1 Introduction

Gallbladder cancer (GBC) was first reported in 1777 (Lazcano-Ponce et al., 2001). Today it is most often found in two situations which are late stage with constitutional symptoms and positive imaging findings, and early stage, incidentally diagnosed after cholecystectomy for other disease. The incidence of GBC has declined over the last few decades and the survival time is also better than in the past. With the improvement of treatment and imaging techniques, the future promises more late stage cases will survive longer and more early stage cases will be discovered. We are optimistic this will lead to increased survival times on the whole and eliminate the current fear of GBC as a highly mortal cancer (Gourgiotis et al., 2008).

2 Epidemiology

Gallbladder cancer is uncommon in the US with an incidence of about 1.2/100,000, according to the Surveillance, Epidemiology, and End Results Program (SEER). The incidence of GBC during the last three decades has declined, primarily due to declining incidence among the elderly aged 65+ (Figure 1); while it has remained unchanged in the younger aged groups. GBC is rarely seen before age 35 and as seen in Figure 3, increases dramatically with age especially after the age of 60. Women are nearly 1.5 times more likely to get GBC than men.

The SEER database just has cancer data from 1975 to the most recent year of 2010. However Siegel et al made an effort to project estimated new cases for most of the cancers which will be diagnosed in 2014. From their efforts, GBC and extrahepatic bile duct cancers in 2014 are the seventh most common cancers in the gastrointestinal tract with a projected 10,650 new cases, higher than cancers in the small intestine and in the anus, anal canal and anorectum, but dwarfed by projected colon cancer cases which are approximately 9.5 times greater than GBC and extrahepatic bile duct cancers, (Figure 2) (Siegel et al., 2014).

In the US, Hispanic and American Indian/Alaska Native have greater incidence rates than other ethnic groups. Some of the reasons potentially behind this variance in ethnic incidence will be discussed below.

Fifty percent of GBC patients had a survival time of only one year after diagnosis. The survival time after 3 years plateaus at less than 20% (Figure 6).
**Figure 1:** Age-Adjusted SEER Incidence Rates Gallbladder All Races, Both Sexes, 1975-2010 (SEER 9).

**Figure 2:** Estimated New Cancer Cases In Digestive System, United States, 2014.
Figure 3: Age-Specific (Crude) SEER Incidence Rates Gallbladder Cancer All Ages, All Races, Both Sexes, 1992-2010.

Figure 4: Age-Adjusted SEER Incidence Rates By Sex Gallbladder, All Ages, All Races, 1975-2010 (SEER 9).
Figure 5: Age-Adjusted U.S. Mortality Rates for GBC, All Ages, All Races, Both Sexes, 1975-2010.

Figure 6: Relative Survival of Gallbladder Cancer By Survival Time – All Ages, All Races, Both Sexes, 1992-2009.
The 5-year relative survival for gallbladder cancer in the US has increased about 5% every 15 years, from 5% in 1975 to 10.2% in 1990, and 14.5% in 2005 (Figure 7).

The age standardized mortality rate of gallbladder cancer across the world also appears to be declining (Hariharan et al., 2008). In the US during a period from 1975 to 2010, there was also a dramatic decline by fifty percent in the mortality rate of GBC from approximately 1.4/100,000 to 0.6/100,000 (Figure 5).

There is, however, a wide variation in the rate of gallbladder cancer globally. This could be due to environmental exposure which is different in parts of the world, and the genetics aspect of different ethnicities such as the Native American variance above (Hundal & Shaffer, 2014). Despite this regional and ethnic variation, GBC generally has similar female to male ratio from 1.5 to 2.5 (Figure 4). Noted exceptions to this are Denmark, Spain, Pakistan, and Colombia where the ratio exceeds 5. Gallbladder cancer incidence is highest in areas of Asia (India, Pakistan, Korea, Japan), followed by Eastern Europe (Slovakia, Poland, Czech Republic, Yugoslavia), South America (Ecuador, Uruguay), Southern and Central Europe, Northern Europe (except Sweden), and Northern America and Australia (Randi et al., 2006).

3 Etiology

3.1 Gallstones

Cholelithiasis is the strongest risk factor for GBC, according to a meta-analysis of studies with relative risk of 4.9% [95% confidence interval (CI): 3.3-7.4] (Randi et al., 2006). This risk factor is supported by many other studies (Hamdani et al., 2012; Scott et al., 1999; Zatonski et al., 1997).
Gallstones greater than 3 cm increased the risk of GBC 9-10 times compared with the size less than 1 cm (Diehl, 1983; Lowenfels et al., 1989).

Frequency of surgical intervention for gallstone disease may account for some of the decline in incidence of GBC, particularly with the advent of improved diagnostic techniques such as nuclear medicine functional scans and ultrasound. Advanced laparoscopic techniques have furthered this. The decrease in incidence of GBC started decades prior to the application of laparoscopic cholecystectomy (Hundal & Shaffer, 2014).

3.2 Polypoid Lesions

Gallbladder polyps are usually found incidentally since they are asymptomatic. Most of them are cholesterol polyps and benign, but the adenoma-carcinoma sequence may occur in some cases. When the diagnosis of polyp is made pre-operatively features predicting malignancy include age >50 years, sessile polyps, size >10 mm and rapid growth on serial ultrasonography (Andrén-Sandberg et al., 2012; Myers et al., 2002).

3.3 Porcelain Gallbladder

Porcelain gallbladder is a term describing diffuse fibrous thickening and calcification of GB wall. It is uncommon with an incidence of no greater than 0.2% (Hayes & Muldoon, 2014; Kim et al., 2008; Towfigh et al., 2001). One study found that there were 2 types of GB calcification: selective mucosal calcification and diffuse intramural calcification, the latter being understood as porcelain GB. Only selective calcification had a significant association with GBC [Odd ratio (OR) =13.89, p<0.01] (Stephen & Berger, 2001). This is supported by other studies finding that no cases of true diffuse porcelain GB had GBC and none of the GBC cases had the features of porcelain GB (Kim et al., 2008; Towfigh et al., 2001).

3.4 Anomalous Junction of the Pancreaticobiliary Duct (AJPBD)

Anomalous junction of the pancreaticobiliary duct is a congenital malformation in which the pancreatic duct and the distal common bile duct unite outside or before they reach the duodenal wall. This anomaly is seen more commonly in Asian than Western patients (Nuzzo et al., 2004). An experimental model showed that the reflux and stasis of pancreatic juice in the bile duct stimulated inflammation and induced chronic cholecystitis with intestinal metaplasia (Nagata et al., 1985). GBC associated with AJPBD were not associated with gallstones (Kang et al., 2007; Nagata et al., 1985). Most of the pathological findings were adenocarcinoma, both poorly-differentiated and well-differentiated. The well-differentiated tumors appeared with intestinal types such as papillary, tubular, and mucinous carcinoma (Nagata et al., 1985; Tanaka et al., 1998). These histological subtypes have been seen with less invasion and longer survival time than other subtypes of adenocarcinoma of GBC, possibly due to earlier symptomatic presentation (Henson et al., 1992; Hundal & Shaffer, 2014; Nuzzo et al., 2004). This association further supports a role for chronic irritation in carcinogenesis in GBC.

3.5 Obesity

Larsson and Wolk identified obesity as a risk factor in GBC. They based this on their meta-analysis of GBC cases from the MEDLINE and EMBASE databases from 1966 to 2007. They
found from eight cohort studies and three case control studies with 3288 cases that the overweight and obese have higher risks of developing GBC with 1.15 (95% CI, 1.01-1.30) and 1.66 (95% CI, 1.47-1.88), respectively. Women had a significantly increased risk (relative risk, 1.88; 95% CI, 1.6-2.13) than did men (relative risk, 1.35, 95% CI, 1.09-1.68). In addition, their data showed that a body mass index (BMI) equal or greater than 25 kg/m$^2$ accounts for 30% of female GBC patients but only 12% of males (Larsson & Wolk, 2007).

In their prospective cohort studies from Norway, Australia, and Sweden with data collected from 1972 to 2006, Borena et al performed a multivariable adjusted analysis of z-score and found that higher BMI and blood glucose increased the risk of developing GBC, with the relative risk per unit increment of z-score for BMI of 1.35 (95% CI, 1.11-1.57) and for blood glucose 1.76 (95% CI, 1.10-2.85) (Borena et al., 2014).

3.6 Genetics

According to Fernandez et al from their study conducted in Milan, the relative risk of developing GBC in patients with a first degree relative who had a history of GBC was 13.9 (95% CI, 1.2-163.9) (Fernandez et al., 1994). The total number of GBC cases in this study was only 58 which explains why the confidence interval is widened. Another study from Sweden also showed an increased risk of familial GBC with the standardized incidence ratio of 5.21 (95% CI, 2.07-10.08) (Hemminki & Li, 2003). Counterbalancing these very high relative risks Goldgar et al found from multigenerational linked genealogical records that familial risk from first degree relatives of GBC in Utah was only 2.1 (95% CI, 0.2-6.1) (Goldgar et al., 1994).

Cholesterol excreted from the liver is regulated by adenosine triphosphate-binding cassette transporter ABCG8. Many data support the hypothesis that the polymorphism D19H of ABCG8 might affect ABCG5/G8 transporter function. 19H carriers had lower serum cholesterol suggesting that D19H increases ABCG5/G8 mediated removal of cholesterol into bile and intestine. The increased secretion of cholesterol from the liver stimulates the saturation of cholesterol in bile and formation of cholesterol gallstones. This is substantiated by studies showing that gallstones are strongly associated with the D19H variant of the ABCG8 gene and cholesterol cholelithiasis is due to hypersecretion of cholesterol from the liver (Grunhage et al., 2007; Buch et al., 2007; Srivastava et al., 2009). Thus gallstone susceptibility, in turn, plays a role in GBC.

3.7 Toxins

Tobacco use is found to be one of the risk factors of GBC in a few studies – Jain et al [OR: 4.1 (1.8-9.7); p<0.001], Yagyu et al [Hazard ratio (HR): 2.00 (0.91-4.42) for women, and HR: 2.27 (1.05-4.90 for men], Dutta et al [OR: 11 (2-71)] (Jain et al., 2013; Yagyu et al., 2008; Dutta et al., 2000). There are also some reports showing no significant increase in risk with smoking (Scott et al., 1999; Zatonski et al., 1997).

Drinking more than 72g per day of alcohol has a hazard ratio of 3.60 (1.29-9.85) for men, while there was no association between alcohol intake and GBC in women according to Yagyu et al (2008). Some occupations with reported high alcohol intake such as cooks, stewards and journalists had a 2-3 fold increase in standardized incidence ratio of GBC according to Ji et al (2005). However, there are other studies that have not found evidence of that association (Scott et al., 1999; Zatonski et al., 1997).
3.8 Micro-Organisms

There are many studies showing that a chronic typhoid carrier state can be an important risk factor for GBC (Dutta et al., 2000; Shukla et al., 2000; Tewari et al., 2010). Nagaraja and Eslick found from their meta-analysis that there was a strong association between chronic S. typhi carrier state and GBC in Southeast Asia (OR: 4.13, 95% CI: 2.87-5.94, P <0.01), and more prominent between GBC and controls without gallstones (OR: 5.86, 95% CI: 3.84-8.95, P <0.01) versus GBC and controls with gallstones (OR: 2.71, 95% CI: 1.92-3.83, P <0.01) (Nagaraja & Eslick, 2014).

Individuals, regardless of gallstones status, with positive typhoid culture have 8.47 times higher risk of developing GBC than ones with negative culture (Shukla et al., 2000).

*Helicobacter* species colonize the gastrointestinal tract of both human and animals. Gastric and enterohepatic *Helicobacter* spp are the two classes of this organism species; while *H. pylori* is included in the former, *H. bilis* belongs to the latter (Fox & Lee, 1997).

The first report of an association between *Helicobacter* sp in the gallbladder and gallstone disease came in 1998 from Fox et al (Fox et al., 1998). The following year Rudi et al reported that there was no evidence of *Helicobacter* sp in the bile of German patients with biliary disease (Rudi et al., 1999), and Bohr et al found only one case with PCR positive *Helicobacter* spp in over 99 cases including GSD, GBC and control group (Bohr et al., 2007). In 2001, Méndez-Sánchez et al showed in their study that there was no association of *Helicobacter* in Mexican patients, but did not exclude the possibility that an uncommon *Helicobacter* sp could be causative (Méndez-Sánchez et al., 2001). From these studies it was concluded that the presence of *Helicobacter* sp in the biliary tract mostly depends on ethnicity and geography (Murata et al., 2004).

This potential association between *Helicobacter* spp and biliary tract malignancies was made clearer when Zhou et al reported from their meta-analysis of ten studies published from 2002 to 2011 about infection of *Helicobacter* spp and biliary tract cancer that *H. pylori*, *H. bilis*, *H. hepaticus* and *H. ganmani* were significantly more highly represented in the biliary tract malignancy group than in the benign disease and normal groups with P=0.0001 (Zhou et al., 2013).

There have been a few studies restricted to *H. pylori* and biliary diseases. Bulajic et al published two studies about the association between *H. pylori* and benign and malignant biliary diseases. In the first study they found that the prevalence of *H. pylori* in benign biliary diseases was not significantly different compared to that of *H. pylori* in the control group (Bulajic et al., 2002). The second study, published the same year, showed that patients with gallstones were 3.5 times more likely to have *H. pylori* than a control group with p value >0.05 (95% CI, 0.8-15.8; P=0.100), and *H. pylori* was 9.9 times more frequent in biliary tract malignancies than in the control group (95% CI, 1.4-70.5; P=0.022) (Bulajic et al., 2002; Cariati et al., 2003).

In GBC patients, 32.5% of patients with gallstones had *H. pylori* versus 35.7% of those patients without gallstones. This suggests there might not be an association between gallstone formation and *H. pylori* per se. Mishra et al also found *H. pylori* present 33% of the time in GBC and 28% in GSD (control group) with P>0.05, suggesting that *H. pylori* has high prevalence in endemic regions without a resultant significant difference in GBC and GSD patients (Mishra et al., 2011). For example, in Africa there is a high rate of *H. pylori* infection, but a low rate of GBC and other biliary tract cancers (Bulajic et al., 2002).

Using PCR only to detect the presence of *Helicobacter* spp in the bile of patients with gallstone disease, Jahani Sherafat et al found that *H. pylori* was only discovered in the absence
of non-\textit{H. pylori} species. They found no association between \textit{H. pylori} and biliary tract diseases though the positive rate was 3.92\% (Jahani \textit{et al.}, 2012).

With IHC and PCR, Yakoob \textit{et al} found that there was a significantly higher incidence of \textit{H. pylori} in benign and malignant gallbladder diseases than the control group, and no positive results for \textit{H. bilis} and \textit{H. hepaticus} (Yakoob \textit{et al.}, 2011).

In conclusion, evidence of an association between \textit{H. pylori} and GBC is not clearly defined.

With other \textit{helicobacter} species however, the story appears to be developing differently. Hamada \textit{et al} found that there was a significantly higher rate (P=0.029) of \textit{H. hepaticus} in cholelithiasis and cholecystitis compared to patients with gallbladder polyps and nonbiliary diseases (Hamada \textit{et al.}, 2009); while it was not found in bile samples from many studies (Kobayashi \textit{et al.}, 2005; Jahani \textit{et al.}, 2012; Yakoob \textit{et al.}, 2011). At the present, few studies show any consistent association between \textit{H. hepaticus} and biliary diseases.

Very careful studies on the association between \textit{Helicobacter bilis} and biliary tract cancer were conducted by using PCR with a primer specific for 16S rRNA of \textit{H. bilis}. These found that \textit{H. bilis} presented at a high incidence in biliary tract and gallbladder cancer patients compared to patients with gallstone or non-biliary disease. As seen in Figure 8 the positive rates from Murata’s study were all lower than those from Matsukura’s, a fact explained by Murata’s \textit{et al} use of DNA extracted from archival specimens rather than collected fresh and stored immediately as in the latter’s study. In addition, Matsukura \textit{et al} controlled for confounding factors due to use of no antibiotics in the week before collecting bile samples. Their data suggests that \textit{H. bilis} could play a role in the pathogenesis of biliary malignancies (Matsukura \textit{et al.}, 2002; Murata \textit{et al.}, 2004).

In 2010, Takayama \textit{et al} made an effort to discover the effect of \textit{H. bilis} infection on a human bile duct cancer cell line. They found that \textit{H. bilis} infection in a human bile duct cancer cell line activates NF-kB, one of the transcription factors which in turn stimulate the production of vascular endothelial growth factor (VEGF) leading to angiogenesis, further supporting the hypothesis of \textit{H. bilis’} role in bile duct cancer (Takayama \textit{et al.}, 2010).
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<th>Tumor type</th>
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<td>Unclassified tumors</td>
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<tr>
<td>Secondary tumors</td>
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Table 1: WHO classification of gallbladder cancer (Albores-Saavedra et al., 1992).

4 Pathology

Current histological classification of GBC is given in Table 1 below. Adenocarcinoma is the most common histological type of GBC (Hamdani et al., 2012; Henson et al., 1992; Yamaguchi & Enjoji, 1988), which together with intestinal and mucinous variants accounts for over 85% of the malignancies in this organ. Neuroendocrine tumors of the gallbladder have not been extensively studied, though presumably their behavior, particularly for the well-differentiated forms, would be as difficult to predict in the GB as they are in other intestinal sites. Metastatic tumors involving the gallbladder are not infrequent, and may occasionally present as the initial presentation, particularly low grade tumors such as well-differentiated lymphoma. Papillary adenocarcinoma has the best prognosis among all histological types of GBC (Henson et al., 1992). Small cell carcinoma and undifferentiated carcinoma have the worst prognosis (Compton et al., 2012).

5 Clinical Diagnosis

Patients with GBC usually are asymptomatic; most cases are found incidentally. When symptomatic, clinical findings include weight loss, right upper quadrant abdominal pain, nausea, vomiting, anorexia, and jaundice (Hamdani et al., 2012; Mitchell et al., 2014). Laboratory findings often include low hemoglobin, high liver enzyme levels and bilirubin. GBC are diagnosed by imaging and endoscopically directed fine needle biopsy (Hamdani et al., 2012). Incidental cases (50-72% of cases) are diagnosed after cholecystectomy for acute cholecystitis or gallstone
diseases or rarely from occult metastatic disease (Compton et al., 2012; Kalita et al., 2013). Preoperative diagnosis is challenging for radiologists and even more so for clinicians. One study reported that of 63 unsuspected GBC cases diagnosed with acute cholecystitis before imaging had only 16 cases switched to GBC after imaging studies (Lam et al., 2005). So while the role of radiology in diagnosis of GBC is imperative, there are many imaging pitfalls reported after retrospective studies of this cancer. In one study with 18 cases of GBC in which 6 cases were incorrectly preoperatively diagnosed, 4 of these 6 cases showed focal wall thickening with jaundice as a symptom, 1 patient with polypoid mass clinically presented as acute cholecystitis, and the remainder with diffuse circumferential wall thickening (Mitchell et al., 2014). Another study showed GBC only correctly diagnosed preoperatively in 20%, mistaken for benign diseases in 50%, and with biliary tract tumors in another 30% (Giang et al., 2012). A study in Italy found that during a period of 8 years, there were 5 patients correctly preoperatively diagnosed with GBC and 6 cases were incidentally recognized following resection (Panebianco et al., 2012). To increase the sensitivity of imaging diagnosis of GBC, it needs to be recalled that GBC can have a variety of radiological appearances, which when combined with clinical findings will help radiologists not exclude GBC prematurely (Mitchell et al., 2014). One of the reported pitfalls is that well-differentiated adenocarcinoma with cystic component within the tumor on MRI may mimic benign adenomyomatosis. The imaging cystic pattern in adenomyomatosis has a linear, flat, round, and regular surface characteristic (Yoshimitsu et al., 2005). Differentiation of areas of wall thickening in order to diagnose GBC or benign lesions is another key in avoiding misdiagnosis. A new technique using contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS) to differentiate wall thickening is superior, and significantly increased the accuracy and the agreement among different observers compared with the conventional harmonic EUS (Imazu et al., 2014). In addition, improved diagnosis with histological evidence via EUS-guided FNA is found to be more sensitive (100%), specific (100%) and accurate (100%) with fewer adverse events than cytological study of bile juice obtained from endoscopic transpapillary gallbladder drainage (Ogura et al., 2014). Among imaging techniques, high resolution ultrasound and magnetic resonance imaging (MRI) with MR cholangiopancreatography (MRCP) were similar in their sensitivity and accuracy, while contrast-enhanced computed tomography (CT) had lower sensitivity (Bang et al., 2014). Positron emission tomography (PET) with fluorine-18-labeled fluoro-deoxyglucose (FDG) was not superior to other imaging techniques with a sensitivity and specificity of 75% and 87.5%, respectively (Koh et al., 2003).

6 Pathological Diagnosis

Macroscopically, finding a GB mass is more common overall than wall thickening, though the latter may be more frequent in occult and potentially overlooked cases. The masses are located mostly in the body and the fundus (Hamdani et al., 2012). A pathologic sampling scheme that includes three random sections representing a full longitudinal sample is more sensitive in finding metaplasia, hyperplasia, and dysplasia than one random section from the fundus or in any other GB site. With only one random section, the likelihood of discovering subtle lesions was less than one third (Duarte et al., 1993). A recent study has shown that careful macroscopic examination with no identifiable lesions had a high negative predictive value in excluding GB dysplasia and GBC (Hayes & Muldoon, 2014).
Several studies suggest a pathogenetic pathway of GBC progressing from intestinal metaplasia to dysplasia, then to carcinoma. A significant association between intestinal metaplasia and dysplasia has been found. Hyperplasia and dysplasia are both seen more frequently in association with GBC both within the neoplasm and in adjacent mucosa (Duarte et al., 1993). (Figure 9)

While making a correct preoperative GBC diagnosis is more challenging than postoperative diagnosis, there are still many pitfalls microscopically in terms of underdiagnosis, overdiagnosis and misinterpretation. For example, Rokitansky-Aschoff sinuses (RAS) often demonstrate deep penetration of the GB wall which may be diagnosed as tumor invasion, or if distended by mucin may be confused with extracellular mucin in mucinous carcinoma. Acute cholecystitis with parietal necrosis can induce epithelial atypia suggestive of an aggressive neoplastic process (Albores-Saavedra et al., 2009). (Figure 10).

Underdiagnosis occurs when a well-differentiated invasive carcinoma is confused with benign disease such as adenomyomatosis, or in a high grade tumor when extensive tumor necrosis with minimal residual viable tumor may mimic acute gangrenous cholecystitis. Thus
Figure 10: Surface Dysplasia involving Rokitansky-Aschoff sinuses, extending fully through gallbladder wall. Note the small benign residual gland of the RAS on the lower right.

thorough histologic sampling including areas without necrosis is critical in cases with extensive necrosis to reveal diagnostic viable tumor (Figure 11). When patients present with other malignancies making the diagnosis of primary GBC less likely, the possibility of a second neoplasm of the GB should not be overlooked if focal high grade glandular dysplasia or intramucosal adenocarcinoma within the gallbladder are present. GBC may also present as seemingly primary tumors metastatic to the other abdominal sites (omentum, ovary, pancreas, etc.) (Giang et al., 2012).

GBC has many modes of spread including direct spread or extension, intra-luminal, lymphatic, vascular, neural and intraperitoneal (Fahim et al., 1962). There are three levels of lymph nodes which drain the GB: 1- the lymph nodes along the biliary duct system which is the initial site of spread, 2- the lymph nodes surrounding the pancreas and the duodenum,
those posterior to the portal vein, and around the common hepatic artery, and 3- the lymph nodes of the para-aortic, superior mesenteric, and celiac vessels which, according to the American Joint Committee on Cancer (AJCC) 7th edition (2009), are considered as distant metastatic disease (Misra et al., 2003). The frequency of lymph nodes spread is strongly correlated with the depth of invasion. Lymph node spread is considered as a prognostic factor in GBC (Tsu-kada et al., 1997).

7 Staging

The AJCC 7th edition was released in 2009 with changes in stage grouping and reclassification of regional lymph nodes compared with the 6th edition, though the primary tumor definitions remained the same. The stage groupings in the 5th and 7th edition are similar, but differ in
TNM definition. In the 7th edition, metastasis to regional lymph nodes plays a big role in treatment and prognosis. N1 is limited to hepatic hilar nodes not excluding nodes adjacent to portal vein, and N2 has nodes in other regions such as periaortic, pericaval, superior mesenteric artery, and/or celiac artery which are considered distant metastatic disease and classified as stage IVB (Compton et al., 2012).

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<td>T1b</td>
<td>Tumor invades muscular layer</td>
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<tr>
<td>T2</td>
<td>Tumor invades perimuscular connective tissue; no extension beyond serosa or into liver</td>
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<td>T3</td>
<td>Tumor perforates the serosa (visceral peritoneum) and/or directly invades the liver and/or one other adjacent organ or structure, such as the stomach, duodenum, colon, pancreas, omentum, or extrahepatic bile ducts</td>
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<td>T4</td>
<td>Tumor invades main portal vein or hepatic artery or invades two or more extrahepatic organs or structures</td>
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<td>Metastases to periaortic, pericaval, superior mesenteric artery, and/or celiac artery lymph nodes</td>
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Table 2: GBC staging in the AJCC 7th edition (2009).

8 Molecular Pathology of GBC

Abundant research has sought to unravel the molecular tumorigenesis of GBC to identify potential targets for treatment and prevention. We will summarize the key learnings to date in this evolving field.
The Cadherins include a group of homologous transmembrane proteins mediating intercellular interaction of variable cell types (Hoschuetzky et al., 1994). E (epithelial)-cadherin is a Ca^{2+} ion-dependent glycoprotein belonging to the cadherin family, critical in epithelial cells adherence, suppressing invasion. Its gene (CDH1) is located on chromosome 16q22.1 (Berx et al., 1995). This protein is absent in GBC and present in chronic cholecystitis (Priya et al., 2010).

Catenins are cytoplasmic anchorage proteins, playing a key role in cadherin function by mediating connection of cadherins to the actin filament network. α-catenin does not bind directly to the cytoplasmic domain of cadherins, but mediates the cadherin-catenin complex connecting with actin filament. But β-catenin, a component of desmosomal plaques and adherens junctions, binds directly to the cytoplasmic domain of E-cadherin (Hoschuetzky et al., 1994). Both reduced E-cadherin and β-catenin expression can be seen with tumor progression and with decreased apoptosis (Hirata et al., 2007). Other studies have found that β-catenin is expressed on the cell membrane in most benign gallbladder tissues, while it appears in the cytoplasm and/or nuclei in cancerous tissues (Ghosh et al., 2013; Kimura et al., 2003; Moon et al., 2005). This suggests that alteration of β-catenin quantitatively or in its expression patterns such as shifting from membranous staining to cytoplasmic and/or nuclear staining could be a factor in GBC carcinogenesis.

Normal microvessels in tissue are composed of endothelial cells and pericytes. Pericytes help mature microvessels to be stable. Their absence is found in the leaky microvessels in tumor tissues. The intercellular complexes adhering endothelium and pericytes contain N (neural)-cadherin. The pericytes in tumor microvessels are poorly attached to the endothelium which suggests dysfunction of N-cadherin (Blaschuk & Devemy, 2009). P (placental)-cadherin is an intercellular adhesion molecule associated with undifferentiated cells in both normal adult epithelia and carcinoma (Albergaria et al., 2011). N-cadherin absence and P-cadherin presence are found to be independent factors predicting poor prognosis in adenocarcinoma, squamous cell/adenosquamous carcinoma type of GBC. They are strongly associated with tumor size, lymph metastasis and invasion (Yi et al., 2014).

P53 overexpression is found in GBC and not seen in chronic cholecystitis (Ghosh et al., 2013; Priya et al., 2010). Mutation in the p53 gene produces in abnormal p53 protein which appears to be antigenic, resulting in detectable circulating antibodies against p53. One study from India showed that one third of GBC patients significantly positive with p53 antibodies compared with the cholelithiasis (Nigan et al., 2010). Loss of the p53 tumor suppressor functionality (due either to a defective protein, or loss of function following Ab-Ag interaction) may also contribute to GBC carcinogenesis.

The tumor suppressor gene Ras association domain family 1A (RASSF1A) is hypermethylated in GB adenocarcinomas. RASSF1A has a Ras association domain; however, loss of RASSF1A expression by methylation in cancerous tissues does not require Ras activation. Hypermethylation of RASSF1A gene could be helpful for early detection as serum methylation analysis is sensitive. It also presents a potential treatment target using demethylation agents (Kee et al., 2007). There have been many studies conducted in several countries with variable findings with most of them showing low frequency of Kras expression in GBC, showing the controversy about the role of Kras in GBC pathway. (Kee et al., 2007; Pai et al., 2011).

COX-2, an isoform of cyclooxygenase enzymes, is induced by mitogens, cytokines, and growth factors. Its function is to produce prostaglandin-related inflammation and cell growth. COX-2 mRNA expression is highly increased in colorectal carcinoma tissues, and also overex-
pressed in 80% of invasive GBC suggesting a potential role of COX-2 in tumorigenesis and tumor progression (Asano et al., 2002; Moon et al., 2005). mRNA levels of COX-2 and concentration of prostaglandin E2 (PGE2), which is a product related to that enzyme, are significantly higher in pT3 and pT4 compared with those in pT1 and pT2 (Asano et al., 2002). COX-2 may be an important factor, when interacting with c-Met, beta-catenin and C-erbB2 proteins, in promoting tumor invasion. COX-2 together with c-Met, beta-catenin were found to be present in cancerous cells at the invasive tumor front but not in the central parts of the tumor, significantly associated with the depth of invasion (Moon et al., 2005).

The S100 protein (SP) family comprises more than 20 members involved in many cellular functions, especially in regulating cell growth, cell cycle, differentiation, transcription and secretion. Overexpression of SPs have been found in many types of tumors. Haptoglobin is an acute-phase protein produced in the liver to respond to inflammation, infection, injuries and also malignancies (Tan et al., 2011). Elevated concentration of haptoglobin and its altered glycoforms is reported to be associated with hepatocellular carcinoma (Ang et al., 2006). Haptoglobin-hemoglobin complex has a cytotoxic effect on liver cancer cells and prevented proliferation of cancerous cell via the apoptotic pathway (Kim et al., 1995). One study showed that increased S100A10 and haptoglobin are found in GBC tissues compared with benign GB tissues, and associated with advanced stage and poor prognosis (Tan et al., 2011).

Connective tissue growth factor (CTGF) is a key element in wound repair processing, promoting myofibroblast differentiation and angiogenesis which are similar to the desmoplastic stromal response in cancer. There is no general consensus about the role of CTGF in tumor behavior since it is associated with both suppression and progression of many types of tumors (Jacobson & Cunningham, 2012). A study in GBC showed CTGF was increased most in advanced stage, followed by early stage, then dysplasia and least in chronic cholecystitis. Ironically, high expression of CTGF was significantly associated with increased 5-year survival time compared with the low or absent expression. The authors suggested that desmoplastic action is usually seen in advanced GBC, and increased expression of CTGF in GBC cells may act as paracrine to the stromal composition that possibly reduces the tumor growth. The suggested these changes are related to the expression of some important cancer progression/suppression-related molecules or pathways, such as TGF-b, HIF-1a, beta-catenin/Tcf/MMP-7, and PI3K/AKT and offer further support for the role of inflammatory responses in GBC pathogenesis (Garcia et al., 2013).

9 Predisposition from Genetic Factors

Xeroderma pigmentosum complementation group C (XPC) is one of the nucleotide excision repair (NER) genes. Its protein recognizes and binds to damaged DNA and initiates the NER pathway. It is thought to play a role in suppression of tumorigenesis (Jiao et al., 2011). Jiao et al showed that the allele T of XPC Ala499Val polymorphism was associated with GBC in the Chinese population (Jiao et al., 2011).

Abnormal apoptosis can also lead to development of cancer. Single nucleotide polymorphisms (SNP) of death receptor DR4 haplotypes play an important role in GBC susceptibility without the influence of their ligands such as FASL (Rai et al., 2014).
ADR is an adrenergic receptor found to be related to hypomotility disorder of GSD, which, in turn, is one of the main risk factors of GBC. ADRβ3 T190C polymorphism is significantly related to GBC and GSD susceptibility with the possible mechanism of both gallstone dependent and independent pathways (Rai et al., 2014a).

CYP17 is an enzyme in sex hormone biosynthesis, and GBC has known female predilection. Rai et al found that CYP17 rs743572 is associated with GBC susceptibility in smokers only (Rai et al., 2014b). Also another polymorphism of CYP1A1-MspI [CT] was shown to increase risk of GBC in those tobacco users and in women (Sharma et al., 2014). CYP1A1 polymorphisms increase the risk of GBC as it possibly impairs xenobiotic metabolism via pathway unrelated to gallstones (Sharma et al., 2014).

10 Prognostic Markers

The markers discussed below are recently studied and mostly for assessing prognosis of GBC rather than exploring pathogenesis. Undoubtedly further investigations may however provide links in that story as well.

Transmembrane protease, serine 4 (TMPRSS4), a member of the transmembrane serine proteases is upregulated in many cancers. It is found to be significantly increased in GBC with a positive correlation with tumor stage, histologic grade, lymph node metastasis and lymphatic invasion. It is also an independent prognostic factor for GBC (Wu et al., 2014).

Reduced E-cadherin expression has a significant correlation with poor prognosis in GBC (Hirata et al., 2007). PML and p53 are also possible independent prognostic factors when both of them are normal; the 5 year survival is longer (60 months vs 11 months) when they are normal (Chang et al., 2007). Another study showed the opposite with p53 expression not correlating with survival period. But they did find mucin correlated with survival time and suggested that mucin could be a more important prognostic marker than p53 (Takagawa et al., 2005).
Mucins are glycoproteins with a mucin core protein and O-linked carbohydrates. MUC1 is thought to be an anti-adhesion molecule promoting metastasis and is expressed in many types of human tumors. Many studies have shown MUC1 expressed in GBC has an association with lymph node metastasis, lymphatic invasion and poor outcome (Ghosh et al., 2005; Kashiwagi et al., 2001; S. M. Kim et al., 2012; Xiong et al., 2012), while MUC5AC and MUC4 are inconsistent prognostic factors in GBC (S. M. Kim et al., 2012; Lee et al., 2012; Sasaki et al., 1999; Xiong et al., 2012).

The ADAMs are a multi-functional gene family of membrane proteins, regulating the extracellular matrix remodeling and cell migration. Among them, ADAM-17, known as tumor necrosis factor-alpha converting enzyme, stimulates the release of many growth factors, namely EGFR ligands, transforming growth factor (TGF-alpha), amphiregulin, heparin-binding epidermal growth factor (HB-EGFR) which are related to tumor behavior (Brou et al., 2000). Significantly increased expression of ADAM-17 is found in tumor tissues with high histological grade and pT stage. Also, over expression of ADAM-17 is associated with short survival compared with low expression. It is found to be an independent prognostic factor in GBC (Wu, et al., 2011).

The high mobility group A2 (HMGA2) protein is a nonhistone chromosomal protein highly expressed during embryogenesis, and minimally expressed or undetectable in normal adult tissues. Overexpression of HMGA2 has been found in some benign tumors such as lipoma, leiomyoma and pituitary adenoma in transgenic mice as well as in several cancers such as lung, breast, pancreatic, thyroid, and ovarian cancers (Motoyama et al., 2008). One study shows the HMGA2 expression rate is higher in poorly differentiated GBC than in the well differentiated and associated with a shorter survival period than cases with negative HMGA2 expression (Zou et al., 2012). Another study shows HMGA2 is a direct target of miR-26a which is a single-stranded, non-coding RNA molecule. It has a negative correlation with HMGA2 in GBC and can be considered as a therapeutic target for GBC patients (Zhou et al., 2014).

Tetraspanins are a family of transmembrane proteins crossing the membrane four times and interacting with many types of molecules especially integrins producing multiple functions related to cancer behavior such as spread, migration and cell adhesion. Two tetraspanins, CD82 and CD9, play roles in tumor suppression, while the other two CD151 and tetraspanin 8 are involved in promoting tumor progression (Zoller, 2009). Recently there are several studies showing the role of CD9 and CD151 in GBC. Low or absent expression of CD9 was found in poorly differentiated GBC associated with short survival period (Zou et al., 2012). Matsumoto et al. found that there was a significant difference between 5 year survival of CD151 positive and negative (P< 0.006) and by using multivariate analysis the authors showed CD151 was an independent prognostic factor with hazard ratio of 2.97 (P< 0.02) (Matsumoto et al., 2014).

CD146 is a member of immunoglobulin (Ig) gene superfamily recognized as a cell adhesion molecule (CAM) and has several names such as MUC18, A32 antigen, MCAM, MelCAM (melanoma cell adhesion molecule due to first being seen in melanoma cell), and S-Endo-1. CD146 is overexpressed in melanoma, while it is absent in carcinoma in situ and invasive carcinoma in breast cancer. Its role in cancer behavior is variable in different types of cancers (Shih, 1999). There have been four CAM families identified including integrins, cadherins, selectins and Ig gene superfamily. Among of them, CD146 has a weaker adhesion activity than the other two families or other CAMs such as ICAMs, VCAM-1, and PECAM-1. Recently CD146 has been found to have more functions beyond cell adhesion which involve development, immune response, signal transduction, mesenchymal stem cell differentiation, cell mi-
migration, and angiogenesis (Z. Wang & Yan, 2013). Wang et al. found in their study that positive expression of CD146 is higher in poorly differentiated GBC than in well differentiated GBC, and overexpression of CD146 along with high average microvessel and lymph vessel counts are correlated with poor overall survival. Moreover, CD146 was an independent prognostic factor in GBC from their study (W. Wang et al., 2012).

Polycomb group (PcG) genes are transcriptional repressors modifying chromatin state during embryogenesis by contributing to maintenance of appropriate spatial and temporal expression of homeotic genes. Those PcG genes transcribe two polycomb repressive complexes PRC1-containing more than 10 subunits including oncoprotein BMI-1 (B cell-specific Moloney murine leukemia virus integration site 1) and HPC protein family and PRC2-containing EZH2 (enhancer of Zeste homolog 2), EED (embryonic ectoderm development), SUZ12 (suppressor of zeste 12 protein homolog) and RbAp48. PRC1, PRC2 and their related product trimethylated histone involve more than 1000 silenced genes which determine cell differentiation during embryogenesis (Bracken et al., 2006). PRC2 is one of the histone methyltransferases functioning in transcriptional silencing of differentiation genes. Among the PRC2 components, EZH2 is a catalytic subunit mainly contributing to the enzyme active site when assembling with at least two other noncatalytic subunits-EED and SUZ12. EZH2 is the first subunit documented as overexpression in prostate and breast cancers compared with the other subunits, leading to hypersilencing of genes that promote differentiation (Simon & Lange, 2008). Based on the positive correlation between overexpression of EZH2 and histologic grade of many cancers, Liu and Yang searched for and found that EZH2 is overexpressed in poorly differentiated GBC with lymph node metastasis and invasion, and there was a correlation between EZH2 overexpression and shorter survival periods. It is also found that EZH2 is an independent prognostic predictor (Liu & Yang, 2011). Inhibition of PRC2 by targeting EZH2, which plays a main part in the enzyme active site or its interaction with other subunits, is a promising treatment approach. There was a study using EZH2 methyltransferase inhibitor combined with histone deacetylase (HDAC) such as suberoylanilide hydroxamic acid (SAHA), one of the most clinically advanced anticancer agent, on GBC cultured cells with a significant decrease in cancer cells (Yamaguchi et al., 2010).

Phosphatase and tensin homolog (PTEN) first discovered in 1997 has been recognized as a tumor suppressor. Its main function is dephosphorylating phosphatidylinositol-3,4,5-trisphosphate leading to activating the phosphoinositide 3-kinase (PI3K)–AKT–mammalian target of rapamycin (mTOR) pathway, which will stimulate cell growth and survival. PTEN was found to be related with PTEN hamartoma tumor syndromes including Cowden syndrome, Bannayan–Riley–Ruvalcaba syndrome, Proteus syndrome, Proteus-like syndrome and autism disorder with macrocephaly. PTEN is one of the most common suppressor genes mutated or suppressed in various cancers such as breast, lung, prostate, colon, endometrial and glioblastoma (Leslie & Downes, 2004; Song et al., 2012). Due to its central role in cancer processes, PTEN has been extensively studied and the increasing knowledge about PTEN promises a hopeful targeted therapeutic treatment. One study shows loss of PTEN is related to high grade, metastatic GBC and short survival period. The authors also found that PTEN was a prognostic predictor for GBC (Liu & Yang, 2011).

Epithelial cell adhesion molecule (EPCAM or MK-1) is a transmembrane protein found in epithelial cells responsible for the function of adhering cell to cell. Two studies show overexpression of EPCAM is related to increased overall survival and is a prognostic predictor (Ikeda et al., 2009; L. Yang et al., 2011).
Excision repair cross-complementation group 1 (ERCC1) is a nucleotide excision repair enzyme which is widely studied in cancer science due to its relationship to survival and chemotherapy treatment. ERCC1 has been studied deeply in lung cancers but with inconsistent findings about the relation between ERCC1 and overall survival in a meta-analysis study (Smirnov et al., 2015). However, a study in Chile, where GBC in women has high incidence rate, reveals that patients with ERCC1 expression have survival period of 6 times longer than do patients without ERCC1 expression (P=0.005) (Roa et al., 2011).

Raf kinase inhibitor protein (RKIP) is an inhibitor of Raf-MEK (Mitogen-activated protein kinase)-ERK (extracellular signal-regulated kinase), binding and inhibiting the kinase activity of Raf-1. Its expression is low in metastatic cancer, and meets the criteria of metastasis suppressor gene. Loss of RKIP has been shown to be related to resistance to chemotherapy and radiotherapy. (Escara-Wilke et al., 2012). A GBC study shows that reduced or loss expression of RKIP was significantly higher in GBC versus non GBC (P< 0.001), lymph node metastasis versus non lymph node metastasis (P= 0.009) and in lower overall survival versus in longer survival period (P=0.011). Also RKIP was found to be a prognostic marker for GBC. (H. S. Kim et al., 2010).

Hypoxia-inducible factor-1 (HIF-1) is a transcription factor mediating cell response to hypoxia. It has subunits alpha and beta, with the former playing a main role in this response. Hypoxia-inducible factor-1 alpha (HIF-1α) is degraded when interacting with von Hippel-Lindau (VHL), a tumor suppressor, under normoxic conditions. During hypoxia, the alpha subunit becomes stable and translocates to the nucleus to dimerize with beta subunit. The heterodimer then binds to the hypoxia response element in the promoters of targeted genes which promote cell proliferation, angiogenesis and metabolic responses to adapt with hypoxic conditions. (Kitajima & Miyazaki, 2013). Studies show that overexpression of HIF-1α and reduced expression of VHL in GBC correlate with tumor stage, lymph node metastasis, lymphatic invasion and lower overall survival period (Batmunkh et al., 2010; Z. Yang et al., 2011). A study suggests a panel using the profile of pVHL−/IMP3+/maspin+/S100P+ by immunohistochemistry staining helps to distinguish between GBC-adenocarcinoma type and benign conditions (Shi et al., 2013). Recently there are two studies using hispidulin and cordycepin as HIF-1α targeted therapeutic agents with positive results which give hope for future GBC treatment. (Gao et al., 2014; Wu et al., 2014).

These prognostic markers in GBC are summarized in Table 3 and 4.

<table>
<thead>
<tr>
<th>Poor Prognostic Markers (Overexpression)</th>
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<tbody>
<tr>
<td>ADAM-17</td>
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<tr>
<td>CD146</td>
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<tr>
<td>CD151</td>
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<tr>
<td>EZH2</td>
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<td>HIF-1α</td>
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<td>HMGA2</td>
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<tr>
<td>MUC1</td>
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<td>TMPRSS4</td>
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**Table 3:** Poor prognostic markers (overexpression)
**Better Prognostic Markers (Overexpression)**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Description</th>
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<tbody>
<tr>
<td>CD9</td>
<td>Mobility related protein 1/tetraspanin protein</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>Transmembrane protein</td>
</tr>
<tr>
<td>ERCC1</td>
<td>Excision repair cross-complementing group 1</td>
</tr>
<tr>
<td>EPCAM</td>
<td>Epithelial cell adhesion molecule (MK-1)</td>
</tr>
<tr>
<td>PTEN</td>
<td>Phosphatase and tensin homologue</td>
</tr>
<tr>
<td>RKIP</td>
<td>Raf-1 kinase inhibitory protein</td>
</tr>
<tr>
<td>pVHL</td>
<td>Von Hippel-Lindau protein</td>
</tr>
</tbody>
</table>

*Table 4: Better prognostic markers (overexpression)*

From a pragmatic standpoint however, the use of these newer markers has not yet penetrated routine clinical practice. Certain patients presenting for treatment at our facility may be tested for one or more of these but this is usually always in the context of a clinical trial or other study, and while a few of the markers (MUC-1, E-cadherin) might be on the routine test menu by IHC or other means, the remainder are not widely available for use.

## 11 Treatment

Treatment of GBC is varied and can involve surgical resection, radiation and/or chemotherapy dependent on the stage of disease at presentation.

### 11.1 Surgical management:

Surgical resection is the only curative treatment in GBC. Patients with stage I and II have potentially resectable disease while stage III or IVA can also occasionally be resected. Stage IVB patients have unresectable disease and are candidates for medical management. Ideally, surgery should be performed at high volume centers with availability of multidisciplinary care. Surgical options include cholecystectomy or extended cholecystectomy (resection of surrounding hepatic tissue segment IVB and V) and are dependent on the Tumor (T) stage of the patient. Cholecystectomy is the treatment of choice in patients with T1 disease with extended cholecystectomy an option in patients with T1b disease. This can be curative for a majority of the patients. Radical cholecystectomy (resection of the gallbladder, gallbladder fossa and lymph node dissection) was found to improve overall survival in patients with T2N0, T2N1, and T3N0 disease, but not in those patients presenting with T3N1 and T4 disease. (Foster et al., 2006). A retrospective analysis of the SEER registry showed that evaluation of lymph node (LN) status in patients with T1b, T2 and T3 disease at surgery was associated with improvement in survival. (Jensen et al., 2009). Major liver and common bile duct resection is associated with significant morbidity with no impact on survival and is recommended only in cases that warrant extensive resection to obtain clear surgical margins. (D’Angelica et al., 2009).

### 11.2 Adjuvant treatment

Patients with resected disease are at a risk of recurrence, especially those with positive surgical margins or node positive disease. They can present with locoregional recurrence involving the resection margin, porta hepatis or retroperitoneal lymph nodes or distant metastasis to the peritoneum, liver and lungs. In a study 66 % of patients with resected GBC recurred, with a
median time to recurrence of 11.5 months (Jarnagin et al., 2003). Thus, adjuvant therapy may have a role in decreasing the risk of recurrence. However, on account of the low incidence and prevalence of GBC, randomized studies are limited and majority of the evidence is based on retrospective analysis that can suffer from bias. Jarnagin et al, in a retrospective analysis of patients demonstrated a worse prognosis for patients with GBC vs. hilar cholangiocarcinoma (85 % vs. 41 %). This study concluded that the use the locoregional therapy like radiation was probably more beneficial in patients with hilar cholangiocarcinoma as they had higher locoregional recurrence rate compared to GBC (Jarnagin et al., 2003). In contrast, a retrospective analysis of the SEER database in patients with locally advanced gall bladder cancer or gall bladder cancer with tumor spread to the regional lymph nodes demonstrated an improvement in overall survival in patients who received adjuvant radiation (14 months vs. 8 months, p≤0.001) (Mojica et al., 2007). The benefit of concurrent chemoradiation with 5-Fluoropyrimidine (5FU) after adequate surgical resection has been evaluated in numerous trials that have demonstrated a possible improvement in survival in patients with locally advanced GBC, though the number of patients enrolled in these trials was small (Czito et al., 2005, Kresl et al., 2002). In addition, a large meta-analysis of adjuvant therapy in the treatment of biliary cancer demonstrated a nonsignificant improvement in overall survival with any adjuvant therapy vs. surgery alone (P = .06). There was no difference between gallbladder and bile duct tumors, with a statistically significant improvement in patients who received chemotherapy or chemo-radiation vs. radiation treatment alone (OR, 0.39, 0.61, and 0.98, respectively; P = .02). Patients with lymph node positive and R1 disease seemed to derive the maximum benefit (OR, 0.49; P = .004 and OR, 0.36; P = .002, respectively) (Horgan et al., 2012).

Adjuvant chemotherapy with gemcitabine or a 5FU backbone has also been evaluated in several trials. A phase III trial randomized patients with gall bladder cancer to adjuvant treatment with 5FU and Mitomycin C or observation after surgery. There was a significant improvement in the 5 year survival rate (26 % vs. 14.4%, p = 0.0367) and the 5-year disease-free survival (DFS) (20.3% vs. 11.6%, p = 0.0210) in patients who received adjuvant therapy vs. observation respectively. Significant improvements were also seen in body weight compared with the control. (Takada et al., 2002). Based on the available data, adjuvant external beam radiation and/or chemotherapy is recommended in patients with stage II and III GBC according to the National Comprehensive Cancer Network (NCCN) guidelines (Category 2A recommendation) (NCCN, 2014).

11.3 Unresectable/Metastatic GBC:

Chemotherapy is the mainstay for patients with metastatic or unresectable disease. The prognosis of such patients is poor and is reported to be less than 1 year across most trials. Chemotherapy has the potential to improve the survival and quality of life in patients with advanced cancer. Gimelus, et al randomized patients with pancreaticobiliary tumors to 5FU/Leucovorin, Etoposide and best supportive care vs. best supportive care. Chemotherapy led to significant improvements in overall survival (6 vs. 2.5 months) and quality-adjusted survival (Glimelius et al.,1996). Based on such studies, it is appropriate to offer chemotherapy to patients with good performance status. The most common chemotherapeutic agents used include 5FU and Gemcitabine. A combination of 5FU and cisplatin in 25 patients with biliary tract tumors resulted in a partial response in 24 % of patients. (Ducreux et al.,1998). Fifty four patients with advanced biliary tract tumors were randomized in a phase II trial
to 5FU, cisplatin and epirubicin or 5FU, etoposide and leucovorin. The patient reported symptom relief and survival was similar in both arms, but patients in the ECF arm reported less toxicities (Rao et al., 2005). Gemcitabine, an analog of deoxycytidine is active in biliary tumors, both as a single agent or in combination with other agents. (Gallardo et al., 2001). A phase III study randomized 410 patients with locally advanced or metastatic cholangiocarcinoma, gallbladder cancer, or ampullary cancer to Cisplatin and Gemcitabine vs. Gemcitabine as single agent therapy. Patients in the combination arm had a statistically significant benefit in the median overall survival (11.7 months vs. 8.1 months), median progression-free survival (8.0 months vs. 5.0 months) and the rate of tumor control (81.4% vs. 71.8%, P=0.049). The patients in the combination group experienced more neutropenia, but the incidence of neutropenia associated infections was similar in both groups (Valle et al., 2010). Based on this data, chemotherapy with Gemcitabine and Cisplatin is the standard of care in front line therapy of patients with advanced biliary cancer. Gemcitabine has also been combined with other chemotherapeutic agents like carboplatin and oxaliplatin with encouraging results.

Cholangiocarcinoma is associated with multiple genetic changes including the RAS-RAF-MEK or the PI3K-AKT-mTOR pathways amongst others. Hence, targeted agents have been used as single agents or combined with chemotherapy in an effort to improve outcomes. Erlotinib, an EGFR inhibitor, was studied as a single agent in patients with advanced biliary tract tumors who had received one prior systemic or locoregional therapy. Patients had a response rate of 8%, with 43% achieving stable disease (Philip et al., 2006). Phase II trials of Bevacizumab (a VEGF inhibitor) in combination with gemcitabine and oxaliplatin demonstrated a partial response of 40% with a median overall survival (OS) of 12.7 months. (Zhu et al, 2010). The BINGO trial randomized 150 patients to chemotherapy (gemcitabine+oxaliplatin) plus cetuximab vs. chemotherapy alone. Median progression-free survival was 6.1 months vs. 5.5 months and the median overall survival was 11.0 months (9.1-13.7) vs. 12.4 months (8.6-16.0) in the chemotherapy plus cetuximab group vs. the chemotherapy alone group (Malka et al., 2009). More data is required to determine the impact of targeted agents on this disease.

12 Conclusions

Because of its worldwide distribution and propensity to present at an advanced stage, or be missed by current diagnostic methods at an early stage, control or prevention of GBC is a challenge. Surgical interventions to remove the GB in cases with problematic stones or other risk factors appears to have had some impact in reducing overall incidence, but there are still cases that present treatment issues. Broader understanding of the molecular pathogenesis and the beginnings of screening programs using circulating biomarkers appear to offer the best hope of further impacting the burden of this malignancy for society.

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