after resection.

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# NEDERLANDSE SAMENVATTING

Galblaaskanker is een zeldzame, agressieve tumorsoort die vaak pas in een laat stadium wordt ontdekt. Patiënten met galblaaskanker overleven gemiddeld tot slechts zes maanden na de diagnose. Hoewel de overleving van kanker in het algeheel de afgelopen decennia duidelijk verbeterd is, is dit bij galblaaskanker helaas niet het geval. Omdat de ziekte erg weinig voorkomt (en vaak ook niet in een stadium waarin het nog te genezen is met een operatie) is het erg moeilijk om er onderzoek naar te doen. Dit proefschrift biedt een overzicht van risicofactoren voor het ontstaan van galblaaskanker, de behandeling ervan en factoren die van invloed zijn op de overleving. Deze kennis zal bijdragen aan de verbetering van de zorg voor patiënten met deze zeldzame vorm van kanker.

## DEEL I: ETIOLOGIE EN DIAGNOSE

Een tijdige diagnose is van het grootste belang voor een goede overleving; tumoren die zich slechts beperken tot de wand van de galblaas hebben de beste prognose. Bij andere vormen van kanker heeft het identificeren van risicofactoren en het opstellen van screeningsprogramma's geleid tot een forse verbetering van de overleving. Een bekend voorbeeld is het nationale screeningsprogramma voor baarmoederhalskanker, dat wordt veroorzaakt door een infectie met het humaan papillomavirus. Hoewel er wordt gedacht dat infectieziekten wereldwijd een op de vijf kankergevallen veroorzaken, is er zeer weinig bekend over hoe bacteriële infecties precies het risico op kanker verhogen. In **Hoofdstuk 2** hebben wij onderzocht of er een verband bestaat tussen een infectie met *Salmonella* en *Campylobacter,* bacteriën die voor darminfecties zorgen, en het ontwikkelen van een galweg- of galblaastumor. Beide bacteriesoorten kunnen het DNA van de binnengedrongen cel beschadigen en zijn reeds geassocieerd met andere vormen van kanker zoals darmkanker. In dit onderzoek werd bij 16.000 patiënten met *Salmonella* en 27.000 patiënten met *Campylobacter* geen verhoogd risico op een galwegcarcinoom gezien. Met name bij patiënten met een *Salmonella* infectie is deze uitkomst opvallend. Veel onderzoeken uit niet-Westerse landen laten namelijk zien dat *Salmonella* juist een significante risicofactor is voor het ontwikkelen van galblaaskanker. Ons onderzoek toont aan dat de ontstaanswijze van galblaas- en galwegkanker per geografische regio aanzienlijk kan verschillen.

Zo'n 20% van de patiënten met galblaaskanker komt in aanmerking voor een operatie. Om te bepalen of een chirurgische resectie daadwerkelijk zinvol is, moet voor een operatie beeldvormend onderzoek verricht worden. Op die manier kan

de uitgebreidheid van de ziekte vastgesteld worden. Als er sprake blijkt te zijn van lymfekliermetastasen of uitgebreide ingroei in omliggende organen is een operatie niet zinvol meer omdat deze behandeling dan geen overlevingsvoordeel biedt. Het is onduidelijk welke beeldvormingstechniek het meest gevoelig is om tumorgroei vast te stellen. **Hoofdstuk 3** is een meta-analyse waarin de gevoeligheid van een CT (beeldvormend onderzoek met röntgenstraling) vergeleken wordt met MRI (beeldvormend onderzoek met magnetische stroom) om lymfekliermetastasen op te sporen. In totaal zijn er negen studies geïncludeerd voor beschrijvende review en werden er vijf geïncludeerd voor meta-analyse; vier MRI-onderzoeken en een CT-onderzoek. De sensitiviteit voor de detectie van lymfekliermetastasen varieerde van 0.25 tot 0.93 bij CT en van 0.60-0.92 bij MRI. Een verschil tussen beide modaliteiten werd niet aangetoond. Door zowel CT als MRI werden kleine (<10mm) lymfekliermetastasen meestal gemist. Nieuwere beeldvormingstechnieken (zoals nano-MRI) zijn wellicht beter in staat om lymfekliermetastasen op te sporen en zo de patiëntselectie voor chirurgie te verbeteren.

## DEEL II: CHIRURGISCHE EN SYSTEMATISCHE BEHANDELING

Richtlijnen voor de behandeling van galblaaskanker zijn vaak gebaseerd op uitkomsten van onderzoeken met kleine aantallen patiënten. Ook zijn deze patiënten vaak behandeld in gespecialiseerde centra en zijn daarmee niet per definitie een accurate reflectie van de algehele klinische praktijk. Landelijke databases bevatten vaker data over veel grotere cohorten met patiënten die op verschillende wijze zijn behandeld. De onderzoeksuitkomsten van deze data zijn vaak beter te vergelijken met de realiteit en zijn essentieel om mogelijkheden voor verbetering te identificeren. Gegevens op nationaal niveau over patiënten met galblaaskanker zijn echter schaars, vooral uit Westerse landen. In **Hoofdstuk 4** beschrijven we de uitkomsten van meer dan 1800 patiënten met galblaaskanker in Nederland. De overleving in twee tijdsperiodes (2005-2009 en 2010-2016) werd met elkaar vergeleken. De voornaamste bevinding is dat er in tien jaar tijd slechts een minieme verbetering van de overleving werd gezien; de mediane overleving steeg van vijf maanden (2006-2009) naar zes maanden (2010-2016). Meer patiënten met vroege galblaaskanker (tot de spierlaag beperkt) ondergingen een radicale operatie (12% vs. 26%), wat betekent dat ook de lymfeklieren en het galblaasbed in de lever zijn verwijderd. Patiënten die een radicale operatie ondergingen leefden gemiddeld significant langer (77 vs. 12 maanden ). Bij patiënten die niet in aanmerking kwamen voor een operatie werd palliatieve chemotherapie vaker toegepast (11% tussen 2007-2009 versus 29% tussen 2010-2016). Patiënten die palliatieve chemotherapie ondergingen leefden langer (7 maanden) dan patiënten

die dit niet kregen (2 maanden). Deze uitkomsten tonen aan dat alhoewel radicale chirurgie en palliatieve chemotherapie mogelijk bijdragen aan een betere overleving, deze behandelmodaliteiten slechts zelden worden toegepast. Een multidisciplinaire benadering in combinatie met het betrekken van een gespecialiseerd behandelteam is essentieel om de uitkomsten van patiënten met galblaaskanker te verbeteren.
Bij patiënten met grote galblaastumoren zijn uitgebreide resecties, een hemi-hepatectomie of een hepatopancreatoduodenectomie, noodzakelijk om de tumor compleet te verwijderen. Het is niet bekend of zulke uitgebreide chirurgie ook daadwerkelijk leidt tot een langere overleving. Gezien de onduidelijke winst en de aanzienlijke risico's zijn chirurgen terughoudend om deze operaties uit te voeren. In **Hoofdstuk 5** beschrijven wij de uitkomsten van 33 Nederlandse patiënten met galblaaskanker die een uitgebreide resectie hebben ondergaan. Bij 16 (48%) patiënten kon de tumor in zijn geheel worden verwijderd. Negentien patiënten (58%) kregen een ernstige complicatie en vier patiënten (12%) overleden binnen 90 dagen na de operatie. De mediane overleving was 13 maanden en tien patiënten (30%) leefden langer dan twee jaar. Slechte prognostische factoren waren tumorgroei in de galwegen of lever, perineurale/perivasculaire groei en geelzucht. Hoewel de risico's hoog zijn, kan uitgebreide chirurgie aan een selecte groep van patiënten toch een kans bieden op overleving op de lange termijn.

Icterus (geelzucht) wordt traditioneel gezien als een uiterst slechte prognostische factor voor overleving in patienten met galblaaskanker. Icterus is een mogelijke contra-indicatie voor chirurgie. Recente studies tonen aan dat bij sommige patiënten met icterus een operatie zinvol kan zijn en de overleving kan vergroten. **Hoofdstuk 6** beschrijft de invloed van obstructieve icterus op de kans op resectie en overleving bij patiënten met galblaaskanker die een chirurgische exploratie hebben ondergaan. Het cohort bestond uit 202 patiënten; 124 patiënten zonder icterus (waarvan 104 geopereerd) en 75 patiënten met icterus (waarvan 44 geopereerd). In de groep icterische patiënten was er vaker sprake van niet-resectabele ziekte, grotere operaties, ernstige postoperatieve complicaties, hogere mortaliteit en irradicaliteit. De overleving van patiënten met icterus was ook slechter dan bij patiënten zonder icterus (8 maanden vs. 26 maanden). Echter, in de subgroep van patiënten die uiteindelijk daadwerkelijk een in opzet curatieve resectie hebben ondergaan was icterus geen onafhankelijke voorspeller van een slechte overleving. Het blijkt dat de aanwezigheid van ingroei in de lymfeklieren of lever en irradicale resectie wel onafhankelijk geassocieerd waren met een slechte prognose. Deze factoren zijn ook geassocieerd met de aanwezigheid van icterus. Bij patiënten met een obstructie icterus kan chirurgie een kans op curatie

bieden indien er sprake is van beperkte verspreiding van de tumor op pre-operatieve beeldvorming en geen ingroei in de ductus choledochus.

Het is onduidelijk of adjuvante chemotherapie (aCT) een overlevingsvoordeel biedt, zeker bij patiënten zonder lymfeklieruitzaaiingen waarbij de tumor geheel is verwijderd. In **Hoofdstuk 7** hebben wij de waarde van aCT bij patiënten met gereseceerde galwegkanker onderzocht. Hiervoor hebben we gebruik gemaakt van het SEER-register, de nationale kankerregistratie van de Verenigde Staten. Deze data hebben wij gelinkt met data van Medicare, de nationale database met verzekeringsdeclaraties. Propensity score matching en de exclusie van patiënten die 30 dagen na de operatie overleden zijn gebruikt om de invloed van bias te verminderen. In totaal zijn er 1554 gematchte patiënten geïncludeerd; 813 met aCT en 741 zonder aCT. De mediane overleving was 24 maanden en verschilde niet tussen beide groepen. Echter, de overleving na chemotherapie was wel significant langer in de subgroep van patiënten met grote (T3/T4) tumoren (19 vs. 12 maanden) en lymfeklieruitzaaiingen (21 vs. 13 maanden). Om klinisch relevante subgroepen te identificeren van patiënten met potentiele goede kansen op een respons hebben we interactieanalyses uitgevoerd, waarin T-stadium, lymfeklierstadium en differentiatiegraad werden gecombineerd. Deze analyse toont aan dat aCT alleen een overlevingsvoordeel biedt aan patiënten met T3/T4 tumoren met lymfekliermetastasen. Onze resultaten tonen aan dat chemotherapie slechts selectief dient te worden gebruikt en zinvol kan zijn in patiënten met uitgebreide ziekte en een hoge kans op recidief.

## DEEL III: HISTOLOGIE EN PROGNOSE

Richtlijnen bevelen een re-resectie aan van het galblaasbed en de lymfeklieren in het hepatoduodenale ligament bij alle patiënten met een per toeval gevonden (incidentele) galblaastumor, die doorgroeit tot ten minste de spierlaag ( stadium ≥T1b). Er is slechts weinig kennis over de overleving en prognostische factoren na een re-resectie. In **Hoofdstuk 8** worden de kansen op de aanwezigheid van tumorrest (residu) en prognostische factoren na een re-resectie beschreven. Patiënten in dit onderzoek zijn geïdentificeerd in het Nederlands Kanker Register. In totaal zijn er 463 patiënten geïncludeerd, waarvan er 110 (24%) een re-resectie hebben ondergaan. De mediane overleving van patiënten met een re-resectie was 53 maanden, vergeleken met 14 maanden bij patiënten zonder een re-resectie. Tumorresidu werd gevonden in 35% van de patiënten die een re-resectie hadden ondergaan en was ook geassocieerd met een slechtere overleving. Preoperatieve factoren die de aanwezigheid van tumorresidu voorspelden waren een hoog T-stadium (T3 ziekte) en positieve lymfeklieren. Dit

onderzoeksresultaat toont aan dat alhoewel re-resectie is geassocieerd met een verbeterde overleving, deze re-resectie slechts in een minderheid van de patiënten wordt uitgevoerd. De aanwezigheid van tumorresidu is de belangrijkste voorspeller voor een slechtere overleving en is geassocieerd met een hoger tumorstadium.
Betrouwbare schatting van de overleving na chirurgie is zeer belangrijk voor goede informatievoorziening, gedeelde besluitvorming met patiënten en ook voor risicostratificatie voor toekomstige klinische trials. Het American Joint Committee on Cancer (AJCC) stadiëringssysteem, momenteel de gangbare methode om een schatting te verkrijgen van de prognose, wordt bekritiseerd omdat het slechts een beperkte discriminerende waarde heeft en slechts gebruik maakt van een klein aantal prognostische factoren. In **Hoofdstuk 9** hebben wij een nieuw, dynamisch instrument ontworpen om de overleving van patiënten met een gereseceerde, niet-gemetastaseerde galblaastumor te voorspellen, gebaseerd op een aantal histopathologische factoren. Een multi-institutioneel cohort van 380 patiënten met galblaaskanker is gebruikt om het model te ontwikkelen. Hierna is het model gevalideerd in een extern cohort van 66 Australische patiënten met galblaaskanker, die behandeld zijn in een tertiair centrum. Leeftijd, T en N classificatie, resectiemarge, differentiatiegraad en angio-invasie waren onafhankelijke voorspellers voor de overleving. De discriminerende capaciteit van het model was superieur aan de capaciteit van het AJCC stadiëringssysteem (C-index 0.71 versus 0.59). Externe validatie toonde ook een goede discriminerende capaciteit (C index 0.74) en een redelijke kalibratie waarbij enige onderschatting van de overlevingskansen van gemiddeld-risico patiënten werd gezien. Dit toont aan dat het model een nuttige modaliteit is voor artsen en patiënten om een goede inschatting van de overleving na een operatie te verkrijgen.

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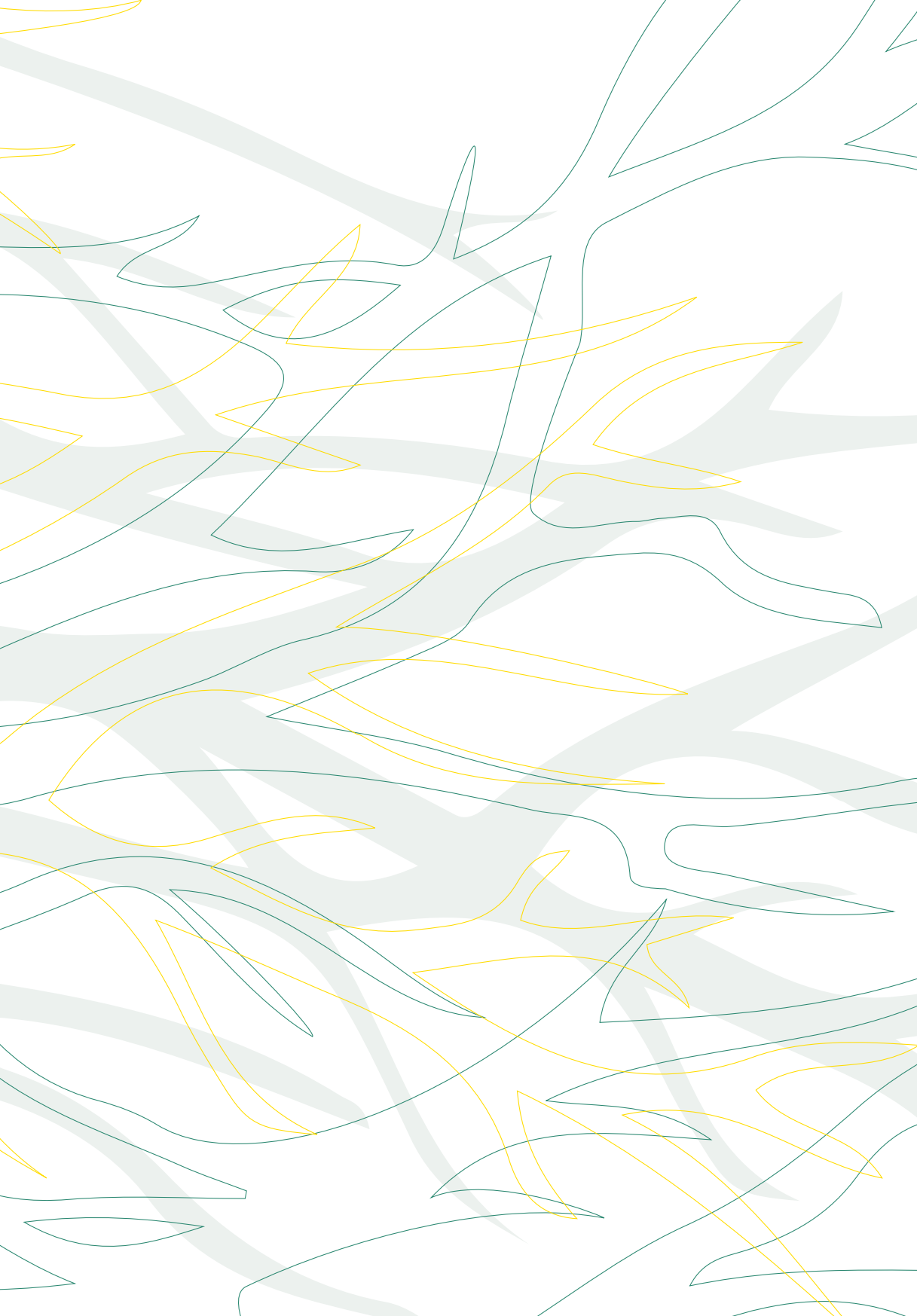

# APPENDICES

List of publications

Data management statement

PhD Portfolio

Dankwoord

About the Author

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**de Savornin Lohman EAJ,** de Bitter TJJ, Hannink G, Wietsma MFT, Vink-Börger E, Nagtegaal ID, Hugh T, Gill AJ, Bhimani N, Seyed Ahadi M, van der Post RS*, de Reuver PR*. Development and external validation of a model to predict overall survival in patients with resected gallbladder cancer: an international validation study. *Submitted.* * Shared senior authorship.

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# DATA MANAGEMENT STATEMENT

This thesis is based on the results of human studies, which were conducted in accordance with the principles of the Declaration of Helsinki. All studies have been approved by the appropriate ethics committee.

Anonymized data used for Chapter 2 is stored in a secure folder on the drives of Statistics Netherlands, accessible only by the (principal) investigator.

The protocol for the systematic review (Chapter 3) is registered with Prospero (record number 83752). Data on the results of the electronic search, screening process and risk of bias assessment is stored on local drives of the department of surgery of the Radboudumc: (H:)HEELdata.

Data from registry studies (Chapter 4, 7 and 8), including the Netherlands Cancer Registry, the nationwide network and registry for histo- and cytopathology in the Netherlands (PALGA) and the Surveillance, Epidemiology and End-Results project (SEER) were received through coded and secured environments, per protocol of the involved parties. Data were converted from excel to SPSS (Chapter 4 and 8) or SAS (Chapter 7) files and stored on the department server of the involved institution. Data from patient cohort studies (Chapter 5, 6 and 9) were anonymized and converted into SPSS files, which are stored on the server of the department of surgery of the Radboudumc.

The data will be saved for 15 years after termination of the research (December 1, 2020). Published datasets and analyses during these studies are available from the corresponding author, after approval of all parties involved, on reasonable request.

# PHD PORTFOLIO

**Name**
PhD candidate: E.A.J. de Savornin Lohman
**Department:** Surgery

**Graduate School:**
Radboud Institute for Health Sciences

**PhD period:**
01-09-2017 – 30-09-2020
**Promotor(s):**
Prof. C.J.H.M. van Laarhoven,
Prof. I.D. Nagtegaal

**Co-promotor(s):**
Dr. P.R. de Reuver,
Dr. R.S. van der Post

| | Year(s) | ECTS |
|---|---|---|
| **TRAINING ACTIVITIES** | | |
| **A) Courses & Workshops** | | |
| - Introduction day Radboudumc | 2017 | 0.5 |
| - RIHS introduction course | 2018 | 1 |
| - BROK course | 2018 | 2 |
| - Biometrics statistics course | 2017-2018 | 5 |
| - Scientific writing course | 2018 | 3 |
| - Basiscursus oncologie | 2018 | 4.5 |
| - The art of finishing up | 2019 | 1 |
| - Cochrane systematic review course | 2019-2020 | 4 |
| **B) Seminars & lectures** | | |
| - Radboud research rounds | 2017-2020 | 1 |
| - Weekly research meeting surgical department (attendance and 3x presentation) | 2017-2020 | 3 |
| - New Frontiers Symposium RIMLS | 2018 | 0.2 |
| - Research meeting pathology department (presentation) | 2018 | 0.5 |
| - Weekly research meeting department of surgery, Michigan Medicine | 2020 | 0.5 |

C) Symposia & congresses

| | | |
|---|---|---|
| Chirurgendagen, Nederlandse Vereniging voor Heelhunde, Veldhoven, including 1 presentation | 2018-2019 | 1.5 |
| RIHS PhD retreat | 2017 | 0.5 |
| Dutch Pancreatic Cancer Group, Pancreassymposium, Utrecht | 2017 | 0.1 |
| IHPBA World Congress, including 4 presentations (3 oral and 1 poster) | 2018-2020 | 2.25 |
| United European Gastroenterology Week, Vienna (including poster presentation) | 2018 | 1 |
| Najaarsvergadering Nederlands Vereniging voor Heelkunde, Ede (including oral presentation) | 2018 | 0.5 |
| Digestive Disease Days, Veldhoven (including oral presentation) | 2019 | 0.5 |
| EAPBBA Regional Congress, Amsterdam (including 3 oral presentations) | 2019 | 1.5 |
| Academic Surgical Congress, Houston (including 2 presentations, one oral and one poster) | 2019 | 1.25 |

D) Other

| | | |
|---|---|---|
| Dutch Hepatocellular and Cholangiocarcinoma group meeting attendance and website hosting | 2018-2020 | 1 |
| Exchange Scholar at Michigan Medicine | 2020 | 3 |

TEACHING ACTIVITIES

E) Lecturing

| | | |
|---|---|---|
| Lecture at the Regional Education meeting for surgical residents | 2018 | 0.2 |

F) Supervision of internships / other

| | | |
|---|---|---|
| - Supervision of Bachelors' students, 2 projects | 2018-2020 | 2 |
| - Supervision of Master's students, Marianne Wietsma, Mike van Dooren and Eva Brekelmans | 2018-2020 | 3 |

# DANKWOORD

Met het schrijven van deze pagina's is het einde dan toch echt daar. Ik had nooit verwacht dat ik van deze periode over zo veel meer zou leren dan wetenschap alleen. Zonder de hulp, inzet en het vertrouwen van vele anderen was dit proefschrift er nooit geweest. Ik wil graag iedereen bedanken die heeft bijgedragen en een aantal mensen in het bijzonder.

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Elise de Savornin Lohman was born on April 3rd, 1992 in Amsterdam. During her childhood, she liked to do acrobatic tricks with her friends whilst performing for circus Elleboog. In 2010, she obtained her Gymnasium degree (cum laude) at the Vossius Gymnasium and started Medical School at the Universiteit van Amsterdam.

During her student years, Elise discovered her love for surfing and joined surf student association D.E.R.M. In addition to early morning surf sessions before class, she became more involved with the society as an active committee-member. After obtaining her bachelor's degree, she wrote her masters' thesis on the treatment of metastatic breast cancer under the supervision of professor Sabine Linn (Antoni van Leeuwenhoek ziekenhuis). Before starting her clinical rotations, she moved to Lappeenranta, Finland for three months to do a clinical internship at the anesthesiology department. Directly after, she went to Malawi and volunteered at a local hospital for two months. Whilst in Malawi she discovered her interest in surgery, which was confirmed during her clinical rotations. In 2017, after finishing her senior internship at the surgical department of the AMC and Flevoziekenhuis, Elise started with her work as a full-time PhD student at Radboudumc under the supervision of prof. dr. Kees van Laarhoven, prof. dr. Iris Nagtegaal, dr. Philip de Reuver and dr. Chella van der Post. Her research focused on the treatment and outcomes of patients with gallbladder and biliary tract malig-nancies. Some of her daily activities included the coordination of the POLYP- and TULYP studies, two prospective registration studies for the collection of data on patients with gallbladder polyps and -malignancies. In addition, she collaborated with the Dutch Hepatocellular and Cholangiocarcinoma Group (DHCG) and also became their website operator.

Furthermore, at the beginning of 2020 Elise was fortunate to reside as a research scholar at Michigan Medicine in Ann Arbor, United States, under the supervision of dr. Hari Nathan. Her research focussed on combining data from the nation-wide cancer registry with insurance data to investigate the value of adjuvant chemotherapy in the treatment of resected biliary tract cancer.
Currently, Elise is working as a surgical resident not in training at the Erasmus MC under the supervision of dr. Sjoerd Lagarde and dr. Bas Wijnhoven. Besides her work, she enjoys running, road biking, climbing, surfing, spending time with friends and family and travelling.